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ORIGINAL ARTICLE

Leucocyte phagocytic activity of treatment-naïve HIV positive patients in Umuahia, Nigeria

Udensi N[1], Onyekwere T.O[2], Okoroiwu L. I[2], Muhibi M.A[3*]

[1]Department of Haematology, Federal Medical Centre, Umuahia, Nigeria.

[2]Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria.

[3]Haematology and Blood Transfusion Science Unit, Department of Medical Laboratory Science, Edo State University Uzairue, Edo State, Nigeria.

Correspondence: muhibudeen@yahoo.com.

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Abstract

Objective: This study was conducted to determine white cell phagocytic activity in Human Immunodeficiency Virus (HIV) infection.

Methods: One hundred HIV-positive patients were enrolled in this study while fifty apparently healthy seronegative individuals served as controls. The haemoglobin estimation, haematocrit, total white blood cell count (TWBC count), platelet count were analysed using conventional manual methods, the TWBC was repeated after incubation with carbonyl iron powder at 37°C to determine the phagocyte count, while CD4 count was analysed using Partec Cyflow counter.

Results: The TWBC count of the test group before incubation with carbonyl iron powder (CIP) was 4839 ± 1686 (/mm^3) while after incubation with CIP was 4451 ± 1657 (/mm3). The TWBC count of the control group before incubation with carbonyl iron powder (CIP) was 6096 ± 2196 (/mm^3) while after incubation with CIP was 4074 ± 1745 (/mm3). The **phagocyte** count of test and control groups were 388±29 and 2022±451, with later being significantly higher than the former (p=0.001). Significant differences were observed in neutrophils, eosinophils, monocytes, lymphocytes, haemoglobin concentration, haematocrit and platelet count of the control 45 ±7.2 (%), 3 ± 1.2 (%), 4 ± 1.4 (%), 41 ± 12 (%),12 (g/dl), 38 ± 4 (%), 211 ±14 (x 10^9/L); when compared with the test 20.7 ± 11.0 (%), 1 ± 1.4 (%), 2 ± 1.7 (%), 28 ± 10(%), 9.2 (g/dl), 27 ± 09 (%), 90± 17 (x 10^9/L) ($p<0.05$). There was equally a significant difference in the value of CD4 count of the controls 864 ± 266 cells/µL when compared with the test 420 ± 203 cells/ µL at $p < 0.05$.

Conclusion: The CD4 and phagocyte counts have a positive correlation with TWBC. All patients living with HIV should have their immune status monitored regularly to forestall inability of the system to perform phagocytosis.

Keywords: Phagocytosis, CD4 cells, leucocytes, immunity, HIV.

Introduction

The Human Immunodeficiency Virus (HIV) is a lentivirus that causes the Acquired Immune Deficiency Syndrome (AIDS), a disorder in humans in which advanced failure of the immune system permits serious opportunistic infections and a type of cancer called Kaposi sarcomas to increase. Infection with HIV happens by the transmission of blood, semen, vaginal fluid, pre-ejaculate, or breast milk (1).

In the broad classification, there are two types of HIV named as HIV-1 and HIV-2; both are clever in initiating AIDS, but HIV-2 is somewhat slower, with respect to disease advancement (2). The virus invades vibrant cells in the human immune system such as helper T-cells (3). HIV infects vibrant cells in the human immune system such as helper T cells (precisely $CD4^+$ T cell), macrophages, and dendritic cells (3).

Phagocytes are cells that defend the body by eating harmful and undesirable foreign elements, bacteria, and lifeless or fading cells. Phagocytes include many types of white blood cells such as neutrophils, monocytes, macrophages, mast cells and dendritic cells (4). The phagocytes move by a process called chemotaxis. When phagocytes come in contact with bacteria, the receptors on the phagocytes's surface will bind to them. The binding will lead to the engulfing of the bacteria by the phagocytes (5). Some phagocytes kill the ingested pathogen with oxidants and nitric oxide (6). After phagocytosis, macrophages **and** dendritic cells can participate in antigen presentation, a procedure in which a phagocyte moves parts of the ingested material back to its surface (6).

Phagocytes have a big desire for food; scientists have even served macrophages with iron fillings and then used a small magnet to separate them from other cells (7).

It has been proven that neutrophil activity can be reduced by HIV infection leading to deficiencies in neutrophil chemotaxis, phagocytosis, intracellular killing, Antigen Presenting Cell (APC) manufacture and cytokine manufacture(8,9). HIV interaction with monocytes/macrophages occurs with viral envelope proteins, directly or indirectly by specific antibodies (10,11). The CD4 and chemokine receptors are implicated in virus adsorption but alternative receptors such as fibronectin and membrane glycolipids can contribute to binding.

