

The Relationship between Haematologic indices/Immunologic markers and HIV disease in Antiretroviral-naïve HIV seropositive Individuals in the Niger Delta Region of Nigeria.

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Abstract: *Aims and objectives:* To determine the relationship between Haematologic indices and immunologic marker in prediction of HIV disease in patients in the Niger delta region of Nigeria

Methodology: Fifty anti-retroviral drugs naïve HIV-infected adult subjects aged 20-55 years and 50 matched controls were recruited from January-December, 2009 at Haematology clinic of the University of Port Harcourt Teaching Hospital. Samples were collected for complete blood count, Erythrocyte Sedimentation Rate, Retroviral screening and confirmation, and CD4 positive T-lymphocyte count.

RESULTS: The mean ESR in HIV-infected patients (97.46 ± 39.9 mm/hr) and that of the control was (10.1 ± 5.7 mm/hr). The mean Haemoglobin concentration, ALC, Platelet count and CD4 positive lymphocyte count of HIV-infected patients were 10.9 ± 2.0 g/dl or 0.33 ± 0.06 L/L, 3283 ± 691 cells/mm³, 157000 ± 81979 cells/ μ l and 217.0 ± 144.8 cells/ μ l respectively and that of the control were 14.3 ± 1.5 g/dl or 0.43 ± 0.045 L/L, 4746 ± 1263 cells/mm³, 197660 ± 48742 cells/ μ l and 896.2 ± 198.0 cells/ μ l respectively. CD4 positive lymphocyte count was weakly inversely correlated to ESR in both patients and control ($r = -0.18$; -0.11 respectively), and weakly directly correlated to ALC ($r = 0.18$; $r = 0.24$), Haematocrit ($r = 0.07$; $r = 0.31$) and platelet count ($r = 0.05$; $r = 0.08$).

Conclusion: Elevated ESR, decreased Haematocrit, ALC and Platelet count are essential haematologic predictive markers of HIV disease progression in resource limited centre's, though they may not be useful surrogate to CD4 count.

Key Words: HIV, Correlational, Haematologic markers, Immune predictive marker.

I. Introduction:

One of the public health problems of this age is HIV/AIDS pandemic. At the end of 2007, the Joint United Nations Program on HIV/AIDS (UNAIDS) estimated that, globally, 33.2 million adults and children were living with HIV/AIDS. Sub-Saharan Africa remains the most seriously affected region with AIDS remaining the leading cause of death there.¹⁻²

Nigeria is Africa's most populous nation with a population estimated at well over 140 million in 2006. The adult HIV prevalence has increased from 0.4% in 1988 to 5.8% in 2001. Thereafter the prevalence decreased from 5.0% in 2003 to 3.1% in 2009. That is to say that about 4.32million Nigerians (3.1% Nigerians) is living with HIV/AIDS even though the 2009 estimate was 2.6 million because the population was based on sentinel surveillance of antenatal clinic attendance. Rivers state, a state in the Niger Delta Region of Nigeria, accounts for about 365,000 (about 7.3% of over 5 million inhabitants of Rivers state are living with HIV/AIDS).^{3,4}

The impact of the epidemic on the social and economic development of Nigeria has been substantial. It has contributed to the present decrease in life expectancy, increased the number of deaths of young adults and increased the number of orphans in the country (estimated at one orphan in every 15-24 HIV/AIDS-related deaths). It has also increased the cost of achieving developmental goals and increased the level of poverty in the country.

The Federal Ministry of Health, as part of measures to respond to HIV/AIDS epidemic in this region, had to scale up the care, treatment and support of people living with HIV/AIDS through various agencies and non-governmental organizations (NGO's).⁵

However, one of the greatest health challenges facing HIV-related care in the Niger Delta region is the evaluation of baseline CD4 positive lymphocyte count of HIV-seropositive individuals, a parameter essential in predicting disease progression and the decision-making process of when to start Highly Active Anti-retroviral Therapy (HAART)

Since investigation facilities determine accreditation for donor driven therapy, there is therefore need to evolve low cost tests in order to access care and treatment of people living with HIV/AIDS in resource constraint settings like those found in the Niger delta region.

The present research is aimed at determining the relationship between CD4 positive lymphocyte count and these essential haematological parameters and to ascertain if they can be used as surrogate for CD4 positive lymphocyte counts in resource limited settings sub-Saharan African countries.

