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Effect of HIV Infection on Some Haematological Parameters and Immunoglobulin Levels in HIV Patients in Benin City, Southern Nigeria

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Abstract

This research is aimed at determining the effect of HIV infections on some haematological and immunological parameters. One hundred and fifty subjects were recruited for the studies (50 HIV seropositive subjects not on ART, 50 HIV seropositive subjects on ART and 50 HIV seronegative controls). Informed consent was obtained from all subjects. Ethical approval was obtained from the Ethics Committee of the Faculty of Health Sciences and Technology, Nnamdi Azikiwe University. Five millilitre of blood was collected from each subject for HIV test by ELISA, CD4 count by Cyflow technique, immunoglobulin test by immunoturbidimetric method, estimation of FBC by automated analyzer and ESR by Westergren method. Statistical package for social science (version 20) was used for the data analysis. Results shows that the mean ± SD of WBC was significantly higher in control than HIV subjects on ART ($p<0.05$). IgG and ESR was significantly increased in HIV positive subjects on ART and non-ART than control ($p<0.05$), while HGB and HCT was significantly lower in HIV positive subjects on ART and HIV subjects not on ART than control ($p<0.05$). However there was a non-significant increase in CD4 count and IgA in control compared to HIV subjects on ART and those not on ART, while there was a non-significant decrease in IgM in control compared to HIV subjects on ART and those not on ART ($P>0.05$). IgG was significantly higher in female HIV subjects on ART compared to the males while IgM was significantly higher in male HIV subjects not on ART than the females ($P<0.05$). There is an elevation of ESR and IgG levels in HIV infected subjects with a decrease in HGB and HCT value. The elevated IgG could be attributed to infection requiring IgG response, while elevated ESR could be an indicator of inflammatory response. Immunoglobulin could be used as a predictive marker for monitoring HIV, alongside haematological parameters.

Keywords:

HIV; Immunoglobulins; Antiretroviral therapy; ESR

Abbreviations:

HIV: Human Immunodeficiency Virus; FBC: Full Blood Count; ART: Antiretroviral Therapy; IgM: Immunoglobulin M; IgG: Immunoglobulin G; ESR: Erythrocyte Sedimentation Rate

Introduction

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) and it causes Acquired immunodeficiency syndrome [1]. HIV infects thymus-derived cells (T cells) by means of a glycoprotein-120 (gp 120) embedded in its envelope. The virus is found in highest concentrations in blood, semen, vaginal and cervical fluids of the human body. Although the primary route of transmission is through sexual intercourse, HIV is also spread by the use of infected needles among intravenous drug users, exchange of infected blood products, and from an infected mother to her foetus during pregnancy [2].

The incidence of HIV/AIDS particularly in Nigeria and the World in general is of major concern to healthcare practitioners. There were 34 million people living with HIV at the end of 2010 and 1.8 million people died from AIDS-related causes in 2010 [3]. Nigeria has the second highest number of people living with HIV in the world after South Africa. Nigeria with about 2.98 million people living with HIV (PLWH) makes about 9% of the global HIV burden. The epidemic in the country can be described as heterogeneous, with various communities in different stages, some declining while others are still rising [4].

Immunoglobulin (Ig) also known as antibody is a large γ-shaped protein produced by B-cells that is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses [5]. There are five different isotypes, namely IgA, IgG, IgM, IgD andIgE. IgM is the first antibody to appear in response to initial exposure to an antigen. They appear early in the course of an infection and usually reappear, to a lesser extent after further exposure. Diagnostically its presence in patient's serum indicates recent infection. IgA on the other hand plays a critical role in mucosal immunity while IgG protects the body from infectious pathogens such as viruses, bacteria and fungi.

Clinically significant haematologic abnormalities are common in persons with HIV infection. Impaired haematopoiesis, immune-mediated cytopenias and altered coagulation mechanisms have all been described in HIV-infected individuals. These abnormalities may occur as a result of HIV infection itself, as sequelae of HIV-related opportunistic infections or malignancies or as a consequence of therapies used for HIV infection and associated conditions.

