IMMUNOGLOBULIN LEVELS CANNOT REPLACE CD4+ CELL COUNT AS MARKERS IN HIV CARE

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ABSTRACT

Background: HIV infection predisposes to AIDS by depleting the immunity of the host. The cellular and humoral immune response markers that can be used to monitor the progress of the disease and treatment are the CD4+ cells and immunoglobulins respectively.

Aim: This work was carried out to determine immunoglobulin levels and CD4+ cell count in HIV negative individuals, HIV positive subjects on HAART and treatment naïve HIV positive subjects to provide useful information for effective management of HIV infection.

Methods: Thirty participants (Female-11; Male-19; aged 18- 60 years) were recruited for each of the groups: HIV infected individuals on HAART, HIV- infected individuals not yet on treatment and HIV- negative adults; to give cumulative number of participants of 90. Cyflometry method was used for CD4 count using Partec CD4 machine, plasma IgA, IgG and IgM were measured by ELISA while HIV tests were carried out using immunochromatographic and ELISA assays.

Results: The participants were adults in the age range of 18 to 60 years and majority in terms of gender were male (63.3%) in all the groups. Data collected through questionnaire revealed that majority of the participants (>50%) in each group had education above secondary school level with 83.3% taking balance diet regularly. In every group as well, some participants (>40%) take both multivitamin supplements and herbal concoction. There was no significant difference in the plasma value of IgA, IgG and IgM in HIV negative, HIV positive participants on HAART and treatment naive participants using ANOVA ($p>0.05$). However, there was a significantly higher CD4+ Cell Count in HIV Negative participants compared with HIV Positive participants on HAART and treatment naïve participants($p<0.05$).

Conclusion: There was no significant difference in plasma immunoglobulins A, G and M in HIV positive patients on HAART, HIV treatment naïve and HIV negative control while CD4+ T-cells count was significantly higher in HIV negative participants compared with HIV positive individuals.

Key words: Immunoglobulins, HIV, HAART, Herbal concoction.

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INTRODUCTION

Socio-demography is an epidemiological index used to stratify people into groups based on factors like wealth, income, race, education, ethnicity, gender, occupation, social status, or derived power, educational institutions, age, place of residence, religion, educational level and marital status (Portal, 2014; Nutter, 1999). Socio-demographic data are helpful in understanding the epidemiology of infectious diseases such as HIV for prevention and control (Grusky, 2011; von-Valtier, 2011). Socio-demography index allows for the study and analysis of the distribution (who, when, and where), patterns and determinants of health and disease conditions for appropriate intervention (Portal, 2014; Grusky, 2011; von-Valtier, 2011; Nutter, 1999).

Immunoglobulins are gamma globulin fraction of plasma/serum proteins used by the immune system to identify and neutralize foreign objects such as pathogenic bacteria and viruses such as HIV (Hague *et al.*, 1989). Though antibodies to HIV are non-neutralizing antibodies but administration of intravenous immunoglobulin in HIV infection has a major clinical benefit, and can reduce viral activity to delay disease progression (Hague *et al.*, 1989). Immunoglobulins mediate several functions which include fixation of complement that results to lyses of cells and release of biologically active molecules, binding of various cells to facilitate specific functions by bound cells e.g. phagocytic cells, lymphocytes and platelets (Hague *et al.*, 1989). Cluster of differentiation 4 (CD4) is a glycoprotein that acts as a co-receptor for the T-cell receptor (Vijayan et al., 2017; MacNeil *et al*., 2007; Isobe *et al.*, 1986). It is found on the surface of immune cells such as T helper cells, monocytes, macrophages, and dendritic cells (Vijayan *et al*., 2017; MacNeil *et al*., 2007). They are white blood cells that constitute essential part of the human immune system (Vijayan *et al*., 2017; Isobe *et al.*, 1986). CD4 cells are called T-helper cells or T4 cells because they send signals to other types of immune cells such as CD8 killer cells to release cytotoxin to destroy infectious agents (Vijayan *et al*., 2017; Isobe *et al.*, 1986). Depletion of CD4 cells such as in untreated HIV infection, or following immune suppression prior to a transplant will make the body vulnerable to a wide range of infections that it would otherwise have been able to fight naturally (Vijayan *et al*., 2017; MacNeil *et al*., 2007; Isobe *et al.*, 1986).

