

Practique Clinique et Investigation

HIV-Associated Thrombocytopenia: Mapping Hematological Changes in the HIV Infected and AIDS Patients

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ABSTRACT

Aim: 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. The first AIDS case in India was detected in the year 1986. Seldom studies have been conducted correlating the CD⁴ cell counts and complete blood picture including the platelet counts in the 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 in the HIV infected and AIDS patients and correlate them with the seronegative controls.

Materials and Methods: The present study was a cross-sectional, hospital-based study on subjects that were divided into 3 groups, Group A consisting of 500 patients who were healthy controls without any systemic illness; Group B consisting of 500 patients who were diagnosed as HIV infected; and Group C consisting of 500 patients diagnosed as AIDS patients depending on their CD4 cell counts. Evaluation of complete blood picture was done using Sysmex XP 100, a fully automated analyzer while CD4 cell counts were evaluated using partec cyflow counter.

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 results were found to be statistically significant with the p-value being <0.001 for hemoglobin (Hb), white cell counts (WBCs) and platelet counts in the HIV infected and AIDS patients when compared with the with the seronegative controls.

Conclusions: Hemoglobin (Hb), white cell (WBCs) and platelet counts were significantly altered in the HIV infected and AIDS patients when compared with the seronegative controls.

Keywords: *CD4 cell counts; Complete blood picture; 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].

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. There is no evidence till date that HIV transmission can occur as a result of exposure to saliva, tears, sweat and urine [4,5].

HIV can infect many tissues, however, 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]. The incidence and severity of several common cutaneous diseases are increased in patients with HIV and this correlates, in many instances, with the absolute number of CD⁴ T-helper cell counts. The cutaneous manifestations can occur in all stages of HIV disease and it is a prognostic indicator for development of AIDS [6].

India carries the third largest number of HIV infected patients in the world after South Africa and Nigeria [7]. The first AIDS case in India was detected in the year 1986 [4].

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. When the counts of granulocytes fall <500 per mm³, 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 [8-10].

Literature is replete with studies reporting several types and varying grades of hematological abnormalities with chronic thrombocytopenia as one of the most common complications of infection with HIV [11]. Also, gradually decreasing platelet counts or, thrombocytopenia develops in approximately one third of the patients infected with HIV during the course of the their infection [12,13]. Different studies have been carried-out on CD⁴ cell counts and hematologic parameters in the HIV infected patients in different parts of the world including Nigeria, Brazil, Thailand, Switzerland and Ghana, though, seldom studies have been conducted correlating the CD⁴ cell counts and complete blood picture including the platelet counts in the 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 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 which was designed to assess the in CD⁴ cell counts and complete blood picture HIV infected and AIDS patients and correlate them with the seronegative controls. The study consisted of 1500 subjects attending the outpatient Department. The said subjects were divided into 3 groups including:

Group A: consisting of 500 patients who were healthy controls without any systemic illness;

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

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

The permission from the Ethical Committee of the Institution was obtained before starting 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. The patients at the extremes of ages, pregnant women and those on chemotherapy were excluded from the study because of possible weakened immune status. All the subjects participating in the study were explained in detail about the intent of the study and a written, informed consent was obtained from each patient. The patients were, then, made to sit in the chair comfortably and a detailed history was elicited followed by clinical examination which was performed keeping in mind the protocols of the Universal Precautions for the infectious diseases in the ART Centre with the help of diagnostic instruments and artificial illumination. The findings were recorded in a specialized proforma and the patients were, then, eventually subjected to the phlebotomy procedure for the drawing of blood for the purpose of analysis.



Figure 1: Partec cyflow counter for evaluation of CD4 cell counts.

Evaluation of CD⁴ cell counts in HIV infected and AIDS patients: For evaluation of CD⁴ cell counts in the samples collected, 50 µl of EDTA anti-coagulated blood was added to 10 µl of monoclonal antibody and after 15 minutes of incubation, 1 ml of No Lyse dilution buffer was added and the sample tubes were attached to the Partec Cyflow Counter (Figure 1) for an automated evaluation of CD⁴ cell counts in the collected samples.