Hence, this present work will investigate the changes in some haematological and immunological parameters in HIV positive subjects on ART and not on ART.

Materials and Methods

Selection of subjects

One hundred and fifty (150) subjects aged 18-67 years were recruited from the HIV unit of Central Hospital, Benin City as follows;

- Fifty HIV positives subjects not on anti-retroviral therapy
- Fifty HIV positives on ART subjects
- Fifty apparently, healthy subjects from the staff and student population of same hospital.

Subjects on antiretroviral therapy were on triple combination therapy consisting of Zidovudine (300 mg), Lamivudine (150 mg) and Nevirapine (200 mg) taken twice daily [2].

Ethical Consideration and Informed Consent

Informed consents were obtained from all the participants. The ethical approval for the study was obtained from the Ethics Committee of the Faculty of Health Sciences and Technology, Nnamdi Azikiwe University.

Sample Collection

Seven millilitres of blood (7 ml) was collected and dispensed as follows; Three (3) ml of blood into tripotassium Ethylene diamine tetra-acetic acid (K_3EDTA) vacutainer for CD4 count by flow cytometric method, and determination of haematocrit, haemoglobin level, total white cell count and differential white cell count using an auto-analyser and ESR using standard routine methods.

Four (4) ml into plain vacutainer. This was used for HIV tests by immunochromatographic methods and immunoglobulin G, A and M analysis by immunoturbidimetric method, using spectrophotometer.

Exclusion Criteria

1. Pregnant women.

2. Subjects with malignancy demanding cytotoxic chemotherapy or radiation therapy.

3. Patients at the extremes of age were excluded since they may naturally have very weakened immune system [6].

Laboratory Methods

HIV-1/2 assay as described by alere medical company limited, Japan

Procedure

The protective foils were removed from each test strip. Fifty microliter (50 µl) of plasma was dispensed into the specimen pad of the test strip with the aid of precision pipette. Chase buffer was added to the sample pad after one minute waiting. The reaction was allowed for 15 min (up to 60 min). The appearance of distinct red lines on test region and control region of the kit suggests positive HIV test while one distinct red line in the region of control suggested HIV negative test. Appearance of the distinct red line on the control region validated the HIV result and without that, the kit was assumed to be invalid (i.e., the HIV result cannot be accepted or it is considered invalid).

HIV-1/2 stat-pak assay as described by chembio diagnostic systems incorporated

Procedure

The Chembio HIV 1/2 STAT-PAK test devices were removed from its pouch and placed on a flat surface. The test device was labeled with the patient's identification number. Five microlitre (5 µl) sample loop was used to collect the specimen. The sample loop was held vertically and; used to touch the sample pad in the sample well and dispensed. 3 drops (about 105 µl) of the running buffer bottle was added drop wise into the sample well.

Uni-Gold HIV test (as described by trinity Biotech Plc, Ireland)

Procedure

The kit was taken to room temperature by allowing it to stand for 20 min on the work bench. The Uni-gold HIV test devices from their protective wrappers was removed and

labeled with appropriate identification number. The disposable pipette was used to transfer the plasma from the bottle. The pipette was held over the sample port and two drops of sample (about 60 µl) was added carefully. 10 min interval was allowed from the time of wash reagent addition for reaction to occur. The result was read at the end of 10 min incubation. Result should not be read after 20 min following sample addition.

Cyflow counter for automated CD4 cell count (as described by partec, Germany)

Procedure

Twenty microliter (20 µl) of K_3ETA whole blood was collected into Partec test tube (Rohren tube). Twenty microliter (20 µl) of CD4+ antibody was added into the tube. The contents were mixed and incubated in the dark for 15 min at room temperature. 800 µl of CD4 buffer was gently added into the mixture and mixed gently. The content of the Partec tube was displayed as peaks and interpreted {i.e., the results of each test will be read by the cyflow counter as CD4+ cells (cell/µl) on the liquid crystal display}.