HIV infection could also lead to anaemia in various ways; variations in cytokine manufacture with attendant effects on haematopoiesis and decreased erythropoietin concentration (12).Thrombocytopenia is a

corporate discovery among HIV-1 infected patients; during all the phases of the infection (13).

Carbonyl iron is an extremely pure iron, prepared by chemical breakdown of purified iron. Most of the contaminations are carbon, oxygen, and nitrogen. In 1925, carbonyl iron was devised whereas in 1934, it turned out to be the first magnetic recording oxide. Units of carbonyl iron powder suspended in a carrier fluid are used as a magneto-rheological fluid. This substance is used in ascertaining the phagocytic activity of white cells in a controlled laboratory procedure (7).

When $CD4^+$ cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections(14). CD4 count as a marker of immunosuppression, is used in observing disease advancement, determining when to initiate therapy, staging the disease and determining treatment failure (15, 16).

The dynamic prevalence of HIV infection in Nigeria has been documented. However, not much work has been done to determine leucocyte phagocytic activity in HIV positive individuals in Umuahia. This work was done to determine the phagocytic activity of leucocytes in treatment-naive persons living with HIV, using both Full Blood Count (FBC) and CD4 Count as surrogate markers of immune status.

Materials and Method

Study Area

The study was conducted at the haematology unit of Federal Medical Centre (FMC), Umuahia. Umuahia is the capital of Abia State,with a population of 359,230 according to the 2006 Nigerian census (17). It covers a land area of about 245km² and lies on latitude 5.5250°N and 7.4622° longitude.

Study Population and Enrolments

After securing ethical approval from the Institutional Review Board of FMC, Umuahia, informed consents were obtained from participants comprising of 100 HIV positive subjects who are not on Anti Retroviral Treatment (ART). Out of 100 participants, 50 were males while 50 were females. Apparently, healthy HIV sero-negative volunteers that served as controls were 50 in number.

Laboratory Analysis

Venous blood was collected from each participant by venepuncture into ethylene diamine tetracetic acid (EDTA) containers for CD4 count, total white blood cell count, differential count, Hb estimation, haematocrit, platelet count and leucocyte phagocytic activity. The specimens were analysed in accordance with manual techniques recommended by Ochei and Kolhatkar with exemption of CD4 count which was done in strict adherence to the Partec Cyflow manufacturer's instruction (18).

Differential Count

A small drop of EDTA anti-coagulated blood was dropped 1cm from the end of the slide .The slide was placed on a flat surface. The spreader was placed in front of the drop of blood at 30° angle. The spreader was drawn back slightly to touch the drop of blood and to allow the blood spread along the contact line. The spreader was pushed forward smoothly and rapidly, maintaining the contact between the slide and the spreader. The smear was covered completely with Leishman stain. It was allowed to fix for 1 to 2mins. The stain was diluted twice the volume of the buffer solution. It was also allowed to stain for 10mins.The slide was washed with the buffer solution, then drain and dried in air in a slanting position. The films were read using X100 objectives and differential white blood cells counts were recorded.

Haemoglobin Estimation

The haemoglobin estimation was done using the Drabkin's solution (cyanmethaemoglobin method) which is a reagent containing potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate. The ferricyanide forms methaemoglobin which is converted to cyanmethaemoglobin by the cyanide. A volume of 20µl of blood was added to 5mls of Drabkin's solution in a test tube. The mixture was well mixed and allowed to stand for 10minutes.The absorbance was read spectrophotometrically at 540nanometers with Drabkin's solution as blank.

Haematocrit

The capillary tubes were filled two-third full with well mixed venous blood. One end of the tubes were sealed with plasticine. The filled tubes were placed in a microhaematocrit centrifuge and spun at 12,.000g for 15 minutes. The tubes were placed into a microhaematocrit reader and the packed cells volume (PCV) was read as a percentage (18).

Platelets Count

Whole blood was diluted appropriately using 1% ammonium oxalate which lyses other cells, fixing the platelets. A volume of 20 µl of blood was added to 380 µl of 1% ammonium oxalate. The improved Neubauer counting chamber was charged with the well mixed diluted blood. The platelets were allowed to settle in a moist chamber for 3 to 5 minutes and the platelets were counted with X40 objective.