II. Materials And Method:

The study was carried out at the University of Port Harcourt Teaching Hospital, Port Harcourt, River state. The study population was HIV-infected, antiretroviral-naïve subjects of both sexes aged 18 years and above enrolled into the antiretroviral therapy program at the Haematology department of the University of Port Harcourt Teaching Hospital (UPTH) from January-December, 2009. Ethical clearance for the study was obtained from the ethical committee of the University of Port Harcourt Teaching Hospital. Written and informed consent was obtained from all the HIV subjects before being enlisted into the study.

A total of fifty (50) HIV- seropositive antiretroviral-naïve adults patients and 50 age and sex matched controls who were healthy HIV seronegative prospective blood donors were studied between January and December, 2009. About ten (10) milliliters of whole venous blood samples was collected by clean venepuncture using a 10millilitres hypodermic syringe and needle from each patient. About two (2) milliliters and five (5) milliliters of the blood were placed inside dipotassium EDTA (ethylene diamine tetra-acetic acid or sequestrene) anti-coagulated plastic bottles for CD4 test and Complete Blood Count (with ESR) analysis respectively. The remaining 3 milliliters of blood was put in a plain tube without anticoagulant; centrifuged at 3000 rpm for 10 minutes and serum samples obtained.

The serum sample was screened for the presence of HIV antibodies using Enzyme Linked Immunosorbent Assay (ELISA) technique. In this study double ELISA technique was used. The first was Gene screen (Sanofi, Pasteur) test kit. All initial reactive samples were re-tested with another ELISA test kit (Biorad, France) for the purpose of eliminating those samples presenting false reaction at the initial stage. Serum samples that were positive by second ELISA technique were confirmed using Immunocomb (Organics, Isreal) or Western blot (Biotech, Ireland).

The EDTA anti-coagulated blood (2 milliliters) was used for CD4 positive lymphocyte evaluation using the flow cytometric technique. Here, 20 microlitre of whole blood was added to 20 microlitre of anti CD4 monoclonal antibody. The mixtures were incubated in dark cupboard for about 10minutes before adding 800microlitre of buffer solution to the tube. Thereafter, the entire mixture was transferred to the flow cytometer for evaluation of the CD4⁺ lymphocyte count of the patient.

The ESR was determined using the Westergren graduated tube method while the complete blood count was done using automated coulter machine

All data were entered and analyzed using EPI info statistical software version 6.02. Statistical analysis of mean and standard deviation were calculated. Student t-test was used to test the significance of differences between mean values. A probability (p) less than 0.05 were taken to indicate statistical significance.

III. Results:

A total of fifty patients (50) aged 20-55 years (mean age 34.1 ± 8.1 years) were studied, out of which 35 (70%) were females and 15 (30%) males (M: F ratio was 1:2). Forty eight (96%) were symptomatic while 2 (4%) were asymptomatic. (See table 1).

The mean ($\pm SD$) CD4 positive lymphocyte count of the HIV- infected patients was 217.7 ± 144.8 cells/ μl while that of control was 896.2 ± 198.0 cells/ μl . The p value was significant ($p < 0.05$).

The mean ($\pm SD$) ESR of the HIV infected patients was 97.4 ± 39.9 mm/hr and that of control was 10.1 ± 5.7 mm/hr, the p value was significant ($p < 0.05$). The mean ESR (86.0 ± 36.8 mm/hr) of asymptomatic and symptomatic (97.8 ± 40.2 mm/hr) patients were significantly higher than those of the control group (10.1 ± 5.7 mm/hr) ($p < 0.05$). There was no significant sex variation of the ESR of both the HIV infected patients ($p = 0.079$) and that of healthy control group (0.607). (Table 1).

The mean ($\pm SD$) Total WBC of HIV infected patients was $5,268 \pm 1,601$ cells/ μl while that of the controls was $5,454 \pm 2,283$ cells/ μl . There was no statistical significant difference between Total WBC of the HIV infected patients and that of healthy control population ($p=0.638$).Table 2.

The mean ($\pm SD$) Absolute Lymphocyte Count (ALC) of HIV infected patient was 3283 ± 691 cells/ μl while that of controls was $4,746 \pm 1,263$ cells/up ($p=0.000$).There was a statistical significant difference between ALC of HIV infected patients and that of healthy control population ($p<0.001$).

The mean (\pm SD) Packed cell volume (PCV/Hemoglobin concentration) of HIV infected patients was 0.33 ± 0.061 L/L (10.9 ± 2.0 g/dl) while that of the controls was 0.43 ± 0.045 L/L (14.3 ± 1.5 g/dl). Similarly, the mean Haemoglobin concentration of HIV infected patients was significantly lower than that of healthy control population ($p<0.001$).

