Haematological Correlate of anti-Phospholipid Syndrome in HIV Patients attending a Tertiary Health Institution in South-Western Nigeria

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.

Aims: To determine if antiphospholipid antibody (aPL) level correlate with CD4 Cell Counts and hematological parameters in HIV positive Subjects. The study also aimed at comparing the serum aPLs level, CD4 Cell Counts and hematological parameters of HIV positive Subjects on antiretroviral therapy (ART) with those not on ART.

Methodology: This is a cross-sectional, as well as institutional-based study. 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 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 by Immunometric Enzyme Immunoassay using the Human Anti-Phospholipid Screen IgG/IgM ELISA kit (Alpha Diagnostic International, Texas, USA). Data generated were analyzed using SPSS-18.0 (Statistical packages for social Sciences – version 18.0).

Results: The result of 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. Howbeit, the CD4 Cell Count (638.89±119.56 cell/μL), WBC (5.38±1.49X10³/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±1.57X10³/μL, 42.23±10.96% and 39.10±7.81%, respectively).

Conclusion: Significant elevated aPLs level is present in HIV infection, howbeit, 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
1.0 INTRODUCTION

Anti-phospholipid syndrome (APS) is an acquired thrombophilic disorder in which autoantibodies are produced to a variety of phospholipids and phospholipid binding proteins [1]. It has also be defined as a multisystem autoimmune disease, as well as hypercoagulable state caused by the presence of antiphospholipid antibodies (aPL) namely: lupus anticoagulant (LA), anticardiolipin antibodies (aCL), or anti-β2 glycoprotein-1 (β2GP1) antibodies which directly react against phospholipids and phospholipid binding proteins including cardiolipin, phosphatidylserine, phosphatidylinositol, phosphatidyethanolamine, 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 [2-4].

According to Wilson et al. [5], a patient with “definite” APS must have persistent high-titer antiphospholipid antibodies (aPL) 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 β2GP1, which are also highly predictive of risk for thrombosis. Patients may be found to have not only aCL or lupus anticoagulant but also aPL or combinations which are not included in the criteria [6].

The primary mechanism behind the hypercoagulable state has been thought to be activation of endothelial cells, monocytes and platelets by antiphospholipid antibodies [7]. This leads to increased expression of adhesion molecules on endothelial cells and upregulation of tissue factor. Activated platelets also synthesize thromboxane A2 altering the prostacyclin-thromboxane balance to favour thrombosis. In addition, aPL may also impair fibrinolysis and interfere with the thrombomodulin-protein C-protein S pathway [8]. Haematological manifestation of anti-phospholipid syndrome include: thrombocytopenia, autoimmune haemolytic anaemia (AIHA), bone marrow necrosis (BMN) and thrombotic microangiopathy among several others [9, 10].

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 [11-14]. 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).

Furthermore, anti-phospholipid syndrome can be diagnosed mainly by Enzyme linked immunobsorbent assays [6] and lupus anticoagulant assays [15]. 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 [16] and is more common in women than men in about a 5:1 ratio [17]. The risk of thrombosis in patients with APS is estimated to range from 0.5% to 30% [18]. Anticardiolipin antibodies (ACA) have been reported in HIV infected patients with a prevalence ranging from 7% to 94% [19, 20]. Antiphospholipid antibodies have also been detected in patients with acute and chronic infections and malignant diseases [4, 21, 22].
While HIV infection is primarily associated with depletion of CD4+ lymphocytes [23, 24], 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 [25]. 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 [26]. 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.

Abuaf [27], 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. [28], studied the prevalence of aPL 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 [28].

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 the levels of anti-phospholipid antibodies with CD4 Cell Counts and hematological parameters in HIV subjects. And to also compare the serum aPLs level, CD4 Cell Counts and hematological parameters of HIV positive Subjects on antiretroviral therapy (ART) with those not on ART.

2.0 METHODOLOGY

2.1 Study Area

The study was carried out at Babcock University Teaching Hospital (BUTH) Ilishan-Remo, Ikenne Local Government Area, Ogun state, South-Western region of Nigeria, coordinates: 6° 52’ N3° 43’ 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. [29]:

\[ n = \frac{Z^2 \times P \times (1-P)}{d^2} \]

Where:

- \( n \) = minimum sample size
- \( Z \) = confidence interval (1.96)
- \( P \) = 5.5%, prevalence of HIV infection from previous study [30].
- \( d \) = desired level of significance (0.05)
N = 1.96² * 0.060 (1-0.055) = 79

The minimum sample size required (N) = 79

However, 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 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.

2.6 Exclusion Criteria

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

2.7 Study Design

This is a cross-sectional hospital based study. Pre-test counseling 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 [31].

2.8 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 cell count and haematological parameters using previously described methods.

2.9 Laboratory Analysis

2.9.1 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 (ChemiBio 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 [24].

2.9.2 CD4+ cell count evaluation

CD4+ cell count was evaluated using Partec® Cyflow Counter (Germany), as described by PCC [32]. The Cyflow Counter was operated as instructed in the user’s operational manual.

