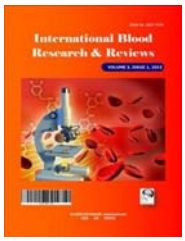

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Assessment of Antiphospholipid Antibodies, CD4 Count and Some Haematological Parameters in HIV Patients attending a Tertiary Health Institution in South-Western Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OAOS and AT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EED and ESS managed the analyses of the study. Author OBO managed the literature searches. All authors read and approved the final manuscript

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ABSTRACT

Background: Antiphospholipid antibodies (aPLs) are the serological markers used in the diagnosis of the antiphospholipid syndrome (APS). HIV infection has been associated with an elevated aPls level, but its link to the APS with clinical thrombosis is still been investigated**.** This study is designed to determine and correlate serum level of antiphospholipid antibodies with CD4 count and some haematological parameters of HIV seropositive subjects in comparison to those of healthy controls

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and also to compare these parameters between antiretroviral therapy (ART) naïve and treated patients.

Methodology: A cohort of 110 patients which consist of 90 HIV positive Patients (22 males and 68 females) and 20 HIV negative patients (10 males and 10 females) which serve as control attending Babcock University Teaching Hospital (BUTH) Ilishan-Remo, Ogun State, Nigeria were recruited for the cross-sectional study. HIV antibodies were detected using 3 rapid diagnostic kits (Determine, Unigold and Stat Pak). CD4+ cells were counted using Partec® Cyflow Counter (Germany). The Full Blood Count was analyzed using the Sysmex® Automated Haematology Analyzer (Kobe-Japan). Antiphospholipid antibodies (aPLs) were assayed using the Human Anti-Phospholipid Screen IgG/IgM ELISA kit (Alpha Diagnostic International, Texas, USA).

Results: The present study showed that the mean serum antiphospholipid antibody level was significantly (P<0.001) higher in HIV positive Patients (11.83±7.36u/ml) compared to the control group (7.30±3.95u/ml). While on one hand, there was a strong positive correlation between serum aPLs level and PLT (r= 0.044), MCHC (r= 0.084) and LYM (r= 0.105) in HIV infection; on the other hand, there was a strong negative correlation with CD4 count (r= -0.094), PCV (r= -0.099), Hb (r= -0.072), RBC (r= -0.003), WBC (r= -0.063), MNO (r= -0.213), GRA (r= -0.003), MCV (r= -0.023) and MCH (r= -0.005). Also, there was no significant differences (P>0.05) between the aPLs level of HIV group on ART (11.44±7.74 u/ml) and those not on ART (12.00±7.24 u/ml). Some haematological parameters like PLT, PCV, Hb, RBC and red cell indices of the HIV group on ART did not differ significantly from those not on ART. However, the CD4 count (638.89±119.56 cell/µL), WBC (5.38±1.49X10^3/µL), LYM (51.43±7.99%) and GRA (46.30±10.18%) of the HIV group on ART were significant higher than those not on ART (465.30±145.92 cell/µL, 4.55±1.57X10^3/µL, 42.23±10.96% and 39.10±7.81%, respectively).

Conclusion: Significant elevated aPLs level is present in HIV infection; however, the information obtained is not sufficient to indicate the occurrence of anti-phospholipid syndrome in HIV infection. There was no strong relationship between aPLs level and indicators of immunohaematological abnormalities in HIV infection. This finding is plausible and would therefore require further investigation.

Keywords: Anti-phospholipid antibodies; anti-phospholipid syndrome; HIV; CD4 cell count; haematological parameters.

1. INTRODUCTION

Antiphospholipid antibodies (aPLs) are a heterogeneous group of autoantibodies that can bind directly to phospholipids, thus allowing them to exert an influence on protein cofactors or complexes containing phospholipids. They are used as the serological markers in the diagnosis of the antiphospholipid syndrome [1].

Anti-phospholipid syndrome (APS) on the other hand, is an acquired thrombophilic disorder in which autoantibodies are produced to a variety of phospholipids and phospholipid binding proteins [2]. It has also be defined as a multisystem autoimmune disease, as well as hypercoagulable state caused by the presence of antiphospholipid antibodies (aPLs) namely: lupus anticoagulant (LA), anticardiolipin antibodies (aCLs), or anti-β_2 glycoprotein-1 (β_2GP1) antibodies which directly react against phospholipids and phospholipid binding proteins including cardiolipin, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylcholine resulting in the occurrence of venous and arterial thrombosis, recurrent fetal loss, thrombocytopenia, and other clinical manifestations in the presence of persistent circulating antiphospholipid antibodies [3-5].

According to Wilson *et al.* [6], a patient with ‘‘definite’’ APS must have persistent high-titer antiphospholipid antibodies (aPLs) associated with a history of arterial or venous thrombosis (or both), or recurrent pregnancy morbidity. Laboratory criteria are well defined and require aCL IgG or IgM or lupus anticoagulant in high titers (>40 IgG phospholipid units [GPL] or IgM phospholipid units [MPL] or >99th percentile), confirmed on repeat testing 12 weeks later). The 2006 International Criteria have included IgG and IgM antibodies to β_2GP1, which are also highly predictive of risk for thrombosis. Patients may be found to have not only aCL or lupus anticoagulant but also other aPLs or combinations which are not included in the criteria [7].