Individual is HIV negative when their test outcome is non-reactive to HIV epitopes using validated assays, while those who have positive test outcome to the laboratory procedures are referred to HIV Positive patients indicating those individuals are infected with HIV (Arachchige and Perere, 2021; Eisinger *et al.*, 2019; Deeks, 2013; Moore and Chaisson, 1999;). Highly Active Antiretroviral Therapy (HAART) is useful in the management of HIV/AIDS as it decreases the patient's total burden of HIV, maintains functions of the immune system, and prevents opportunistic infections that often lead to death (Arachchige and Perere, 2021; Eisinger *et al.*, 2019; Deeks, 2013; Moore and Chaisson, 1999). A person is considered to be HIV treatment-naive if they have never taken any antiretroviral therapy for their infection (Nada *et al.*, 2017). Measurement of plasma immunoglobulins and CD4 count in HIV infection are useful in monitoring both disease progression and response to ARV therapy in HIV positive patients on HAART and Treatment Naïve patients (Lugada *et al.*, 2004).

This work was designed to determine socio-demographic pattern, immunoglobulin levels and CD4+ cell count in HIV Negative, HIV positive patients on HAART and treatment naïve patients to provide useful information for effective management of HIV/AIDS infection.

MATERIALS AND METHODS

Study Area

This study was carried out in Osogbo, the capital city of Osun State, located in the southwest Nigeria.

It was an urban setting with a population of 395,500. The city covers an area of 47km^2 with an elevation of 320 meters. The average temperature is 26^{0}C with 82% humidity. The study sites included Ladoke Akintola University of Technology Teaching Hospital, Idi Seke; State Hospital, Asubiaro and Saint Mary Catholic Hospital, Jaleiyemi; all in Osogbo. The health institutions chosen represent public hospitals with established ARV programme in Osogbo, an urban city in Osun State. The residents are majorly Yorubas, however, there are other tribes including Hausas, Igbos and those of Edo origin. The weather is typically tropical with periods of heavy rainfall alternating with the dry season.

Study Design and Subjects

This was a cross-sectional study. All patients attending ARV clinics at these centers who consented were recruited to participate in this study. The Informed Consent Form was discussed with every participant in both English and Yoruba as appropriate for proper understanding of the objectives and procedures of the study. A research assistant who has been trained to do this per site also assisted in obtaining demographic data and other pertinent information on nutritional attitudes of the patients by administering the structured questionnaire for the study.

The participants were adults (aged 18 years and above), who tested positive to HIV by national screening algorithm including those that were on HAART and those that were treatment naive. The control group was made up of adults who tested negative to HIV screening. Pregnant women and patients who received fluid intervention as part of standard care less than or equal to one month were excluded.

Sample Size Determination

The sample size was determined using the statistical formula (Hulley and Cummings, 2001)

$n=pq\ (z)^2/e^2$;

and with prevalence of HIV in Osun State reported as 1.6% (OSSACA, 2016).

Where

n = Sample Size

P = Prevalence 1.6 % (0.016)

q= (1-P) = 0.984

z= Standard Deviation (1.96) at 95% confidence interval

e= Margin of 5 % (0.05)

By substitution;

$n=0.016*0.984(1.96)^2/(0.05)^2$

=24.193 which is approximately 25 in a demographic study.

To make provision for minimum of 10% attrition, 30 participants were recruited for each of the groups: HIV infected individuals on HAART, HIV- infected individuals not yet on treatment and HIV- negative adults; to give cumulative number of participants of 90.