Evaluation of complete blood picture, hemoglobin (Hb), packed cell volume (PCV), red cell counts (RBCs), white cell counts (WBCs) and platelet counts, in HIV infected and AIDS patients: For evaluation of complete blood picture, Sysmex XP 100 (Figure 2) was used. Sysmex XP 100 was a compact, fully automated 3 part differential hematology analyzer. 50 µl of blood was taken as the sample. The process was 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 the populations of white blood cells (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 was analyzed by a sophisticated software giving a numerical representation of the likely overall distribution of the cell populations.



Figure 2: Sysmex XP 100 for evaluation of complete blood picture including the platelet counts.

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 distribution of patients based on age and gender as well as the distribution of male and female patients based on age is shown in Table 1 - Table 3.

Age group (in years)	Control Group	%	HIV Group	%	AIDS Group	%
10 - 20	40	8%	31	6.20%	16	3.20%
21 - 30	127	25.40%	193	38.60%	150	30%
31 - 40	99	19.80%	161	32.20%	181	36.20%
41 - 50	126	25.20%	79	15.80%	102	20.40%
51 - 60	73	14.60%	21	4.20%	38	7.60%
61 - 70	35	7%	15	3%	13	2.60%

Table 1: Distribution of patients based on age groups.

Gender	Control Group	HIV Group	AIDS Group
Males	291	235	259
Females	209	265	241

Table 2: Distribution of patients based on gender.

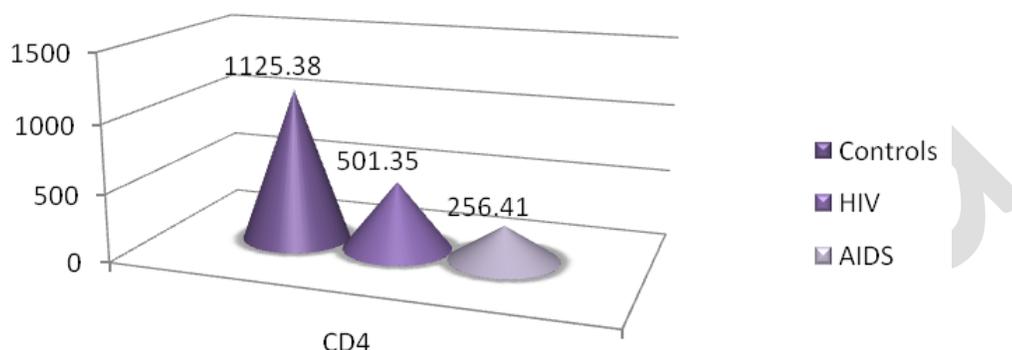
Age group (in years)	Control Group		HIV Group		AIDS Group	
	Male	Female	Male	Female	Male	Female
10 - 20	29	11	11	20	06	10
21 - 30	79	48	79	114	70	80
31 - 40	52	47	81	80	90	91
41 - 50	71	55	41	38	55	47
51 - 60	38	35	14	07	31	07
61 - 70	22	13	09	06	07	06

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

CD4 cell counts in HIV infected and AIDS patients: The mean CD⁴ cell counts in the controls was 1125.38 with a standard deviation of 154.73, in the HIV group was 501.35 with a standard deviation of 140.20 and in the AIDS group was 256.41 with a standard deviation of 67.05. The results were found to be statistically significant with the p-value being <0.001. (Table 4) (Graph 1).

	Group						p-value	Post-hoc test
	Control		HIV		AIDS			
	Mean	SD	Mean	SD	Mean	SD		
CD4 cell counts	1125.38	154.73	501.35	140.20	256.41	67.05	<0.001; Sig	C>H>A

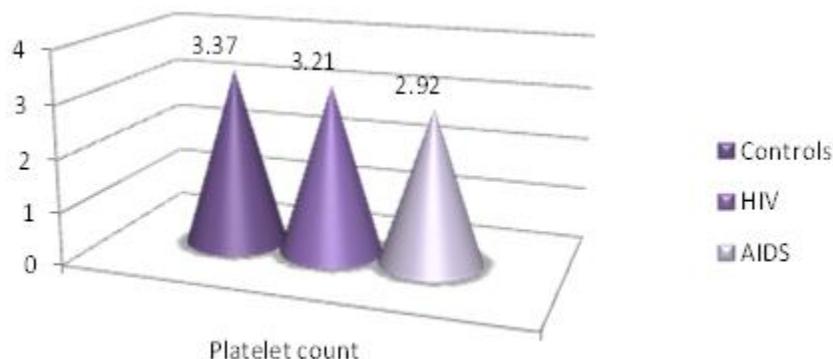
Table 4: Evaluation of CD⁴ cell counts in the three groups.