The primary mechanism behind the hypercoagulable state has been thought to be activation of endothelial cells, monocytes and platelets by antiphospholipid antibodies [8]. This leads to increased expression of adhesion molecules on endothelial cells and upregulation of tissue factor. Activated platelets also synthesize thromboxane A_2 altering the prostacyclin-thromboxane balance to favour thrombosis. In addition, aPLs may also impair fibrinolysis and interefere with the thrombomodulin-protein C-protein S pathway [9]. Haematological manifestations of APS include: thrombocytopenia, autoimmune haemolytic anaemia (AIHA), bone marrow necrosis (BMN) and thrombotic microangiopathy among several others [10,11].

The syndrome has been classified into two main classes: primary and secondary. In the former, anti-phospholipid syndrome occurs in the absence of any known autoimmune disorder as seen in patients having neither clinical nor laboratory evidence of another definable condition. In the latter, anti-phospholipid syndrome is seen in conjunction with other autoimmune diseases such as systemic lupus erythematosus. In rare cases, anti-phospholipid syndrome leads to rapid organ failure due to generalized thrombosis and a high risk of death; this is termed as “catastrophic antiphospholipid syndrome” (CAPS).

Precise cause of primary anti-phospholipid syndrome is unknown. However, some factors are associated with developing anti-phospholipid antibodies though not necessarily the syndrome as noted by previous Researchers [12-16]. These include: Infections (e.g HIV infection, Hepatitis C Virus infection, Syphilis and malaria, with a higher incidence of positivity of anti-phospholipid antibodies), medications (consumption of certain drugs, such as hydralazine, quinidine, phenytoin, and antibiotics such as amoxicillin may lead to an increased risk of APS) and genetic predispositions (although the disorder is not considered hereditary, research indicates that relatives of people with anti-phospholipid syndrome are more likely to have the antibodies).

An association between antiphospholipid antibodies (aPLs) and infectious agents in particular has been reported in several epidemiologic and experimental studies [16]. The connection between infections and aPLs has been supported by some indirect evidences, such as the seasonal distribution of aPLs [17] and high frequency of aCLs in healthy children who frequently suffer from a wide range of common viral infections [18]. Infection-induced aPLs have been traditionally regarded as transient and were generally not associated with clinical features of antiphospholipid syndrome. Several reports demonstrated that some patients can produce pathogenic antibodies in response to infection [15].

Viral agents most frequently associated with antiphospholipid syndrome include: HIV, Parvovirus B19, Cytomegalovirus, Varicella-Zoster Virus, Hepatitis C Virus, Epstein-Barr Virus, HTLV-1, Adenovirus, Influenza Virus, Mumps Virus and Rubella Virus [15,19,20].

HIV is a pandemic and has remained a public health concern for many decades. HIV infection, though associated with many opportunistic infections and neoplasms, it is further complicated with marked depletion of the CD4 Cells and haematological abnormalities [21,22]. It has also been associated with an increased prevalence of anti-phospholipid antibodies, but its link to the anti-phospholipid syndrome with clinical thrombosis remains focus of research [23]. A 2-10 fold increased incidence of venous thromboembolism (VTE) has been reported among HIV infected persons compared with the HIV-negative population of the same age. While most abnormal coagulation factors/markers improve after starting HAART, the disturbances fail to normalize completely in some [24]. HIV patients have also been reported to have a higher incidence of the lupus anticoagulant and antiphospholipid antibodies than the general population, which may contribute to a hypercoagulable state. HIV-infected patients may produce aPLs, in particular of IgM isotype, but full-blown clinical features of APS are distinctly uncommon [25].

Furthermore, anti-phospholipid syndrome can be diagnosed by lupus anticoagulant assays [3] and Enyzme linked immunoabsorbent assays [7]. The presence of antiphospholipid antibodies (aPLs) has been detected in approximately 5–20% of the healthy population. The prevalence increases with age, especially in elderly individuals with chronic disease [26] and is more common in women than men in about a 5:1 ratio [27]. The risk of thrombosis in patients with APS is estimated to range from 0.5% to 30% [28]. Anticardiolipin antibodies (ACAs) have been reported in HIV infected patients with a

prevalence ranging from 7% to 94% [29,30]. Antiphospholipid antibodies have also been detected in patients with acute and chronic infections and malignant diseases [5,31,32].

Abuaf [33], investigated the prevalence of aPLs in HIV infection. Anticardiolipn antibodies (aCLs) were reported to be present in 0-94%, anti-β2-glycoprotein-1 (anti-β2-GP1) in 4-47%, anti-prothrombin (aPT) in 2-12% of patients with lupus anticoagulant (LA) found in 0-53.5%. Similarly, Loizou et al. [34], studied the prevalence of aPLs in 100 black South African HIV positive patients. Their results show that there was a low prevalence of anti-β2-GP1 (6%), all exclusively belonging to the IgA isotype, as well as aCL (7%), which were mainly positive for IgG. A prevalence of 43% (mainly IgG) aPT was found showing that the pattern of aPLs in black South Africans differs from that found in caucasians [34].

