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.

17 **1.0 INTRODUCTION**
18
19 Anti-phospholipid syndrome (APS) is an acquired thrombophilic disorder in which autoantibodies are
20 produced to a variety of phospholipids and phospholipid binding proteins [1]. It has also be defined as a
21 multisystem autoimmune disease, as well as hypercoagulable state caused by the presence of
22 antiphospholipid antibodies (aPL) namely: lupus anticoagulant (LA), anticardiolipin antibodies (aCL), or
23 anti-β_2 glycoprotein-1 (β_2GP1) antibodies which directly react against phospholipids and phospholipid
24 binding proteins including cardiolipin, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine,
25 phosphatidylglycerol, and phosphatidylcholine resulting in the occurrence of venous and arterial
26 thrombosis, recurrent fetal loss, thrombocytopenia, and other clinical manifestations in the presence of
27 persistent circulating antiphospholipid antibodies [2-4].
28
29 According to Wilson *et al.* [5], a patient with ''definite'' APS must have persistent high-titer
30 antiphospholipid antibodies (aPL) associated with a history of arterial or venous thrombosis (or both), or
31 recurrent pregnancy morbidity. Laboratory criteria are well defined and require aCL IgG or IgM or lupus
32 anticoagulant in high titers (>40 IgG phospholipid units [GPL] or IgM phospholipid units [MPL] or >99th
33 percentile), confirmed on repeat testing 12 weeks later). The 2006 International Criteria have included
34 IgG and IgM antibodies to β_2GP1, which are also highly predictive of risk for thrombosis. Patients may be
35 found to have not only aCL or lupus anticoagulant but also other aPL or combinations which are not
36 included in the criteria [6].
37
38 The primary mechanism behind the hypercoagualable state has been thought to be activation of
39 endothelial cells, monocytes and platelets by antiphospholipid antibodies [7]. This leads to increased
40 expression of adhesion molecules on endothelial cells and upregulation of tissue factor. Activated
41 platelets also synthesize thromboxane A_2 altering the prostacyclin-thromboxane balance to favour
42 thrombosis. In addition, aPL may also impair fibrinolysis and interefere with the thrombomodulin-protein
43 C-protein S pathway [8]. Haematological manifestation of anti-phospholipid syndrome include:
44 thrombocytopenia, autoimmune haemolytic anaemia (AIHA), bone marrow necrosis (BMN) and
45 thrombotic microangiopathy among several others [9, 10].
46
47 The syndrome has been classified into two main classes: primary and secondary. In the former, anti-
48 phospholipid syndrome occurs in the absence of any known autoimmune disorder as seen in patients
49 having neither clinical nor laboratory evidence of another definable condition. In the latter, anti-
50 phospholipid syndrome is seen in conjunction with other autoimmune diseases such as systemic lupus
51 erythematosus. In rare cases, anti-phospholipid syndrome leads to rapid organ failure due to generalized
52 thrombosis and a high risk of death; this is termed as "catastrophic antiphospholipid syndrome" (CAPS).
53
54
55 Precise cause of primary anti-phospholipid syndrome is unknown. However, some factors are associated
56 with developing anti-phospholipid antibodies though not necessarily the syndrome as noted by previous
57 Researchers [11-14]. These include: Infections (e.g HIV infection, Hepatitis C Virus infection, Syphilis and
58 malaria, with a higher incidence of positivity of anti-phospholipid antibodies), medications (consumption of
59 certain drugs, such as hydralazine, quinidine, phenytoin, and antibiotics such as amoxicillin may lead to
60 an increased risk of APS) and genetic predispositions (although the disorder is not considered hereditary,
61 research indicates that relatives of people with anti-phospholipid syndrome are more likely to have the
62 antibodies).
63
64 Furthermore, anti-phospholipid syndrome can be diagnosed mainly by Enyzme linked immunoabsorbent
65 assays [6] and lupus anticoagulant assays [15]. The presence of antiphospholipid antibodies (aPLs) has
66 been detected in approximately 5–20% of the healthy population. The prevalence increases with age,
67 especially in elderly individuals with chronic disease [16] and is more common in women than men in
68 about a 5:1 ratio [17]. The risk of thrombosis in patients with APS is estimated to range from 0.5% to 30%
69 [18]. Anticardiolipin antibodies (ACA) have been reported in HIV infected patients with a prevalence
70 ranging from 7% to 94% [19, 20]. Antiphospholipid antibodies have also been detected in patients with
71 acute and chronic infections and malignant diseases [4, 21, 22].
72