CD4 Count

A volume of 20 µl of EDTA whole blood was dispensed into a glass test tube then 20 µl of CD4 antibody was added, while the mixture was incubated at room temperature in the dark for 15 minutes. A volume of 800 µl of no-lyse buffer was added and mixed and analyzed on the Partec CyFlow regular counter.

Counting Total White Blood Cells

A volume of 380 µl of Turks fluid was dispensed into test tubes for the test and control samples and 20 µl of blood was added and mixed with Pasteur pipette. A clean dry improved Neubauer counting chamber, with its cover glass already in position was charged with the aid of capillary tube that has been allowed to take up the suspension by capillary action. The white cells on the four squares of the chamber were counted with x10 objective.

Counting Total White Blood Cell after Incubation with Carbonyl Iron Powder

A volume of 1 ml of blood samples of test and controls were dispensed into different test tubes, then, 0.5g of carbonyl iron powder was added into each of the test tubes and mixed by tilting the test tubes. The tubes were incubated for 1 hour in a water bath at 37^{0}C and mixed at intervals of 15 minutes. A magnet was used to separate the phagocytes after 1 hour. This was done by pouring the content of the test tubes on a cellophane while the magnet was under it. A volume of 380 µl of Turk's fluid was dispensed into other test tubes, and 20 µl of blood from which the phagocytes were separated from, was pipetted and mixed. A clean dry improved Neubauer counting chamber with its cover glass already in position was charged with the mixture with the aid of capillary tube that has been allowed to take up the suspension by capillarity. The white cells on the four squares were recounted.

Statistical Analysis

Statistical analysis was performed using SPSS version22.0. The comparison between the test and the control was done using the students t-test while Pearson correlation analysis was applied to determine the relationship between CD4 count and full blood count. *P*-values < 0.05 were considered statistically significant.

Results

The total white blood cell count of the test before incubation with carbonyl iron powder (4839 ± 1686) showed a significant difference when compared with the control (6096 ± 2196) with p value of 0.032. Also the phagocyte count in the control groups (2022±451) was significant higher than that of the test group (388 ± 29) with p value as 0.001 . These are as depicted in Table 1.

As shown in Table 2, the mean values of the CD4 count of the test : 420 ± 203 cells/µL showed significant changes when compared with the controls : 864 ± 266 cells/µL at 95% Confidence Interval (p= 0.04). Also, statistical differences were observed in neutrophils, eosinophils, monocytes, lymphocytes, haemoglobin concentration, haematocrit and platelet count ($p < 0.05$). Positive correlation between phagocyte count and CD4 count was equally established (Table 3)

Table 1: Mean ± SD of WBC and Phagocytes in Research Participants

Parameter	PLWH n=100	Reduction of WBC in PLWH (%)	Control n=50	Reduction of WBC in HIV Negative group(%)	P-value
TWBC before incubation with CIP (per mm^3)	4839 ± 1686	8.0 ± 2.0	6096± 2196	33.2 ±92	0.032
TWBC after incubation (per mm^3)	4451 ± 1657	8.7 ± 1.7	4074 ±1745	49.6 ± 31	0.047
Phagocyte Count	388 ± 29		2022± 451		0.001

Note: PLWH= People living with HIV

Table 2: Mean ± SD Of CD4+ Count and Full Blood Count Parameter of Participants

Parameters	Test (n= 100)	Control (n= 50)	P value
CD4 + (Cells/µL)	420 ± 203*	864 ± 266*	0.014
Neutrophils (%)	61.2 ± 11.0	45.5 ± 7.2	0.035
Eosinophils (%)	1.1 ± 1.4	3 ± 1.2	0.019
Monocytes (%)	2.0 ± 1.7	4 ± 1.4	0.032
Lymphocytes (%)	28.8 ± 02	41 ±3.6	0.029
Basophils (%)	0	0	N/A*
Platelet count ($x10^9$/L)	90 ± 29	211 ± 42	0.012
Hb estimation (g/dl)	9.2 ± 2.4	12.5 ± 3.1	0.045
Haematocrit (%)	27.4 ± 9.5	39.7 ± 4.4	0,040