Graph 1: Mean comparison of CD⁴ cell counts between the groups.

Complete blood picture, hemoglobin (Hb), packed cell volume (PCV), red cell counts (RBCs), white cell counts (WBCs) and platelet counts, in HIV infected and AIDS patients: A mean Hb value of 13.75 with a standard deviation of 1.76 was observed in the controls while a mean value of 13.38 with a standard deviation of 1.87 was observed in the HIV group and a mean value of 12.37 with a standard deviation of 1.18 was observed in the AIDS group. The results were found to be statistically significant in this case either with the p-value being <0.001. (Table 5) A mean packed cell volume (PCV) of 37.88 with a standard deviation of 3.18 was observed in the controls while a mean PCV value of 38.23 with a standard deviation of 21.24 was observed in the HIV group and a mean PCV value of 37.63 with a standard deviation of 5.46 was observed in the AIDS group. The p-value, though, was not found to be statistically significant. (Table 5) A mean red cell count (RBCs) of 4.59 with a standard deviation of 0.43 was observed in the controls while a mean value of 4.57 with a standard deviation of 0.74 was observed in the HIV group and a mean value of 4.64 with a standard deviation of 0.73 was observed in the AIDS group. The p-value in this case, too, was not found to be statistically significant. (Table 5) A mean white cell count (WBCs) of 8134.84 with a standard deviation of 3988.69 was observed in the controls while a mean value of 9688.40 with a standard deviation of 2813.78 was observed in the HIV group and a mean value of 10264.00 with a standard deviation of 5819.57 was observed in the AIDS group. The p-value in this case was found to be statistically significant with it being <0.001. (Table 5) A mean platelet count of 3.37 with a standard deviation of 0.66 was observed in the controls while a mean count of 3.21 with a standard deviation of 0.64 was observed in the HIV group and a mean count of 2.92 with a standard deviation of 1.91 was observed in the AIDS group. The p-value in this case, too, was found to be statistically significant being <0.001. (Table 5) (Graph 2) To summarize, the levels of hemoglobin (Hb), white cell counts (WBCs) and platelet counts showed statistically significant results with the levels of hemoglobin (Hb) and platelet counts significantly decreased in the AIDS group when compared with the HIV group and the controls while on the other hand, the

levels of white cell counts (WBCs) were significantly increased in the HIV and AIDS groups as against the controls (Table 5) (Graph 2).



Graph 2: Mean comparison of platelet counts between the groups.

	Group						p-value	Post-hoc test
	Control		HIV		AIDS			
	Mean	SD	Mean	SD	Mean	SD		
Hemoglobin (Hb)	13.75	1.76	13.38	1.87	12.37	1.18	<0.001; Sig	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; Sig	H,A>C
Platelet counts	3.37	0.66	3.21	0.64	2.92	1.91	<0.001; Sig	C>H>A

Table 5: Evaluation of complete blood picture and their mean comparison between the groups.

DISCUSSION

Human immunodeficiency virus infection (HIV)/acquired immune deficiency syndrome (AIDS) is the most deadly disease which causes devastation to the body by affecting the host's immune system [14]. The pathogenesis of HIV infection is largely attributed to the decrease in the number of T cells (a specific type of lymphocytes) that bear the CD⁴ cell surface receptors (CD⁴⁺). The immune status of a child and/or, adult with HIV can be assessed by measuring the absolute number (per mm³) or, percentage of CD⁴⁺ cells and this is considered as the standard way to assess and characterize the severity of HIV-related immunodeficiency. Progressive depletion of CD⁴⁺ T cells is associated with progression of HIV disease and an increased likelihood of opportunistic infections and other clinical events associated with HIV including wasting and death. The normal absolute CD⁴ cell counts in adolescents and adults range from 500 cells per mm³ to 1500 cells per mm³ of blood with the CD⁴ count progressively decreasing with increasing severity of the infection.