73 While HIV infection is primarily associated with depletion of CD4+lymphocytes [23, 24], it has also been
74 associated with an increased prevalence of anti-phospholipid antibodies, but its link to the anti-
75 phospholipid syndrome with clinical thrombosis remains focus of research [25]. A 2-10 fold increased
76 incidence of venous thromboembolism (VTE) has been reported among HIV infected persons compared
77 with the HIV-negative population of the same age. While most abnormal coagulation factors/markers
78 improve after starting HAART, the disturbances fail to normalize completely in some [26]. HIV patients
79 have also been reported to have a higher incidence of the lupus anticoagulant and antiphospholipid
80 antibodies than the general population, which may contribute to a hypercoagulable state.

81
82 Abuaf [27], investigated the prevalence of aPLs in HIV infection. Anticardiolipn antibodies (aCLs) were
83 reported to be present in 0-94%, anti-β2-glycoprotein-1 (anti-β2-GP1) in 4-47%, anti-prothrombin (aPT) in
84 2-12% of patients with lupus anticoagulant (LA) found in 0-53.5%. Similarly, Loizou *et al.* [28], studied the
85 prevalence of aPL in 100 black South African HIV positive patients. Their results show that there was a
86 low prevalence of anti-β2-GP1 (6%), all exclusively belonging to the IgA isotype, as well as aCL (7%),
87 which were mainly positive for IgG. A prevalence of 43% (mainly IgG) aPT was found showing that the
88 pattern of aPLs in black South Africans differs from that found in caucasians [28].

89
90 Even though anti-phospholipid syndrome has been reported to be associated with HIV infections, the
91 evaluation of markers predictive for this disease is not done routinely. This is worrisome because the
92 development of antiphospholipid antibodies in HIV infected individuals can further worsen the
93 deteriorating immune responsiveness. Therefore, the knowledge of the presence of these antibodies will
94 aid appropriate clinical intervention. To the best of our knowledge, no work has been done on the
95 haematological correlate of anti-phospholipid syndrome in HIV infected Patients attending Babcock
96 University Teaching Hospital (BUTH) Ilishan-Remo, Ogun State, Nigeria. Lack of data in this regard,
97 necessitates this research. The aim of this study is therefore to determine and correlate the levels of anti-
98 phospholipid antibodies with CD4 Cell Counts and hematological parameters in HIV subjects. And to also
99 compare the serum aPLs level, CD4 Cell Counts and hematological parameters of HIV positive Subjects
100 on antiretroviral therapy (ART) with those not on ART.

101
102 **2.0 METHODOLOGY**

103
104 **2.1 Study Area**

105
106 The study was carried out at Babcock University Teaching Hospital (BUTH) Ilishan-Remo, Ikenne Local
107 Government Area, Ogun state, South-Western region of Nigeria, coordinates: 6° 52' $N3^O$ 43° E.

108
109 **2.2 Study Duration**

110
111 This study was carried out between January and April, 2017.