The mean (\pm SD) platelet count of HIV infected patients was $157,000 \pm 81,979$ cells/ μ l while that of controls was $197,660 \pm 48,742$ cells/ μ l ($p=0.003$). The mean platelet count of HIV infected patients was significantly lower than that of the healthy control population ($p=0.01$) (Table 2).

There was a poor inverse correlation between the CD4 + lymphocyte count and ESR of both the HIV infected and control ($r = -0.18$; $r = -0.11$) respectively and a weak direct correlation between the CD4 of both HIV-infected and control with their respective ALC ($r = 0.18$; $r = 0.24$), Haematocit ($r = 0.07$; 0.31) and Platelet count ($r = 0.05$; 0.08) (table 3).

DISTRIBUTION OF ESR AND CD4+ LYMPHOCYTE COUNT AMONG STUDY GROUP.

[Table 1]

	GROUP	NUMBER (n)	CD4 (cells/ μ l) (Mean \pm SD)	ESR (mm/1 st hr) (mean \pm SD)	p-value
CONTROL		50	896.2 ± 198.0	10.1 ± 5.7	
	Male	41	903 ± 210.4	9.6 ± 5.4	0.607
	Female	9	865 ± 132.7	12.4 ± 6.5	
PATIENT		50	217.7 ± 144.8	97.36 ± 39.8	
	Female	35	236.4 ± 118.9	95.2 ± 40.7	0.079
	Male	15	174.1 ± 94.3	102.5 ± 38.7	
	Asymptomat ic	2	330.0 ± 169.7	86.0 ± 36.8	

Table 2
Comparative table of Haematological Parameters of HIV- infected and healthy control group.
[Table 2]

Measured Parameters	Control Subjects Means \pm SD (N=50)	HIV-infected Patients Means \pm SD (N=50)	p-value	Statistical Inference
ESR(mm/hr)	10.1 ± 5.7	97.4 ± 39.9	<0.05	Significant
Total WBC(cellsmm ³)	$5,454 \pm 2,283$	$5,268 \pm 1,601$	0.63	Not significant
ALC (cells/mm ³)	$4,746 \pm 1,263$	$3,500 \pm 1,131$	0.000	Significant
PCV (L/L)	0.43 ± 0.045	0.33 ± 0.061	0.000	Significant
Platelets (cells/mm ³)	$197,660 \pm 48,742$	$157,000 \pm 81,979$	0.003	Significant

NB: ESR= Erythrocyte Sedimentation Rate; PCV= Packed Cell Volume; WBC=White Blood Count; TLC=Total Lymphocyte Count; BMI=Body Mass Index.

Table 3.

Pattern of statistical correlation between CD4+ count,	Measured parameters	CD4+ counts(/ μ l)	r-value HIV-infected	r-value control
				and

Haematological parameters in HIV infected and healthy control group.

ESR	CD4+	-0.18	-0.11
PCV	CD4+	0.07	0.31
WBC	CD4+	-0.04	-0.08
ALC	CD4+	0.18	0.24
PLATELET COUNT	CD 4+	0.05	0.08

[Table 3]

IV. Discussion

From this study, the patients' absolute mean CD4 positive lymphocyte counts (217.1 ± 114.8 cells/ μ l) appeared to be relatively higher than values obtained in previous studies by Ndakotsu and coworkers⁶ among their cohort of 104 HIV-infected subjects in Ife where a mean value of 155.4 ± 90.6 cells/ μ l was obtained from patients. This may be attributable to increased level of awareness of HIV infection in this region as the patients present early in our clinic.

We observed a control mean CD4 positive lymphocyte count of 896.2 ± 198.0 cells/ μ l among HIV-negative healthy blood donors residing in Port-Harcourt in the Niger Delta region of Nigeria. This was consistent with values reported in previous studies by Idoko and coworkers⁷ (821 ± 12 cells/ μ l) in Jos, North Central Nigeria, but relatively higher than values reported in previous studies by Ejele and co-workers⁸ (685 ± 99 cells/ μ l) in Port-Harcourt, South-South Nigeria in 2005 and Ndakotsu and co-workers⁶ (655.7 ± 17.6 cells/ μ l) in Ife, South-West Nigeria in 2008. This may be due to improved nutritional status among the healthy HIV-negative controls coupled with increase awareness of the populace.⁹

The data showed that the mean ESRs of the symptomatic (97.8 ± 40.2 mm/hr) and asymptomatic (86.0 ± 36.8 mm/hr) patients were significantly higher than that of the control (10.0 ± 5.7 mm/hr) ($p < 0.001$). This was consistent with the previous studies by Ndakotsu and coworkers⁶ where the mean ESR of the symptomatic and asymptomatic patients were higher than that of the controls. The increase in ESR is because infection by Human Immune deficiency virus elicits an increase in the whole body's protein (including acute phase reactant proteins and lipoproteins) turnover. This increase in body's proteins is applicable to both symptomatic and asymptomatic HIV infected persons.⁹

There was a weak inverse relationship between ESR and CD4 positive lymphocyte count in both patients and controls ($r = -0.18$ and $r = -0.11$ respectively) (Table 5). This finding was in accordance with those of some previous studies^{6,10-16} where ESR was found to be a sensitive predictive marker of HIV disease progression.