The proposed immunological classification outlines four bands of HIV-related immunodeficiency as none, mild, advanced to severe immunodeficiency. The likelihood of disease progression to AIDS or, death without anti-retroviral therapy (ART) increases with increasing immunodeficiency (decreasing CD⁴ cell counts), opportunistic infections and other HIV-related

conditions increasingly likely with falling CD⁴ cell counts, especially, below 200 cells per mm³ of blood. Response to ART is affected by the immune stage at which it is started with individuals commencing ART with advanced immunodeficiency (CD⁴ cell counts >200-350 per mm³) to have better virological outcomes than those who commence with more severe immunodeficiency. Adults starting ART with CD⁴ cell counts <50 per mm³ have a much greater risk of death while on the contrary, adults who commence ART with mild immunodeficiency do not appear to obtain any additional benefits. Pregnancy does affect the CD⁴ cell counts although the significance of these changes is not clearly understood and for practical purposes, the immunological classification remains the same. The present study was carried-out to evaluate the CD⁴ cell counts, complete blood picture and lipid profile in HIV infected and AIDS patients and correlate them with the seronegative controls.

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 [15]. Different methods have been implemented in evaluating the CD⁴ cell counts by different authors. Chanarat et al. [16] used Coulter manual CD⁴ kit for evaluating the CD⁴ cell counts. Ghate et al. [17] estimated the CD⁴ cell counts by using a formula where total leucocyte count was multiplied by lymphocyte percentage and divided by 100 and then, multiplied by 100th part of CD⁴ percentage. Pasupathi et al. [12] and Srirangaraj S and Venkatesha D [18] estimated the CD⁴ cell counts by using Fluorescence Activated Cell Sorter (FACS) count system.

On the contrary, Sharma et al. [9] estimated the CD4 cell counts using flow cytometry (SRL, Ranbaxy). Ana Luiza Dias Angelo et al. [19] estimated the CD⁴ cell counts using automated flow cytometer software (multi-set). Tiwari et al. [20] estimated the CD⁴ cell counts using flow cytometry absolute cell count system at NPHL. Dora Mbanya et al. [21] estimated the CD⁴ cell counts using conventional flow cytometry using a Becton-Dickinson FACScount. Sen et al. [22] estimated the CD⁴ cell counts using FACS Counter. Edathodu et al. [23] estimated CD⁴ cell counts by standard flow cytometry using FACS Calibur. Pranitha SS and Kulkarni MH [15] estimated CD⁴ cell counts in BD FACS Calibur flow cytometer, an automated multi-color system. In the present study, Partec Cyflow counter 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.

The mean CD⁴ cell counts was found to be 1125.38 with a standard deviation of 154.73 in the controls, 501.35 with a standard deviation of 140.20 in the HIV group and 256.41 with a standard deviation of 67.05 in the AIDS group in the present study. The results were found to be statistically significant with the p-value being <0.001. A gradual decrease in the CD⁴ cell counts observed in HIV infected and AIDS patients in the present study when compared to the controls were still found to be higher than the mean values observed in the 2 studies conducted by Pasupathi et al. [12,24] who recorded a mean CD⁴ cell count of 394 in HIV infected and 191 in AIDS patients and 375 in HIV infected and 150 in AIDS patients although the results obtained were found in accordance with the results obtained in the studies conducted by Tiwari et al. [20] who recorded a mean value of 281 cells per mm³ and Sharma et al. [25] who observed a mean CD⁴ cell count of 622.4 in HIV infected and 245.39 in AIDS patients as against 798.81 in the control group.

The values obtained in the present study were, also, found to be slightly higher than the values obtained in the study conducted by Sharma et al. [9] who divided the patients based on their CD⁴ cell counts into 3 groups with group I (10-300), group II (301-600) and group III (>600 cell counts) and obtained a mean of 163.43 in group I, 325 in group II and 502.33 in

group III. 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, HIV infected and AIDS patients were categorized based on their CD⁴ cell counts with 10 cells per mm³ - 350 cells per mm³ and 350 cells per mm³ - 500 cells per mm³ of blood.

Tiwari et al. [20] 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, Tiwari et al. [20], also, 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. Pasupathi et al. [26] estimated the RBC, WBC and platelet counts and hemoglobin using fully automated hematology 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 patients compared to the HIV infected patients and controls. De Santis et al. [27] estimated blood cell counts by using an ABX Pentra 120 DX automated hematology analyzer. Sen et al. [22] estimated blood cell counts by hematology analyzer and hemoglobin by cyanmethemoglobin method. Aryee Tagoe and Asantewaa [28], 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 MH [15] 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 patients when compared to the HIV infected and controls.