Even though anti-phospholipid syndrome has been reported to be associated with HIV infections, the evaluation of markers predictive for this disease is not done routinely. This is worrisome because the development of antiphospholipid antibodies in HIV infected individuals can further worsen the deteriorating immune responsiveness. Therefore, the knowledge of the presence of these antibodies will aid appropriate clinical intervention. To the best of our knowledge, no work has been done on the haematological correlate of anti-phospholipid syndrome in HIV infected Patients attending Babcock University Teaching Hospital (BUTH) Ilishan-Remo, Ogun State, Nigeria. Lack of data in this regard, necessitates this research. The aim of this study is therefore to determine and correlate serum level of antiphospholipid antibodies with CD4 count and some haematological parameters of HIV seropositive subjects in comparison to those of healthy controls and also to compare these parameters between antiretroviral therapy (ART) naïve and treated patients.

2. METHODOLOGY

2.1 Study Area

The study was carried out at the HIV Clinic, Babcock University Teaching Hospital (BUTH) Ilishan-Remo, Ikenne Local Government Area, Ogun State, South-Western region of Nigeria, coordinates: 6^{O} 52' N3^{O} 43^{O} E.

2.2 Study Duration

This study was carried out between January and April, 2017.

2.3 Sample Size Calculation

The minimum sample size (n) required for the study was estimated using the population proportion formula described by Naing et al. [35]:

$$N = \frac{Z^2 \times P (1-P)}{d^2}$$

Where:

N = minimum sample size
Z = confidence interval (1.96)
P = 5.5%, prevalence of sero-positivity of HIV infection from previous study [36].
d = desired level of significance (0.05)

$$N = 1.96^2 \times 0.060 \frac{(1-0.055)}{(0.05)^2} = 79$$

The minimum sample size required (N) = 79

However, to make our work more robust, we decided to screen a total of 90 test Subjects and 20 control Subjects.

2.4 Subjects

Ninety (90) HIV Patients attending HIV Clinic, Babcock University Teaching Hospital (BUTH) Ilishan-Remo, Ogun State were recruited as Test Subjects, while 20 apparently healthy HIV negative individuals were used as Controls.

2.5 Eligibility of Subject

2.5.1 Inclusion criteria

Consenting HIV positive Patients without any other immunosuppressive disease/condition except HIV/AIDS attending HIV Clinic, Babcock University Teaching Hospital (BUTH) Ilishan-Remo, Ogun State were recruited for the study.

2.5.2 Exclusion criteria

Non-consenting HIV positive Patients, as well as HIV positive Patients with other immune suppressive disease/condition were excluded from the study.

2.6 Study Design

This is a cross-sectional hospital-based study. Pre-test counselling was instituted in which the purpose, benefit and procedures of the study were explained to the participants. A brief structured questionnaire was used to obtain demographic information from consenting subjects. Interpreter was provided for translation in local dialect where necessary. Informed consent was obtained from each patient and all participants were requested to voluntarily sign the consent forms in their own handwriting. The study groups were stratified by sex, level of education, occupation and marital status. All data were kept confidential in accordance with World Medical Association Declaration of Helsinki [37].

2.7 Sample Collection and Handling

Using standard aseptic procedures, ten (10) milliliters of blood was collected from each participant and 5 ml was dispensed into a container having 0.08 ml of ethylene diamine tetra-acetic acid (EDTA) and the remaining 5 ml was placed in a plain container, allowed to clot and separated by centrifugation at room temperature. Sera were immediately assayed. Caution was also taken not to use highly lipemic or hemolyzed or heat inactivated samples throughout the study. The sera obtained were used for the detection of HIV and anti-Phospholipid antibodies, while the anti-coagulated blood was used for the determination of CD4 count and full blood count using previously described methods.

2.8 Laboratory Analysis

2.8.1 HIV detection

HIV detection was carried out using the current National algorithm for HIV sero-diagnosis. This involved the use of 3 rapid diagnostic kits, following their manufacturer's instructions. Briefly, each patient's serum was screened for the presence of HIV antibodies using Determine (Abbott Laboratories, Tokyo, Japan) and Unigold HIV (Trinity Biotech Plc Bray, Co. Wicklow, Ireland). When both kits showed positivity, the patient was regarded as positive for HIV infection and vice versa. However, when test results were discordant, a third kit, which is the Tie breaker, 1/2 Stat Pak (Chembio Diagnostic Systems, New York, USA) was used. The HIV serostatus of the patient was taken as the result of either of the first two kits that agree with that of the third kit [22].

2.8.2 CD4+ cell count evaluation

CD4+ cell count was evaluated using Partec® Cyflow Counter (Germany), as described by PCC [38]. The Cyflow Counter was operated as instructed in the user's operational manual.

2.8.3 Haematological Parameters Analysis

Full Blood Count was analyzed using the Sysmex® Automated Haematology Analyzer KX-21N, Sysmex Corporation, (Kobe-Japan) as described by Samuel et al. [38].

2.8.4 Antiphospholipid antibodies Assay

The Antiphospholipid antibodies (aPLs) were assayed by Immunometric Enzyme Immunoassay using the Human Anti-Phospholipid Screen IgG/IgM ELISA kit supplied by Alpha Diagnostic International, Texas, USA as described by Banzato *et al.* [39]. The ELISA plate washer and ELISA reader were operated as instructed in the user's operational manual.

2.9 Statistical Analysis

Data generated are presented as mean±SEM using tables and analyzed using Statistical Packages for Social Sciences – Version 18.0 (SPSS-18.0). Student's t-test was used to compare two variables and one-way-analysis of variance (ANOVA) for more than two variables. P values<0.05 were considered statistically significant. Data were also subjected to Pearson correlation analysis using Graphpad INSTAT® Software Package to determine the relationship between aPLs and CD4 Cell count, as well as haematological parameters. An association was established between two variables when an OR value ≥1.00 was obtained [40].