*N/A-Not applicable

Table 3: Correlation Analysis of CD4 Count and Full Blood Count

	CD4t	TWBC	TWBC After	% reductn of phago	Hb	Neutro	Eosino	Mono	Baso	Plt	Lym
CD4	1	279	023	524	055	165	098	016	-	125	216
Pearson Cor		.001	.782	.000	.000	.506	.231	.848	-	.128	.008
N	150	150	150	150	150	150	150	150	150	150	150
TWBC	279	1	885	114	097	086	078	120	-	139	029
Pearson Cor	.001		.000	.166	.237	.295	.340	.144	-	.090	.721
N	150	150	150	150	150	150	150	150	150	150	150
TWBC after	028	885	1	325	025	041	073	145	-	071	027
Pearson Cor	.782	.000		.000	.765	.621	.376	.077	-	.388	.745
N	150	150	150	150	150	150	150	150	150	150	150
Phagocyte	524	114	325	1	.160	.087	.660	.009	-	.130	.112
Pearson Cor	.000	.166	.000		.051	.288	.419	.915	-	.112	.171
N	150	150	150	150	150	.150	150	150	150	150	150
Hb	.055	.097	.025	.160	1	.077	.008	.007	-	.077	.046
Pearson Cor	.506	.237	.765	.051	-	.352	.927	.933	-	.835	.577
N	150	150	150	150	150	150	150	150	150	150	150
Neutrot	.165*	.086	.041	.087	.077	1	.003	.066	-	.065	-.070
Pearson Cor	.044	.237	.621	.288	.352		.969	.426	-	.426	.391
N	150	150	150	150	150	150	150	150	150	150	150
Eosino	.098	.078	.73	.066	.008	.003	1	.074	-	.058	.018
Pearson Cor	.231	.340	.376	.419	.927	.969		.365	-	.482	.826
N	150	150	150	150	150	150	150	150	150	150	1250
Mono	.016	.120	-.145	.009	.007	.066	.074	1	-	.034	098
Pearson Cor	.848	.144	.077	.915	.933	.426	.365	-	-	.677	.233
N	150	150	150	150	150	150	150	150	150	150	150
Baso	-	-	-	-	-	-	-	-	-	-	-
Pearson Cor	-	-	-	-	-	-	-	-	-	-	-
N	150	150	150	150	150	150	150	150	150	150	150
Plt	125	139	.071	.130	.017	.065	.058	.034	-	1	109
Pearson Cor	.128	.090	.388	.121	.835	.426	.482	.677	-	-	.186
N	150	150	150	150	150	150	150	150	150	150	150
Lym	216	029	027	112	046	.070	.018	.098	-	.109	1
Pearson Cor	.008	.721	.745	.171	.577	.391	.826	.233	-	.186	
N	150	150	150	150	150	150	150	150	150	150	150

Note
Pearson cor = Pearson Correlation. (2-tailed)

Discussion

In this study, it was observed that the phagocyte count was higher in the HIV negative subjects than in the people living with HIV (PLWH), who are treatment-naive. This agrees with what was previously reported that, mononuclear phagocytes from HIV infected patients have reduced Fc gamma (Fc ¥) in vitro (19). Receptors for the Fc portion of IgG (Fc receptors) delay in phagocytosis of IgG-opsonized particles and clearance of immune complexes(19).

The positive correlation between phagocyte count and CD4 count reported in this study is in keeping with the other researchers' reports where HIV was equally reported to manifest itself by causing reduction in CD4+T cell, cytokine deregulation and immune dysfunction (9, 20).

It was also reported in this study that the CD4 count of the test group was significantly lower than that of the control group. HIV infection leads to low levels of $CD4^+$ T cells through either apoptosis of uninfected bystander cells or direct viral killing of infected $CD4^+$ T cells by CD8 cytotoxic lymphocyte that recognize infected cells (1, 14).An essential cause of the degeneration of $CD4^+$ T cell counts is the disaster of the recreating capacity of the immune system to produce antibodies.

Neutropenia reported in this study corroborates reports that HIV-infected individuals do experience decreased peripheral blood neutrophil counts compared to uninfected individuals. Additionally, HIV infection of stromal cells can disrupt the bone marrow microenvironment, thus reducing support for progenitor development and decreasing factors important for granulocyte development such as Neutrophil (21).

In this study, there was thrombocytopenia in the tests which is in line with outcome of research carried out by other researchers(13). Platelets was established to always have direct interaction with HIV through pathogenic devices like binding, engulfment, internalization of HIV-1, playing a role in host defence during HIV-1 infection (13).

Conclusion

In was established that HIV infection impairs phagocytic activity of leucocytes of treatment-naive HIV positive individuals. It is recommended that HIV-positive individuals should be sustained on highly active antiretroviral therapy (HAART) to produce a significant improvement in phagocytosis, chemotaxis and anti-microbial activities of monocyte and neutrophils, with regular monitoring of the immune systems.

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Conflict of interest

Authors declared no conflict of interest

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