Ethical Consideration

Participants' data were handled with the utmost care and confidentiality it deserves. The data were identified on Excel spread sheet with unique identifier in form of enrollment number allotted to every participant, age and sex as recommended for health data storage. The privacy and confidentiality of every participant were maintained during data processing and dissemination of research outcome. All participants and their health care providers will benefit directly by having timely access to their laboratory parameters at no cost to them. The researcher was responsible for all costs of analyzing the parameters stated in this study. Every participant gave informed consent to be enrolled for the study. Also, ethical clearance was obtained from Ladoke Akintola University of Technology Teaching Hospital Research Ethics Committee in Osogbo.

Sample collection and processing

Five milliliters (5ml) of venous blood were collected aseptically from each subject by venepuncture and dispensed into K_2EDTA bottle for absolute CD4 + cell count by flow cytometry using cyflow green from Partec, Germany, immunoglobulins concentration by ELISA technique and HIV screening was done in adherence to national algorithm for control group only.

HIV status determination:

Determine HIV-1/2 which is an immunochromatographic test for qualitative detection of antibodies to HIV-1 and HIV-2 was used as the first kit to screen all participants. Serum was added to the sample pad. As the sample migrates through the conjugate pad, it reconstitutes and mixes with the selenium colloid-antigen conjugate. The mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient window site. If antibodies to HIV-1 and/or HIV-2 are present in the sample, the antibodies bind to the antigen-selenium colloid and to the antigen at the window, forming a red line at the patient window site. If antibodies to HIV-1 and HIV-2 are absent, the antigen-selenium colloid flows past the patient window, and no red line is formed at the patient window site. To insure assay validity, a procedural control bar is incorporated in the assay device. All sample that test negative by this assay was considered as Negative, while positive samples were subjected to Unigold Screening methods, to rule out false positive results.

Recombinant proteins representing the immunodominant regions of the envelope proteins of HIV-1 and HIV-2, glycoprotein gp41, gp120 (HIV-1) and glycoprotein gp36 (HIV-2) respectively are immobilized at the test region of the Unigold cassette. These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of nitrocellulose membrane is also sensitized as a control region. During the testing two drops of serum was applied to the sample port, followed by two drops of wash buffer. Antibodies of any immunoglobulin class, specific to the recombinant HIV-1 or HIV-2 proteins will react with the colloidal gold linked antigens. The antibody protein-colloidal gold complex moves chromatographically along the membrane to the test and control regions of the test device. A positive reaction is visualized by a pink/red band in the test region of the device. A negative reaction occurs in the absence of human immunoglobulin antibodies to HIV in the analyzed specimen. Consequently no visually detectable band develops in the test region of the device. Excess conjugate forms a second pink/red band in the control region of the device. The appearance of this band indicates proper performance of the reagents in the kit. Every sample found positive by this assay was considered positive, while negative result results into ties which were resolved using STAT-PAK kit.

The Chembio HIV 1/2 STAT-PAK assay employs a unique combination of a specific antibody binding protein, which is conjugated on colloidal gold dye particles, and antigens to HIV 1/2, which are bound to the membrane solid phase. The sample was applied to the sample (S) well followed by the addition of a running buffer. The running buffer facilitates the lateral flow of the released products as well as promoting the binding of antibodies and antigen. If present, the antibodies bind to the gold conjugated-antibody binding protein. In a positive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the test (T) area producing a pink/purple line in the test area. The sample continues to migrate along the membrane and produces a pink/purple line in the control (C) area, demonstrating that the reagents are functioning properly. In a negative sample the dye conjugated-immune complex migrates on the nitrocellulose membrane and flow past the test area and no color is produced. Any sample found to be positive at this stage was considered positive, while negative results were also considered negative, accordingly.

Operations Procedures

Twenty microliter (20 µl) of K3EDTA whole blood was collected into partec test tube (Rohen tube). Twenty microliter of CD4+ antibody was added into the tube. The contents were mixed and incubated in the dark for fifteen minutes at room temperature.

A total of 300 µl of no-lyse buffer was added into the mixture and mixed gently. The contents of the partec tube was displayed as peaks and interpreted.