2.9.3 Hamatological 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. [33].

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. [34]. The ELISA plate washer and ELISA reader were operated as instructed in the user’s operational manual.

2.10 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 [35].

3.0 RESULTS AND DISCUSSION

3.1 Results

The socio-demographic characteristics of the test subjects is 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 response towards routine clinics and routine medications is presented in Table 2. 63% (70%) out of the 90 HIV Subjects are on antiretroviral therapy while the remain 27% (30%) were not. They all attend routine clinic. Also 70% of these Patients take herbs while 30% don’t. 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 aPL (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 that 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 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 hematological 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 Cell Count and other haematological parameters are represented using scatter plot graphs (Figure 1-12). There was a strong positive correlation between serum antiphospholipid antibody (aPL) level and PLT (0.044), MCHC (0.064) 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).
3.5 Discussion

Antiphospholipid antibodies (aPLs) are the serological markers used in the diagnosis of the antiphospholipid syndrome (Soto-vega et al., 2012). These are heterogeneous group of autoantibodies can bind directly to phospholipids, thus allowing them to exert an influence on protein cofactors or complexes containing phospholipids. The factors causing production of aPLs remain largely unknown, and growing evidence suggests that infectious agents such as viruses, bacteria and parasites can induce aPLs [37]. An association between infectious agents and antiphospholipid antibodies (aPLs) has been reported in several epidemiologic and experimental studies.

The connection between infections and aPL has been supported by some indirect evidence, such as the seasonal distribution of aPLs [38] and high frequency of aCL in healthy children who frequently suffer from a wide range of common viral infections [39]. 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 [14].

Viral agents most frequently associated with antiphospholipid syndrome include: HIV, parvovirus B19, cytomegalovirus, varicella-zoster virus, Hepatitis C, Epstein-Barr virus, HTLV-1, Adenovirus, Influenza virus, Mumps virus and Rubella virus [14, 40, 41].

HIV is pandemic and remains as 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 hematological abnormalities. It has also been associated with an increased prevalence of anti-phospholipid antibodies, but its link to the anti-phospholipid syndrome with clinical thrombosis is still been investigated. HIV-infected patients may produce aPL, in particular of IgM isotype, but full-blown clinical features of APS are distinctly uncommon [42].

The present study was designed to determine and correlate the level of Antiphospholipid antibodies with CD4+ cell Count and haematological parameters in HIV patients attending HIV Clinic, Babcock University Teaching Hospital (BUTH), Ilishan-Remo, Ogun State. A cohort of 110 participants consisting of 90 HIV positive cases and 20 healthy controls were recruited for the study and examined. Out of the 90 HIV positive Participants examined, 68 (75.6%) were females while 22 (24.4%). This does not agree with the work of Abdollahi and Morteza [43], who reported no significant differences in the male-to-female ratio.

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±7.36u/ml) compared to the control group (7.30±3.95u/ml). This agrees with the work of Soto-vega et al. [36] and Abdollahi and Morteza [43], 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. [14], 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. Why 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 level of antiphospholipid antibody and CD4 Cell Count, as well as haematological parameters in HIV patients. This agrees with the report of Palomo et al. [44], who observed no correlation between the presence of aPL and reduced platelet count (thrombocytopenia) in Chilean HIV-infected patients and Soto-vega et al. [36], who also reported no correlations between antiphospholipid antibody titers and specific clinical manifestations in HIV positive patients.

Table 1: Socio-demographic Characteristics of the HIV Positive Participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
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<tr>
<td>GENDER</td>
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<td></td>
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<tr>
<td>Male</td>
<td>22</td>
<td>24.4</td>
</tr>
<tr>
<td>Female</td>
<td>68</td>
<td>75.6</td>
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Table 2: Risk factors, routine clinic and medication of the HIV Positive Participants

<table>
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<tr>
<th>Risk factors</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
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<tr>
<td>Yes</td>
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<td>0</td>
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<tr>
<td>Total</td>
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<td>100</td>
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<tr>
<td><strong>DRINK ALCOHOL</strong></td>
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<tr>
<td>No</td>
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<td>100</td>
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<tr>
<td>Yes</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
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<td>100</td>
</tr>
<tr>
<td><strong>ON ROUTINE CLINIC</strong></td>
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<td></td>
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<td>0</td>
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<tr>
<td>Yes</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
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<td>100</td>
</tr>
<tr>
<td><strong>ON ART</strong></td>
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<td></td>
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<tr>
<td>No</td>
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<td>70</td>
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<td>0</td>
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<tr>
<td>Total</td>
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<tr>
<td><strong>HERBAL REMEDIES</strong></td>
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<td>30</td>
</tr>
<tr>
<td>Yes</td>
<td>63</td>
<td>70</td>
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</tbody>
</table>
| Total              | 90        | 100            

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HIV Positive (N=90)</th>
<th>Control (N=20)</th>
<th>T-Value</th>
<th>P-Value</th>
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<tbody>
<tr>
<td>Age (Yrs)</td>
<td>42.47±10.35</td>
<td>36.65±13.80</td>
<td>1.777</td>
<td>0.088</td>
</tr>
</tbody>
</table>
Furthermore, the Total White Blood Cell Count (4.80±1.59 \times 10^3/μL) and CD4 Cell Count (607.38±276.67 cell/μL) of the HIV positive group were found to be significantly lower (P<0.001) than of the healthy controls (7.36±0.97 \times 10^3/μL, 994.60±293.32 cell/μL, respectively). This is in agreement with the work of Abdollahi and Morteza [43], 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 is probably due to destruction of white cells by the HIV, as well as perturbed bone marrow cytokine homeostasis [45].