112
113 **2.3 Sample Size Calculation**

114
115 The minimum sample size (n) required for the study was estimated using the population proportion
116 formula described by Naing *et al.* [29]:

117
118 $$N = \frac{Z^2 \times P (1-P)}{d^2}$$
119
120 Where:

121 N = minimum sample size

122 Z = confidence interval (1.96)

123 P = 5.5%, prevalence of HIV infection from previous study [30].

124 d = desired level of significance (0.05)

3

125 $N = \dfrac{1.96^2 \times 0.060\ (1-0.055)}{(0.05)^2} = 79$
126

127

128 The minimum sample size required (N) = 79

129

130 However, we decided to screen a total of 90 test Subjects and 20 control Subjects.

131

132 **2.4 Subjects**

133

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

137

138 **2.5 Eligibility of Subject**

139

140 **2.5.1 Inclusion Criteria**

141

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

145

146 **2.6 Exclusion Criteria**

147

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

150

151 **2.7 Study Design**

152

153 This is a cross-sectional hospital based study. Pre-test counseling was instituted in which the purpose,
154 benefit and procedures of the study were explained to the participants. A brief structured questionnaire
155 was used to obtain demographic information from consenting subjects. Interpreter was provided for
156 translation in local dialect where necessary. Informed consent was obtained from each patient and all
157 participants were requested to voluntarily sign the consent forms in their own handwriting. The study
158 groups were stratified by sex, level of education, occupation and marital status. All data were kept
159 confidential in accordance with World Medical Association declaration of Helsinki [31].

160

161 **2.8 Sample Collection and Handling**

162

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

170 **2.9 Laboratory Analysis**

171 **2.9.1 HIV detection**

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

178 USA) was used. The HIV serostatus of the patient was taken as the result of either of the first two kits that
179 agree with that of the third kit [24].

180 **2.9.2 CD4+ cell count evaluation**

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

183 **2.9.3 Hamatological Parameters Analysis**

184 Full Blood Count was analyzed using the Sysmex® Automated Haematology Analyzer KX-21N, Sysmex
185 Corporation, (Kobe-Japan) as described by Samuel *et al.* [33].

186 **Antiphospholipid antibodies Assay**
187
188 The Antiphospholipid antibodies (aPLs) were assayed by Immunometric Enzyme Immunoassay using the
189 Human Anti-Phospholipid Screen IgG/IgM ELISA kit supplied by Alpha Diagnostic International, Texas,
190 USA as described by Banzato *et al.* [34]. The ELISA plate washer and ELISA reader were operated as
191 instructed in the user's operational manual.

192 **2.10 Statistical Analysis**

193 Data generated are presented as mean±SEM using tables and analyzed using Statistical packages for
194 social Sciences – version 18.0 (SPSS-18.0). Student's t test was used to compare two variables and one-
195 way-analysis of variance (ANOVA) for more than two variables. P values<0.05 were considered
196 statistically significant. Data were also subjected to Pearson correlation analysis using Graphpad
197 INSTAT® Software Package to determine the relationship between aPLs and CD4 Cell count, as well as
198 haematological parameters. An association was established between two variables when an OR value
199 ≥1.00 was obtained [35].
200
201 **3.0 RESULTS AND DISCUSSION**

202 **3.1 Results**

203 The socio-demographic characteristics of the test subjects is presented in Table 1. Sixty-eight (68)
204 females and twenty- two (22) males participated in this study. The level of education with the highest
205 percentage was Bachelor's degree with 63.3%. Most of the participants were self-employed (54.4%) and
206 married (53.3%). The risk factors of the test subjects and their response towards routine clinics and
207 routine medications is presented in Table 2. 63 (70%) out of the 90 HIV Subjects are on antiretroviral
208 therapy while the remain 27 (30%) were not. they all attend routine clinic. Also 70% of these Patients take
209 herbs while 30% don't. Table 3 shows the comparison of Mean±SEM Age, aPLs, CD4 Count and
210 Haematological parameters of HIV Positive and control: CD4 count (0.001), PCV (0.001), WBC (0.001),
211 MCV (0.012), MCH (0.001), MCHC (0.001) and aPL (0.088). All have a P-value that is statistically
212 significant (P<0.05). While the p-value for AGE, HB, RBC, PLT, LYM, MNO, GRA are greater that 0.05
213 (P>0.05) which were considered statistically not significant. The comparison of mean±SEM aPLs, CD4
214 Count and Haematological parameters of HIV Positive Participants on ART and those not on ART is
215 shown in Table 4. CD4 count (0.001), WBC (0.021), LYM (0.001) and GRA (0.001) have a P-value that is
216 statistically significant (P<0.05).