ELISA Techniques

Principle : Sandwich ELISA method was employed, in which polystyrene microwell strips are pre-coated with monoclonal antibodies specific to the analyte (Immunoglobulins A, G and M). Patient's plasma sample is added to the microwell together with a second antibody conjugatedthe enzyme horse adish peroxidase (the HRP-Conjugate) and directed against the analyte. During incubation, the specific complex formed in is captured on the solidphase. After washing to remove sample serum proteins and unbound HRP-conjugate antibody, Chromogen solutions containing tetramethyl-benzidine(TMB) and urea peroxide are added to the wells. In presence of the antibody-antigen-antibody(HRP) "sandwich" immuno-complex, the colorless TMB Solution are hydrolyzed by the bound HRP-conjugate antibody to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antigen captured in the wells, and to its amount in the sample respectively. Plasma harvested from the same sample was used to quantify IgG, IgM and IgA immunoglobulin by ELISA technique using ELISA microplates specifically produced by DIAPRO (Italy). Addition of samples, controls, gradient standards, conjugation, washing, incubation, chromogen addition, stoppage of colour development and reading of optical density were carried out in strict adherence to the manual validated by DIAPRO.

Statistical Methods

The data generated were coded, entered, validated and analyzed using Statistical Package for Social Science (SPSS) version 22.0. The mean and standard deviation of the parameters (CD4 cell, IgG, IgM and IgA) were expressed for the entire study subjects and by age, sex, other socio demographic parameters and comparisons between groups were done using ANOVA. Values below 0.05 were considered significant.

RESULTS

The participants were age and sex matched in the groups of HIV negative individuals, PLWHA on HAART and treatment naïve HIV positive participants. The participants were adults in the age range of 18 to 60 years and majority in terms of gender were male (63.3%) in all the groups. Data collected through questionnaire revealed that majority of the participants (>50%) in each group had education above secondary school level (Table 1) A proportion of 83.3% of the participants takes balance diet regularly. In every group as well, some participants (>40%) take both multivitamin supplements and herbal concoction (Table 2).

TABLE 1: Demographic Characteristics of HIV Negative, HIV Positive Participants on HAART and Treatment Naïve Participants

Variables	HIV Negative (n = 30)	PLWHA On HAART (n = 30)	HAART Naïve (n = 30)
Age			
≤20 years	05(16.7%)	05(16.7%)	05(16.7%)
21-30 years	12(40.0%)	12(40.0%)	12(40.0%)
31–40 years	05(16.7%)	05(16.7%)	05(16.7%)
41–50 years	04 (13.3%)	04 (13.3%)	04 (13.3%)
51-60 years	04 (13.3%)	04 (13.3%)	04 (13.3%)
Sex			
Male	11 (36.7%)	11 (36.7%)	11 (36.7%)
Female	19 (63.3%)	19 (63.3%)	19 (63.3%)
Marital status			
Single	03 (10%)	05 (16.7%)	02 (6.7%)
Married	21 (70%)	19 (63.3%)	14 (46.7%)
Divorced	06 (20%)	04 (13.3%)	13 (43.3%)
Widowed	0 (0%)	02 (6.7%)	01 (3.3%)
Educational status			
Illiterate	4 (13.3%)	01 (3.3%)	3 (10%)
Elementary school	5 (16.7%)	06 (20%)	8 (26.7%)
Secondary school	13 (43.3%)	12 (40%)	12 (40%)
Diploma and above	8 (26.7%)	11 (36.7%)	07(23.3%)

TABLE 2: Nutritional and Herbs Consumption Habits of HIV Negative, HIV Positive Participants on HAART and Treatment Naïve Participants