3. RESULTS AND DISCUSSION

3.1 Results

The socio-demographic characteristics of the test subjects are presented in Table 1. Sixty-eight (68) females and twenty- two (22) males participated in this study. The level of education with the highest percentage was Bachelor's degree with 63.3%. Most of the participants were self-employed (54.4%) and married (53.3%). The

risk factors of the test subjects and their responses towards routine clinics and medications are presented in Table 2. 63 (70%) out of the 90 HIV Subjects are on antiretroviral therapy while the remaining 27 (30%) were not. However, they all attend routine clinic. Also 70% of these Patients take herbs while 30% do not. Table 3 shows the comparison of Mean±SEM Age, aPLs, CD4 Count and Haematological parameters of HIV Positive and control: CD4 count (0.001), PCV (0.001), WBC (0.001), MCV (0.012), MCH (0.001), MCHC (0.001) and aPLs (0.088). All have a P-value that is statistically significant (P<0.05). While the p-value for Age, Hb, RBC, PLT, LYM, MNO, GRA are greater than 0.05 (P>0.05) which were considered statistically not significant. The comparison of mean±SEM aPLs, CD4 count and haematological parameters of HIV Positive Participants on ART and those not on ART is

Table 1. Socio-demographic Characteristics of the HIV positive participants

Characteristics	Frequency	Percentage (%)
GENDER		
Male	22	24.4
Female	68	75.6
Total	90	100
LEVEL OF EDUCATION		
Primary	1	1.1
Secondary	14	15.6
Graduate	57	63.3
Postgraduate	18	20.0
Total	90	100
OCCUPATION		
Student	2	2.2
Self Employed	50	55.1
Civil Servants	38	42.7
Total	90	100
MARITAL STATUS		
Single	26	28.9
Married	48	53.3
Divorced	6	6.7
Widow	8	8.9
Widower	2	2.2
Total	**90**	**100**

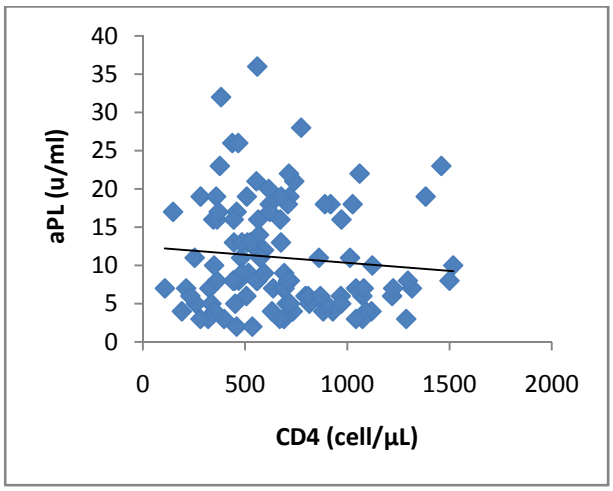

Fig. 1. Scatter Plot showing relationship between aPL and $CD4^+$ Cells. There is a negative correlation between the levels of aPl and $CD4^+$ Cells (r= -0.094)

Table 2. Risk factors, routine clinic and medication of the HIV Positive Participants

Risk factors	Frequency	Percentage (%)
SMOKING		
No	90	100
Yes	0	0
Total	90	100
DRINK ALCOHOL		
No	90	100
Yes	0	0
Total	90	100
ON ROUTINE CLINIC		
No	0	0
Yes	90	100
Total	90	100
ON ART		
No	27	30
Yes	63	70
Total	90	100
OTHER MEDICATION		
No	90	100
Yes	0	0
Total	90	100
HERBAL REMEDIES		
No	27	30
Yes	63	70
Total	90	100

Table 3. Mean±SEM Age, aPLs, CD4 Count and Haematological parameters of HIV Positive and control

Parameters	HIV Positive (N=90)	Control (N=20)	T-Value	P-Value
Age (Yrs)	42.47±10.35	36.65±13.80	1.777	0.088
aPLs (u/ml)	11.83±7.36	7.30±3.95	3.858	0.001*
CD4 Count (cell/µL)	607.38±276.67	994.60±293.32	-5.395	0.001*
PCV (%)	38.69±7.01	46.19± 5.67	-5.108	0.001*
Hb (g/dl)	12.17± 1.97	14.67±1.77	-1.118	0.272
RBC ($X10^6$/µL)	4.16± 0.86	4.26±0.72	-0.563	0.577
PLT ($X10^3$/µL)	245.49±98.88	253.75±71.76	-0.432	0.668
WBC ($X10^3$/µL)	4.80±1.59	7.36±0.97	-5.710	0.001*
LYM# (%)	44.99±10.97	45.44±12.50	-0.150	0.882
MNO# (%)	10.88±5.77	11.31±3.78	-0.413	0.682
GRA# (%)	44.14±10.05	43.50±15.00	0.181	0.858
MCV (fl)	78.35±6.22	94.52±13.52	-2.601	0.012*
MCH (Pg)	24.46±1.92	29.77±4.88	-8.026	0.001*
MCHC (g/dl)	27.95±2.60	31.56±0.90	-6.140	0.001*