There was a statistically significant difference between the mean haematocrit (PCV) of HIV infected patients (0.33 ± 0.06 L/L or 10.9 ± 2.0 g/dl) and that of controls (0.43 ± 0.045 L/L or 14.3 ± 1.5 g/dl) ($p < 0.001$). However there was a significant inverse correlation between patients' ESR and their haematocrit ($r = 0.46$; $p < 0.05$). The inverse correlation was weak in the control group ($r = -0.15$). This was in keeping with previous findings^{6,17} in which significant inverse relation was found between ESR and haematocrit ($p < 0.001$). Low haematocrit (anaemia), a common finding in HIV disease progression has a negative influence on ESR and could have contributed to the elevated ESR level in our patients. In addition to this, hypergammaglobulinemia, malnutrition, tropical diseases, which are also common findings in HIV/AIDS patients may contribute to the elevated ESR levels^{18,19}. Therefore, HIV related anaemia increases the need for blood transfusion.

The data showed that there was no statistical significant difference between the total WBC of HIV infected patients ($5,268 \pm 1,601$ cells/ μ l) and the controls ($5,454 \pm 2,283$ cells/ μ l) ($p > 0.05$). There was a weak inverse correlation between CD4 positive lymphocyte count and total WBC in both the controls and the patients. Although leukocyte proliferation and the synthesis of cytokines, immunoglobulin and positive acute phase proteins contribute to protein turnover in HIV infection, this may not be the finding in all cases. HIV patients may also present with leucopenia which could be immune-mediated especially that resulting from anti neutrophil antibodies in some HIV- antibody positive patients²⁰. Hence total WBC may not give good prediction of disease progression.

Though the mean ALC of HIV infected patients (3283 ± 691 cells/ μ l) was statistically lower than that of controls ($4,746 \pm 1263$ cells/ μ l) there was a weak direct correlation between CD4 positive T- lymphocyte counts and ALC of both HIV-infected patients ($r = 0.18$; $p < 0.05$) and the controls ($r = 0.24$; $p < 0.01$). This was similar to previous studies by Shapiro and Ejele and co-workers which showed that ALC could have a minimal predictive value to CD4 positive lymphocyte counts.

There was a weak direct correlation between CD4 positive lymphocyte counts and platelet counts of the patients ($r = 0.05$; $p < 0.05$) and their controls ($r = 0.08$; $p < 0.05$). This decrease in platelet count with disease progression may be immune-mediated as immune thrombocytopenic purpura is a common finding in AIDS^{20,21}.

ESR was relatively higher in female (12.4 ± 6.5 mm/hr) than male (9.6 ± 5.4 mm/hr) in the controls but the difference was not statistically significant ($p = 0.607$). (Table 1). This was similar to previous study where ESR was shown to be higher in female irrespective of infection or immune status⁶.

V. Conclusion/Recommendation

The findings from this study include the following;

1. Human immunodeficiency virus alters the immunological status of an individual, leading to deterioration of the clinical status and haematological parameters.
2. ESR of HIV-seropositive individuals irrespective of their clinical status (symptomatic or asymptomatic) are usually higher than that of healthy HIV-seronegative individuals.
3. Elevated ESR, decreased haematocrit, decreased absolute lymphocyte count (ALC), decreased platelet count (thrombocytopenia) and CD4 positive lymphocytopenia are useful indicators of HIV disease progression.

Simple low cost haematologic tests such as ESR, Haematocrit, WBC, ALC, and platelet counts should be used as indispensable accreditation baseline tests to access care and treatment of HIV/AIDS (HAART) in resource-limited settings such as those in the Niger Delta Region of Nigeria. Apart from their roles in decision-making on when to start anti-retroviral therapy, they may also proffer prognosticating role, hence they may be invaluable in monitoring the HIV disease progression and the response of patients to antiretroviral therapy in situations where immunophenotyping (CD4 count) are not available.

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