Determination of immunoglobulin G, A, and M by using immune turbidimetric methods (as described by linear chemicals, Spain)

Procedure

The immunoglobulin reagent and the Chemwell autoanalyser were pre-warmed to 37°C. Ten microlitre (10 µl) of reagent was used for IgM while 7 µl was used for IgG and IgA respectively. This was added to 1 ml of sample.

The computer interphase attached to the Chemwell autoanalyser was programmed to suite our analytical requirement and the process for analysis of IgA, IgG and IgM were undertaken according to predefined program. The results obtained were recorded in mg/dl.

Determination of full blood count using automated analyser (as described by sysmex, Germany)

Procedure

The automatic voltage regulator, the uninterrupted power supply unit and the power button on the Sysmex KX-21N Haematology analyser were turned on. The "ready" for analysis was allowed to appear and there was a temporary wait for the analyser to come to "ready state". Then, the sample numbers of the subject entered into the device. Five millilitres (5 ml) of blood collected in K_3EDTA vacutainer was mixed gently. The stopper of the vacuutainer was opened to start analysis. The tube was set to the sample probe and the start switch was pressed so that the device can aspirate the required volume. The tube was held against the sample probe until the buzzer sounds twice. At the end of each analysis, the device displays and prints a hard copy of the report for onward documentation. After the printing, it goes to ready state so that it can analyse another sample.

Erythrocyte sedimentation rate

Procedure

Trisodium citrate solution (0.4 ml) was dispensed into a unit with the help of a pipette. 1.6 ml of K_3EDTA blood was dispensed into the tube containing the trisodium citrate solution. The blood and trisodium citrate solution was mixed properly [7]. Then, a clean and dry Westergren (ESR) tube was inserted into the plastic tube containing the mixture of blood and trisodium citrate until it gets to the zero (0) mark. The tube was placed firmly on the tube holder. The timer was set for 60 min before reading.

Statistical Analysis

Statistical package for social science (version 20) was used for the statistical analysis. The variables were expressed in means and standard deviation. The parameters of control subjects were compared with HIV positives and HIV positives on ART by using ANOVA. The relationship between groups were done by comparing control and HIV positives, controls and HIV positives on ART, HIV positives and HIV positives on ART, male versus female using student t test. Level of significance was considered at $p<0.05$.

Results

Table 1 shows that mean ± SD value of ESR was significantly higher in HIV positives not on ART and HIV positives on ART when compared with control subjects in each case ($p<0.05$).

Mean value of HGB (g/dl) and HCT (%) were significantly higher in control subjects when compared with the HIV positive on ART and HIV positive subjects not on ART in each case ($p<0.05$).

Table 1 Mean ± SD of parameters in control subjects, HIV positive subjects on ART and HIV positive not on ART ($p<0.05$ is significant, while $p>0.05$ is not significant. F (p) value=mean ± SD of the parameters in control subjects, HIV positive subjects on ART and HIV positive subjects compared (using ANOVA); p value of 1 vs. 2=mean ± SD of parameters between control and HIV positive subjects on ART compared (using t test); p value of 1 vs. 3=mean ± SD of parameters between control and HIV positive subjects compared (using t test); p value of 2 vs. 3=mean ± SD of parameters between HIV positive subjects on ART and HIV positive subjects compared (using t test)).