In the present study, Sysmex XP 100 automated analyzer was used for the evaluation of complete blood picture as it has been said to be more reliable, accurate and less time taking than other methods. The results of the present study were in accordance with the results of the studies conducted by Pasupathi et al. [12] and Mbanya et al. [21] who observed decreased levels of RBC counts, platelets and hemoglobin and increased levels of WBC counts in the HIV infected and AIDS patients. The mean value of hemoglobin was 12.37 in AIDS patients in the present study which was in close relation to the hemoglobin level of 11.34 reported in the study conducted by Treacy et al. [29] while slightly higher than the mean value of 10.20 as reported by Pranitha SS and Kulkarni MH [15], a mean value of 10.20 as reported by Daniel Nii Aryee Tagoe and Evelyn Asantewaa [28] and 10.8 as reported by Kaloutsi et al. [30]. 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 was, also, found to be in accordance with the result reported by Erhabor O et al. [31]. Furthermore, the degree of thrombocytopenia observed was found to be directly related to the degree of immunosuppression as was confirmed in the study conducted by Jost et al. [32]. Pranitha SS and Kulkarni MH [15], Costello [33] and Karcher and Frost [34], also, reported the prevalence of thrombocytopenia in their respective studies. According to Pranitha SS and Kulkarni MH [15], 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.

Akinbami et al. [35], also, reported a high prevalence of thrombocytopenia in their study. The possible mechanisms for the same as reported by them in their study included an increased destruction of the blood platelets or, thrombocytes either due to non-specific deposition of the circulating immune complexes (CICs) on platelets or, by the presence of specific anti-platelet antibodies directed against the platelets. Also, direct infection of the megakaryocytes by the human immunodeficiency virus was hypothesized as one of the possibilities for a considerable decrease in the platelet counts during the HIV infection affecting platelet production.

The results of the present study were, also, found to be in accordance with the results of study conducted by Mir et al. [36] who reported anemia, thrombocytopenia and various permutations in majority of the HIV infected patients. According to the results obtained by Walsh et al. [37], Karpatkin [38] and Harbol et al. [39], chronic thrombocytopenia develops in approximately one third of the individuals infected with the human immunodeficiency virus during the course of acquired immune deficiency syndrome (AIDS).

The findings of the present study, though, were not found to be in accordance with the study conducted by Aryee Tagoe and Asantewaa [28] who observed higher platelet counts in HIV positive patients compared to the HIV 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 HIV infection, opportunistic infections or, lymphoma as well as the side effects of the drugs used to treat HIV itself or, the compounding infections and other factors and/or, lymphomas usually seen in this set of 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, also, lead to a subsequent decrease in the WBC counts with associated risk of increased infections in these patients.

CONCLUSIONS

Hemoglobin (Hb), white cell (WBCs) and platelet counts were significantly altered in the HIV infected and AIDS patients when compared with the controls. Further studies are, thus, mandated from across the country with correlation analyses to come to valid conclusions and manage this deadly infectious disease process.

STRENGTHS OF THE STUDY

The potential points of the present study included the participation of equal number of controls, HIV infected and AIDS cases. Furthermore, automated analyzers were used to evaluate the CD⁴ cell counts and complete blood picture in the HIV infected and AIDS patients which were more exact than the traditional methods that have been followed before. Till date, very few studies included these three different parameters in one study. The statistical analysis of the data was, also, done using appropriate statistical tests including ANOVA and post-hoc Games-Howell test for the sake of comparison of the studied parameters in between the groups. All the required standardized precautions were taken during the conduct of the entire study protocol while the ethical concerns were, also, taken into consideration maintaining the privacy of the controls

and the patients included. The inclusion and exclusion criteria were stringently followed while a systematic methodology was followed throughout the study protocol starting from sample selection to doing the statistical analysis of the findings obtained.

LIMITATIONS OF THE PRESENT STUDY

The major limitations of the study were in the facts that the duration of the disease process was not taken into consideration while the present study did not take into consideration the duration of the ART taken by the patients, too, distinguishing between the patients as this was not a longitudinal study where a patient follow-up could be done.

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