***Keys**: aPLs = antiphospholipids antibodies, CD4 = cluster of differentiation 4, PCV = Packed Cell Volume, Hb = Haemoglobin concentration, RBC = Red Blood Cell Count, WBC = White Blood Cell Count, PLT = Platelet count, MCV = Mean Cell Volume, MCH = Mean Cell Haemoglobin, MCHC = Mean Cell Haemoglobin Concentration, LYM# = Absolute Lymphocytes count, GRA# = Absolute Granulocytes Count. Test values differ significantly from control at P<0.05*

shown in Table 4. CD4 count (0.001), WBC (0.021), LYM (0.001) and GRA (0.001) have a P-value that is statistically significant (P<0.05), while other haematological parameters have P-value greater than 0.05 (P>0.05) which were considered statistically not significant. The relationship between the level of antiphospholipid antibodies and CD4 count and other haematological parameters are represented using scatter plot graphs (Figs. 1-12). There was

a strong positive correlation between serum antiphospholipid antibodies (aPls) level and PLT (0.044), MCHC (0.084) and LYM (0.105); on the other hand, there was a strong negative correlation with CD4 COUNT (-0.094), PCV (-0.099), Hb (-0.072), RBC (-0.003), WBC (-0.063), MNO (-0.213), GRA (-0.003), MCV (-0.023) and MCH (-0.005).

Table 4. Mean±SEM aPL, CD4 Count and some Haematological parameters of HIV Positive ART treated and naïve Patients

Parameters	On ART (N=63)	Not on ART (N=27)	T-Value	P-Value
aPLs (u/ml)	11.44±7.74	12.00±7.24	0.318	0.752
CD4 Count (cell/µL)	638.89±119.56	465.30±145.92	10.277	0.001*
PCV (%)	38.62±7.74	36.87±5.03	0.180	0.859
Hb (g/dl)	12.16±2.14	11.19±1.57	0.069	0.945
RBC ($X10^6$/µL)	4.17±0.91	4.13±0.76	0.181	0.857
PLT ($X10^3$/µL)	268.64±91.17	231.48±95.18	0.915	0.366
WBC ($X10^3$/µL)	5.38±1.49	4.55±1.57	2.375	0.021*
LYM (%)	51.43±7.99	42.23±10.96	1.841	0.001*
MNO (%)	11.48±6.36	9.48±3.81	0.69	0.069
GRA (%)	46.30±10.18	39.10±7.81	3.644	0.001*
MCV (fl)	79.78±14.05	75.98±13.35	0.563	0.567
MCH (Pg)	25.79±4.62	24.72±5.24	0.057	0.955
MCHC (g/dl)	28.65±0.92	27.35±0.83	1.507	0.138

***Keys**: aPLs = antiphospholipids antibodies, CD4 = cluster of differentiation 4, PCV = Packed Cell Volume, Hb = Haemoglobin concentration, RBC = Red Blood Cell Count, WBC = White Blood Cell Count, PLT = Platelet count, MCV = Mean Cell Volume, MCH = Mean Cell Haemoglobin, MCHC = Mean Cell Haemoglobin Concentration, LYM# = Absolute Lymphocytes count, GRA# = Absolute Granulocytes Count. Test values differ significantly from control at P<0.05*

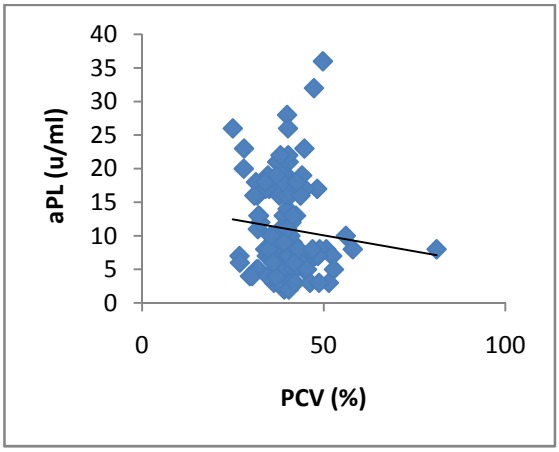

Fig. 2. Scatter Plot showing relationship between aPL and PCV. There is a negative correlation between the levels of aPl and PCV (r= -0.099)

3.2 Discussion

The present study was designed to determine and correlate serum level of antiphospholipid antibodies with CD4 count and some haematological parameters of HIV seropositive subjects in comparison to those of healthy controls and also to compare these parameters between antiretroviral therapy (ART) naïve and treated patients. Out of the 90 HIV positive Participants examined, 68 (75.6%) were females while 22 (24.4%) were males. This does not agree with the work of Abdollahi and Morteza [41], who reported no significant differences in the male-to-female ratio.