Parameter	ESR (mm/h)	HGB (g/dl)	HCT (%)	CD4 (cells/µl)
(1) Control subjects (n=50)	22.70 ± 12.59	12.32 ±2.07	39.02 ± 5.65	605.73 ± 406.13
(2) HIV positive on ART(n=50)	49.50 ± 26.67	10.87 ± 1.72	34.45 ± 4.57	468.73 ± 252.15
(3) HIV positive subjects(n=50)	44.03 ± 24.19	10.60 ± 1.54	34.11 ± 5.35	435.23 ± 238.03
F (p) value	16.54 (0.00)	10.80 (0.00)	11.08 (0.00)	3.43 (0.04)
p value of 1 vs. 2	0.00*	0.00*	0.00*	0.17
p value of 1 vs. 3	0.00*	0.00*	0.00*	0.06
p value of 2 vs. 3	0.6	0.74	0.95	0.82

The CD4+ T cell count showed no significant difference among these groups. **Table 2** shows that the mean ± SD value of WBC (× 10^3/µl) was significantly higher in control subject when compared with HIV positive subjects on ART. However no significant difference existed in the valuables amongst the groups compared in the table.

Table 2 Mean ± SD of parameters in controls, HIV positives on ART and HIV positives not on ART (Key: p< 0.05 is significant, while p>0.05 is not significant (F (p) value=mean ± SD of the parameters in control subjects, HIV positive subjects on ART and HIV positive subjects compared (using ANOVA); p value of 1 vs. 2=mean ± SD of parameters between control and HIV positive subjects on ART compared (using t test); p value of 1 vs. 3=mean ± SD of parameters between control and HIV positive subjects compared (using t test); p value of 2 vs. 3=mean ± SD of parameters between HIV positive subjects on ART and HIV positive subjects compared (using t test)).

Groups	IgA (mg/dl)	IgG (mg/dl)	IgM (mg/dl)
(1) Control subjects (n=50)	350.25 ± 107.45	1853.48 ± 666.10	196.80 ± 87.02
(2) HIV positive subjects on ART(n=50)	319.00 ± 163.71	2955.53 ± 607.62	233.25 ± 118.23
(3) HIV positive subjects (n=50)	288.23 ± 156.29	2580.23 ± 1059.69	247.08 ± 132.45
F (p) value	1.84 (0.16)	19.46 (0.00*)	2.07 (0.13)
p value of 1 vs. 2	0.57	0.00*	0.27
p value of 1 vs. 3	0.1	0.00*	0.12
p value of 2 vs. 3	0.67	0.14	0.88

Table 3 shows that the mean ± SD value of IgG (mg/dl) of HIV positives on ART and HIV positive subjects were significantly higher when compared with control subject (p<0.05). The other valuables compared this table, showed no significant difference.

Table 3 Mean ± SD of parameters in controls, HIV positives on ART and HIV positives not on ART (Key: p<0.05 is significant, while p>0.05 is not significant. F (p) value=mean ± SD of the parameters of controls, HIV positive subjects on ART and HIV positive subjects compared (using ANOVA); p value of 1 vs. 2=mean ± SD of parameters between control and HIV positive subjects on ART compared (using t test); p value of 1 vs. 3=mean ± SD of parameters between control and HIV positive subjects compared (using t test); p value of 2 vs. 3=mean ± SD of parameters between HIV positive subjects on ART and HIV positive subjects compared (using t test)).

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F (p) value	1.84 (0.16)	19.46 (0.00*)	2.07 (0.13)
p value of 1 vs. 2	0.57	0.00*	0.27
p value of 1 vs. 3	0.1	0.00*	0.12

p value of 2 vs. 3	0.67	0.14	0.88

Table 4 shows the mean value of IgG was significantly higher in female HIV subjects on ART when compared to the males, while IgM was significantly higher in male HIV subjects not on ART when compared to the females (P<0.05). However all the other valuables compared amongst the groups were statistically similar in values (p>0.05).

Table 4: Mean ± SD of parameters compared in controls, HIV positives on ART and HIV positives not on ART based on gender (Key: p<0.05 is significant, while p>0.05 is not significant).