An autoimmune mechanism involving antigranulocyte antibodies and impaired granulopoiesis and any infiltrative process involving the bone marrow (infection, malignancy) has also been postulated [46]. 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 [8, 47, 48].

**Table 4:** Mean±SEM aPL, CD4 Count and Haematological parameters of HIV Positive Participants on ART and those not on ART

<table>
<thead>
<tr>
<th>Parameters</th>
<th>On ART (N=63)</th>
<th>Not on ART (N=27)</th>
<th>T-Value</th>
<th>P-Value</th>
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<tbody>
<tr>
<td>aPL (u/ml)</td>
<td>11.83±7.36</td>
<td>12.00±7.24</td>
<td>0.318</td>
<td>0.752</td>
</tr>
<tr>
<td>Test</td>
<td>Mean Value</td>
<td>Standard Deviation</td>
<td>t-Value</td>
<td>P-value</td>
</tr>
<tr>
<td>--------------</td>
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<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>CD4 Count (cell/μL)</td>
<td>638.89±119.56</td>
<td>465.30±145.92</td>
<td>10.277</td>
<td>0.001*</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>38.62±7.74</td>
<td>36.87±5.03</td>
<td>0.180</td>
<td>0.859</td>
</tr>
<tr>
<td>HB (g/dl)</td>
<td>12.16±2.14</td>
<td>11.19±1.57</td>
<td>0.069</td>
<td>0.945</td>
</tr>
<tr>
<td>RBC (X10⁶/μL)</td>
<td>4.17±0.91</td>
<td>4.13±0.76</td>
<td>0.181</td>
<td>0.857</td>
</tr>
<tr>
<td>PLT (X10³/μL)</td>
<td>268.64±91.17</td>
<td>231.48±95.18</td>
<td>0.915</td>
<td>0.366</td>
</tr>
<tr>
<td>WBC (X10³/μL)</td>
<td>5.38±1.49</td>
<td>4.55±1.57</td>
<td>2.375</td>
<td>0.021*</td>
</tr>
<tr>
<td>LYM (%)</td>
<td>51.43±7.99</td>
<td>42.23±10.96</td>
<td>1.841</td>
<td>0.001*</td>
</tr>
<tr>
<td>MNO (%)</td>
<td>11.48±6.36</td>
<td>9.48±3.81</td>
<td>0.69</td>
<td>0.069</td>
</tr>
<tr>
<td>GRA (%)</td>
<td>46.30±10.18</td>
<td>39.10±7.81</td>
<td>3.644</td>
<td>0.001*</td>
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<tr>
<td>MCV (fl)</td>
<td>79.78±14.05</td>
<td>75.98±13.35</td>
<td>0.563</td>
<td>0.567</td>
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<tr>
<td>MCH (Pg)</td>
<td>25.79±4.62</td>
<td>24.72±5.24</td>
<td>0.057</td>
<td>0.955</td>
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<tr>
<td>MCHC (g/dl)</td>
<td>28.65±0.92</td>
<td>27.35±0.83</td>
<td>1.507</td>
<td>0.138</td>
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</tbody>
</table>

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.

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 [43]. The clinical values of measuring haemoglobin concentration include: to screen blood donor prior to blood donation, detect anemia and its 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. [49], 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 [45]. 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.
Figure 1: Scatter Plot showing relationship between aPL and CD4⁺ Cells. There is a negative correlation between the levels of aPL and CD4⁺ Cells ($r = -0.094$).

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

Figure 3: Scatter Plot showing relationship between aPL and HB. There is a negative correlation between the levels of aPL and HB ($r = -0.097$).

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

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

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

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

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

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

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

Figure 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).
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. [49] and Raman et al. [50]. Thrombocytopenia in HIV infection, is due to increased platelet destruction by deposition of circulating immune complexes on platelets. Presence of specific antiplatelet antibodies and direct infection of megakaryocytes by HIV are also being hypothesized [51].

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. This is in agreement with the work of Raman et al. [52], who reported no significant differences among HIV patients on ART and those not on ART with regard to these parameters. Howbeit, the CD4 Cell 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 in this present study 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). This does not agree with the work of Raman et al. [52], who reported otherwise.

ART is an important intervention in leucopenic HIV patients, as it alleviates the cytokine disturbances. An increase in CD4 cell count ranging from 154cellmm\(^-3\) to 262 cellmm\(^-3\) following exposure to ART have been reported by Akele et al. [53]. No doubt, elevated CD4 Cell 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 [48].

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 favor the development of autoimmune manifestations [54].

**4.0 CONCLUSION**

Significant elevated aPLs level is present in HIV infection, howbeit, 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 counseling 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.
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