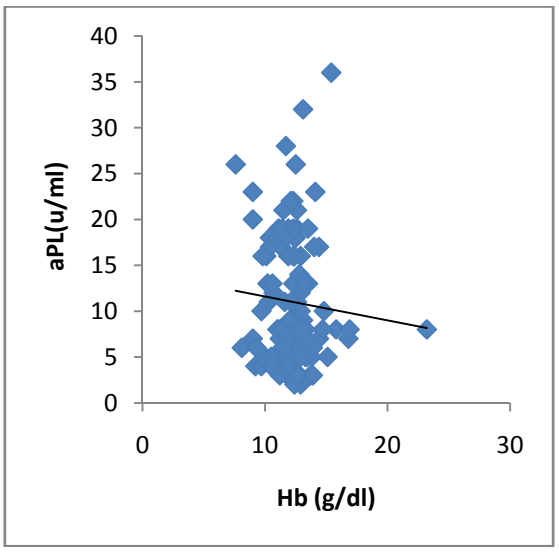

Fig. 3. Scatter Plot showing relationship between aPL and Hb. There is a negative correlation between the levels of aPl and Hb (r= -0.097)

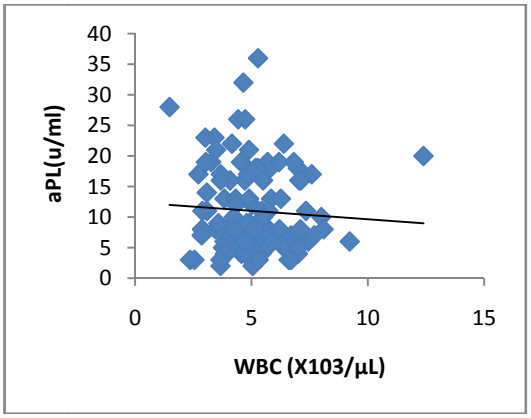

Fig. 4. Scatter Plot showing relationship between aPL and WBC. There is a negative correlation between the levels of aPl and WBC (r= -0.063)

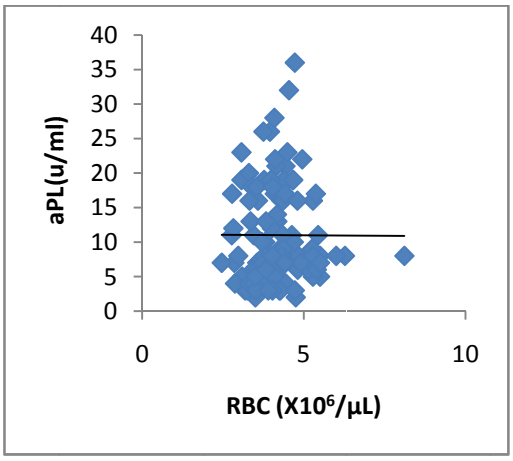

Fig. 5. Scatter Plot showing relationship between aPL and RBC. There is no correlation between the levels of aPl and RBC (r= -0.003)

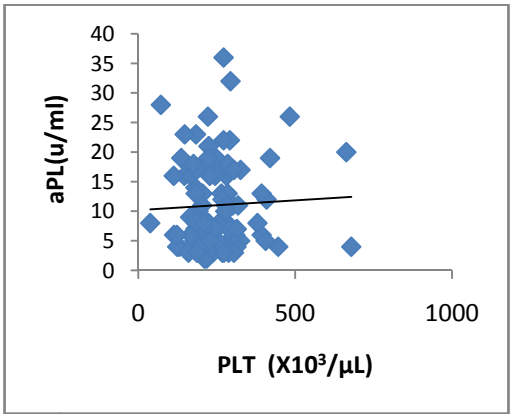

Fig. 6. Scatter Plot showing relationship between aPL and PLT. There is a positive correlation between the levels of aPl and PLT (r= 0.044)

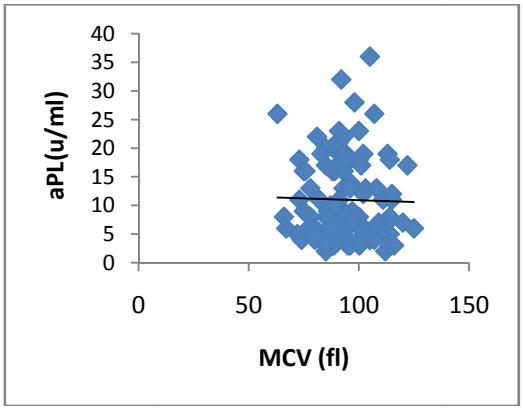

Fig. 7. Scatter Plot showing relationship between aPL and MCV. There is a weak negative correlation between the levels of aPl and MCV (r= -0.023)

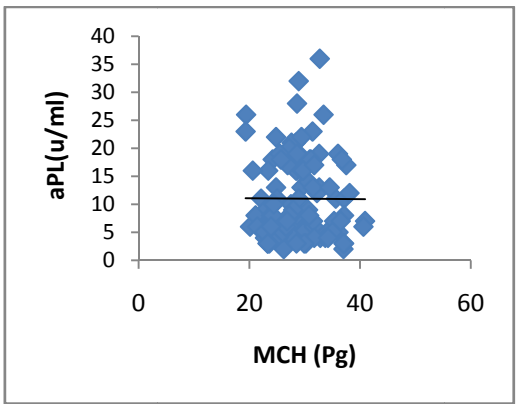

Fig. 8. Scatter Plot showing relationship between aPLand MCH. There is a very weak negative correlation between the levels of aPl and MCH (r= -0.005)