Groups	Gender	IgA (mg/dl)	IgG (mg/dl)	IgM (mg/dl)
HIV positive on art	Male	291.45 ± 118.88	2625.45 ± 429.98	220.27 ± 114.34
	Female	391.73 ± 220.20	3221.45 ± 770.95	273.45 ± 152.04
P-value		0.18	0.04*	0.44
HIV positive subjects	Male	230.38 ± 167.87	2633.75 ± 1159.99	357.75 ± 173.47
	Female	236.75 ± 109.90	2177.13 ± 1009.42	153.13 ± 53.04
P-value		0.35	0.38	0.02*
Control subjects	Male	360.92 ± 91.62	1883.69 ± 575.76	187.69 ± 95.63
	Female	376.70 ± 94.78	2011.62 ± 776.64	218.23 ± 93.64
P-value		0.66	0.71	0.45

Discussion

In this study, erythrocyte sedimentation rate (ESR) obtained for control was low compared to HIV positives on ART as well as HIV positive not on ART. This agrees with the knowledge that, ESR is often raised in infections and inflammatory conditions like HIV. The increase in ESR in these conditions is attributed to increased production of acute phase proteins and releases of proteins by the causative organism into the circulation. Hence, ESR can be used as sensitive index of plasma protein changes, which result from inflammation or tissue damage in HIV infection [8]. However this finding of a raised ESR is at variance with a previous study [9, 10].

Likewise, HGB and HCT were higher in the control subjects when compared with HIV positives on ART and HIV positive subjects in each case. This agrees with the work of Moore [11] which explained that anaemia has been shown to be a risk factor for early death in patients with AIDS, and that the causes of HIV-related anaemia are multifactorial [12-14]. This might also be due to generalized pancytopenia usually caused by chronic condition like HIV infection.

This research found that the WBC ($\times 10^3/\mu l$) for HIV positive on ART was low compared to the controls. This supports a previous work by Anyaechie [14] which revealed that there was a definite pattern of leucopenia in HIV subjects and concluded that reduced leucocyte profile might thus be an additional indication of HIV infection. The reduced levels of leucocytes observed in this work might also be due to generalized pancytopenia due to chronic infections like HIV.

The mean IgG level was higher in control subjects than HIV positive subjects on ART and HIV positives (in each case). This increase could have a link with the increased need for IgG to clear the HIV virus from the system. However, Ifeanyichukwu [15] pointed out that the increase in IgG concentration in HIV infected participants may suggest evidence of increased opportunistic infection requiring IgG response. They also noted that the observed increase in serum IgG suggest the involvement of this class of immunoglobulin in possible protective immunity. This research also agrees with the previous findings by Panagiotis [16] that significant proportions of HIV positive patients have elevated level of one or more immunoglobulin, usually IgG. They also emphasized that the magnitude of the IgG elevation is modest in most patients. In their work, 10% of patients had IgG levels more than double the upper limit of the reference range. This varies with a previous study by Redgrave [17] that reported that a viraemic HIV-infected patients receiving HAART have lower plasma levels of IgG and IgA than viraemic HIV-infected patients.

IgM showed a non-increase in HIV subjects than control. These affirm the findings of Chess [18] that elevated levels of immunoglobulin have been described in patients with AIDS since at least 1984. This also agrees with the notion that IgM is frequently associated with the immune response to antigenically complex blood-born infectious organisms, hence, the significant increase in HIV seropositive patients.

IgM was higher in HIV positive male subjects compared to the females while IgG was significantly higher in HIV positive females on ART compared to the males. The reason for this gender differences was not obvious.

Conclusion

There is an elevation of ESR and IgG levels in HIV infected subjects with a decrease in HGB and HCT values.

Recommendation

Future research should focus on ascertaining the role of viral load in the haematological and immunological variations observed in this study.

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This research did not receive any specific grant from any funding agency in the public, Commercial or non-profit sector.

Consent

The aim and details of the study was explained to the subjects and a written informed consent obtained before they were recruited for the study.

Ethical Approval

Ethical approval was sought and obtained from the ethics committee of the Faculty of Health Science and Technology, Nnamdi Azikiwe University, Nnewi Campus.

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