Variables	HIV Negative (n = 30)	PLWHA On HAART (n = 30)	HAART Naïve (n = 30)
Balanced Dietary Habits			
Regularly	25 (83.3%)	25 (83.3%)	25 (83.3%)
Occasionally	05 (16.7%)	4 (13.3%)	5 (16.7%)
Rarely	0 (0%)	1 (3.4%)	0(0%)
Food supplement intake			
Yes	16 (53.3%)	12 (40%)	14 (46.7%)
No	14 (46.7%)	18 (60%)	16 (53.3%)
Herbs intake (Concoction for infection)			
Yes	18(60%)	19(63.3%)	14 (46.7%)
No	12(40%)	11(36.7%)	16 (53.3%)

The mean immunoglobulin levels and CD4 cell count of HIV negative, HIV positive on HAART and HIV positive treatment naïve are shown in Table 2. The normal expected values for IgG, IgA and IgM are (576 – 1004 mg/dl), (97 – 163 mg/dl) and (45 – 66 mg/dl) respectively. The participants on HAART had the highest mean immunoglobulin, although their levels are not significantly higher because p-values were 0.081, 0.589 and 0.113 for IgG, IgA and IgM respectively. The CD4+ cell count had the highest value in HIV negative participants which is significant at a p-value of 0.038 (< 0.05).

TABLE 3: Mean Immunoglobulin Levels and CD4+ Cell Count of HIV Negative, HIV Positive Participants on HAART and Treatment Naïve Participants

Parameters	HIV Negative (Mean ± SD) (n = 30)	PLWHA On HAART (Mean ± SD) (n = 30)	HAART Naïve (Mean ± SD) (n = 30)	P-value
IgG (mg/dL)	840.73 ± 164.2	909.2 ± 341.9	679.3 ± 135.3	0.081
IgA (mg/dL)	130.0 ± 32.9	153.9 ± 69.9	140 ± 26.9	0.589
IgM (mg/dL)	55.73 ± 10.6	123.4 ± 19.4	81.1 ± 34.7	0.113
CD4 (Cells/µl)	309.1 ± 132	215.4 ± 118.8	224.4 ± 61.1	0.038

DISCUSSION

This study was carried out on 90 participants to determine the applicability of immunoglobulin A, G, and M levels in assessing the immune status of PLWHA, in Osogbo, South-West Nigeria, as against the CD4+ T-cell count which has been adopted by the Natrional Guidelines for treatment of PLWHA (Ifeanyichukwu *et al.*, 2009).

The participants were age and sex matched in the groups of HIV negative individuals, PLWHA on HAART and treatment naïve HIV positive participants. The participants were adults in the age range of 18 to 60 years and majority in terms of gender were male (63.3%) in all the groups. Data collected through questionnaire revealed that majority of the participants (>50%) in each group had education above secondary school level with 83.3% taking balance diet regularly. This diversity observed in terms of socio-demographics and nutritional behaviour is a further confirmation that HIV does not respect any stratum of life. In every group as well, some participants (>40%) take both multivitamin supplements and herbal concoction. These reports are similar to data previously reported in Africa where 34% of PLWHA smoke and 42.7% of Africans living with HIV/AIDS who are on HAART consume herbal concoction regularly (Fanmi *et al.*, 2018).

The mean serum level of immunoglobulin IgA, IgG and IgM were significantly elevated both in HIV positive on HAART and HIV positive naïve. This increase in immunoglobulin levels may be due to polyclonal B-cell activation with advancing disease (Arinola *et al.*, 2015), which may be induced by one or more components of the virus generating pool of neutralising antibodies and auto-antibodies. These findings tally with the previous researchers on this subject (Akinpelu *et al.*, 2012).

The increase in immunoglobulin levels were higher in Africa compared to non-African countries which may be due to genetic differences in B-cell response of Africa origin compared to other race(Fanmi *et al.*, 2018). The findings were in agreement with study of Arinola *et al.* (2015) and corroborated with other researchers.

CONCLUSION

There was no significant difference in plasma immunoglobulins A, G and M in HIV positive patients on HAART, HIV treatment naïve and HIV negative control while CD4+ T-cells count was significantly higher in HIV negative participants compared with HIV positive individuals. Total immunoglobulins G, M and A cannot serve as a marker of immune suppression in HIV diagnosis and treatment monitoring.

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