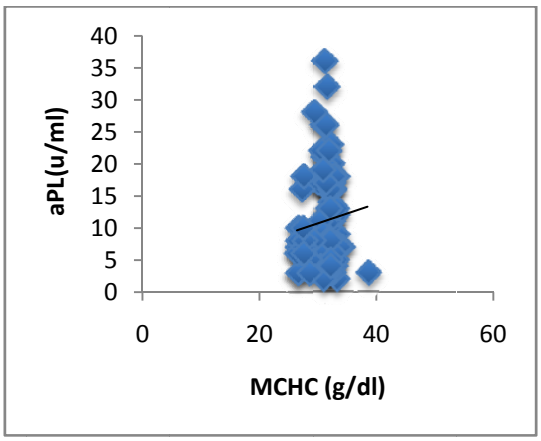

Fig. 9. Scatter Plot showing relationship between aPL and MCHC. There is a positive correlation between the levels of aPl and MCHC (r= 0.084)

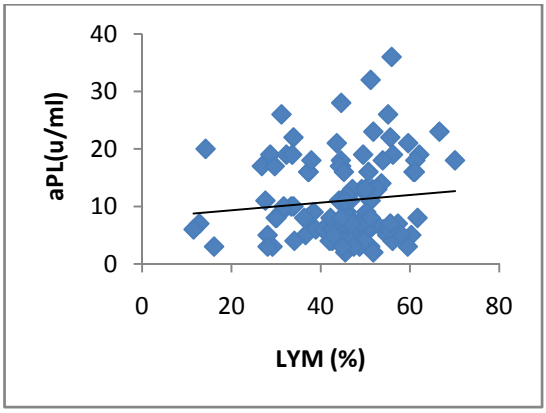

Fig. 10. Scatter Plot showing relationship between aPL and LYM. There is a positive correlation between the levels of aPl and LYM (r= 0.105)

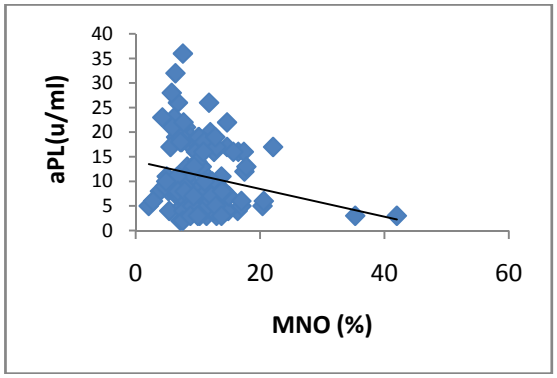

Fig. 11. Scatter Plot showing relationship between aPL and MID. There is a negative correlation between the levels of aPl and MNO (r= -0.213)

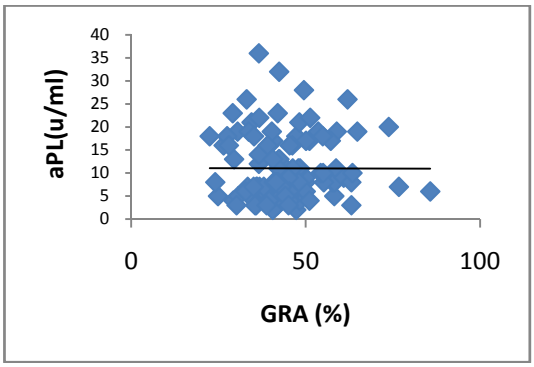

Fig. 12. Scatter Plot showing relationship between aPL and GRA. There is a weak negative correlation between the levels of aPl and GRA (r= -0.003)

The result of the present study showed that the mean serum antiphospholipid antibodies level was significantly (P<0.001) higher in HIV positive Patients (11.83±7.36 u/ml) compared to the control group (7.30±3.95 u/ml). This agrees with the work of Soto-vega et al. [1] and Abdollahi and Morteza [41], who both reported similar findings among HIV-positive patients compared to the healthy controls. It also agrees with the work of Abdel-Wahab et al. [15], who reported that viral infections like HIV can increase the risk of developing elevated aPLs and associated thromboembolic events. Detection of aPLs in the controls agrees with previous reports that aPLs is present in apparently healthy population. While a higher mean serum aPLs in HIV cases confirms earlier claims that HIV infection is not unconnected with elevated aPLs levels.

The present study further revealed that there was no significant correlation between levels of antiphospholipid antibody and CD4 Cell Count, as well as some haematological parameters in HIV patients. This agrees with the report of Palomo et al. [42], who observed no correlation between the presence of aPLs and reduced platelet count (thrombocytopenia) in Chilean HIV-infected patients and Soto-vega et al. [1], who also reported no correlations between antiphospholipid antibody titers and specific clinical manifestations in HIV positive patients.

Furthermore, the Total White Blood Cell Count (4.80±1.59 $X10^3$/µL) and CD4 count (607.38±276.67cell/µL) of the HIV positive group were found to be significantly lower (P<0.001) than of the healthy controls (7.36±0.97 $X10^3$/µL, 994.60±293.32 cell/µL, respectively). This is in agreement with the work of Abdollahi and Morteza [41], who obtained similar results among 58 HIV-infected patients examined. White blood cell count is used to monitor treatment which can cause leucopenia, investigate HIV/AIDS, infections and unexplained fever. Leucopenia is one of the most common complications of HIV and may be broadly classified as being due either to a bone marrow production defect or to increased peripheral loss or destruction of blood cells. The causes for leucopenia in HIV infection are probably due to destruction of white cells by the HIV, as well as perturbed bone marrow cytokine homeostasis [43].

An autoimmune mechanism involving antigranulocyte antibodies and impaired granulopoiesis and any infiltrative process involving the bone marrow (infection, malignancy) has also been postulated [44]. HIV

is cytotoxic to T-helper lymphocytes, which in turn leads to dysregulation of B cells and altered release of cytokines. HIV-infected T cells directly suppress growth of bone marrow progenitors, thus suppressing haemopoiesis. CD4, the cell-surface receptor target of HIV, is carried by T-helper lymphocytes, monocytes and microvascular endothelial cells which are prevalent in marrow. The infection of monocytes in the marrow further alters release of cytokines, which indirectly suppress the capacity for haemopoietic progenitor cells to adequately respond to peripheral leucopenia [9,45,46].

Still, the Hb levels of the HIV patients (12.17± 1.97 g/dl) in this study, were not significantly different (P>0.05) from that of the healthy controls (14.67±1.77 g/dl). This is in accordance with the work of Abdollahi and Morteza [41]. The clinical values of measuring haemoglobin concentration include: to screen blood donor prior to blood donation, detect anemia and it severity and to monitor an anaemic patient's response to treatment. It is also useful in monitoring patients with HIV disease receiving drugs such as AZT.

The mean PCV value of the HIV positive Patients (38.69±7.01%) were found to be significantly (P<0.001) lower than the healthy controls (46.19± 5.67%). This partly support the results of previous study by Osime et al. [47], who reported a non-significant (P>0.05) reduction in the PCV value among HIV positive Patients compared to those of the healthy controls. PCV is used to screen for anaemia when it is not possible to measure haemoglobin concentration accurately. And together with haemoglobin, PCV value is useful in the calculation of MCHC [43].

The red cell indices: MCV (78.35±6.22fl), MCH (24.46±1.92pg) and MCHC (27.95±2.60g/dl) of the HIV positive patients were found to be significantly lower than those of the healthy controls (94.52±13.52fl, 29.77±4.88pg and 31.56±0.90g/dl, respectively). Red cell indices are particularly important for the diagnosis of anemia and since the values obtained for the HIV positive group are less than the reference range (MCV: 80-98fl, MCH: 27-32pg and MCHC: 31.5-36.0g/dl), it could be said that anaemia exist among them, no matter how mild.

It was also observed in this study that the Platelet count (245.49±98.88 $X10^3$/µL) of the HIV groups were lower than the healthy controls (253.75±71.76 $X10^3$/µL), although not statistically significant (P>0.05). This is in accordance with the reports of Osime et al. [47] and Raman et al. [48]. Thrombocytopenia in HIV infection is due to increased platelet destruction by deposition of circulating immune complexes on platelets. Presence of specific anti-platelet antibodies and direct infection of megakaryocytes by HIV are also being hypothesized [49].

Furthermore, there was no significant difference (P>0.05) between the anti-phospholipid antibodies of HIV group on ART (11.44±7.74 u/ml) and those not on ART (12.00±7.24 u/ml). It was also observed that some haematological parameters like PLT, PCV, Hb, RBC and red cell indices, of the HIV group on ART did not differ significantly (P>0.05) from those not on ART. These are in agreement with the work of Raman *et al.* [50], who reported no significant differences among HIV patients on ART and those not on ART with regard to these parameters. However, the CD4 count (638.89±119.56 cell/µL), WBC (5.38±1.49$X10^3$/µL), LYM (51.43±7.99%) and GRA (46.30±10.18%) of the HIV group on ART in this present study were significant higher than those not on ART (465.30±145.92 cell/µL, 4.55±1.57$X10^3$/µL, 42.23±10.96% and 39.10±7.81%, respectively). This does not agree with the work of Raman *et al.* [50], who reported otherwise.

ART is an important intervention in leucopenic HIV patients, as it alleviates the cytokine disturbances. An increase in CD4 count ranging from 154 cell mm^{-3} to 262 cell mm^{-3} following exposure to ART has been reported by Akele *et al*. [51]. No doubt, elevated CD4 count, have been suggested to be a strong indicator of a promising life expectancy as the immune cells which are the major target of the virus appreciated in number. However, if patients develop a new leucopenia while taking ART, then a drug-induced leucopenia must be considered and a change of ART regimen may be necessary [46].

The use of ART therapy is giving rise to a substantial change in the clinical spectrum of HIV infection, in which an increasing frequency of associated autoimmune and lymphomatous processes is being described. The persistent stimulation of the immune system triggered by HIV infection may favour the development of autoimmune manifestations [52].

4. CONCLUSION

The significant elevated aPLs level is present in HIV infection; however, the information obtained

is not sufficient to indicate the occurrence of anti-phospholipid syndrome in HIV infection. There was no strong relationship between aPLs level and indicators of immunohaematological abnormalities in HIV infection. This finding is plausible and would require further investigation. Nevertheless, it is still needful for Clinicians to have a high index of suspicion of APS in HIV patients who present with a thrombotic episode whether they are on antiretroviral therapy or not. They should investigate for the presence of antiphospholipid antibodies; as early diagnosis may influence the course of the HIV/AIDS disease. Finally, resources for the detection of antiphospholipid antibodies should be made readily available in resource-limited settings.

CONSENT

All authors declare that 'written' informed consent was obtained from the participants and pre-test counselling was instituted in the course of the conduct of this study.

ETHICAL APPROVAL

Ethical approval for the study was obtained from the Babcock University Health Research Ethics Committee (BUHREC), Babcock University, Ilishan-Remo, Ogun State, Nigeria, with the ethical approval registration number: BUHREC223/17 (29th March 2017).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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