217 while other hematological parameters have P-value greater than 0.05 (P>0.05) which were considered
218 statistically not significant. The relationship between the level of Antiphospholipid antibodies and CD4 Cell
219 Count and other haematological parameters are represented using scatter plot graphs (Figure 1-12).
220 There was a strong positive correlation between serum antiphospholipid antibody (aPl) level and PLT
221 (0.044), MCHC (0.084) and LYM (0.105); on the other hand, there was a strong negative correlation with
222 CD4 COUNT (-0.094), PCV (-0.099), HB (-0.072), RBC (-0.003), WBC (-0.063), MNO (-0.213), GRA (-
223 0.003), MCV (-0.023) and MCH (-0.005).

224 **3.5 Discussion**

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

232 The connection between infections and aPL has been supported by some indirect evidence, such as the
233 seasonal distribution of aPLs [38] and high frequency of aCL in healthy children who frequently suffer
234 from a wide range of common viral infections [39]. Infection-induced aPLs have been traditionally
235 regarded as transient and were generally not associated with clinical features of antiphospholipid
236 syndrome. Several reports demonstrated that some patients can produce pathogenic antibodies in
237 response to infection [14].

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

241 HIV is pandemic and remains as a public health concern for many decades. HIV infection, though
242 associated with many opportunistic infections and neoplasms, it is further complicated with marked
243 depletion of the CD4 Cells and hematological abnormalities. It has also been associated with an
244 increased prevalence of anti-phospholipid antibodies, but its link to the anti-phospholipid syndrome with
245 clinical thrombosis is still been investigated. HIV-infected patients may produce aPL, in particular of IgM
246 isotype, but full-blown clinical features of APS are distinctly uncommon [42].

247 The present study was designed to determine and correlate the level of Antiphosholipid antibodies with
248 $CD4^+$ cell Count and haematological parameters in HIV patients attending HIV Clinic, Babcock University
249 Teaching Hospital (BUTH), Ilishan-Remo, Ogun State. A cohort of 110 participants consisting of 90 HIV
250 positive cases and 20 healthy controls were recruited for the study and examined. Out of the 90 HIV
251 positive Participants examined, 68 (75.6%) were females while 22 (24.4%). This does not agree with the
252 work of Abdollahi and Morteza [43], who reported no significant differences in the male-to-female ratio.

253 The result of the present study showed that the mean serum antiphospholipid antibodies level was
254 significantly ($P<0.001$) higher in HIV positive Patients (11.83±7.36u/ml) compared to the control group
255 (7.30±3.95u/ml). This agrees with the work of Soto-vega *et al.* [36] and Abdollahi and Morteza [43], who
256 both reported similar findings among HIV-positive patients compared to the healthy controls. It also
257 agrees with the work of Abdel-Wahab *et al.* [14], who reported that viral infections like HIV can increase
258 the risk of developing elevated aPLs and associated thromboembolic events. Detection of aPLs in the
259 controls agrees with previous reports that aPLs is present in apparently healthly population. Why a higher
260 mean serum aPLs in HIV cases confirms earlier claims that HIV infection is not unconnected with
261 elevated aPLs levels.
262
263 The present study further revealed that there was no significant correlation between level of
264 antiphospholipid antibody and CD4 Cell Count, as well as haematological parameters in HIV patients.
265 This agrees with the report of Palomo *et al.* [44], who observed no correlation between the presence of
266 aPL and reduced platelet count (thrombocytopenia) in Chilean HIV-infected patients and Soto-vega *et al.*
267 [36], who also reported no correlations between antiphospholipid antibody titers and specific clinical
268 manifestations in HIV positive patients.
269 **Table 1:** Socio-demographic Characteristics of the HIV Positive Participants
270

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**

271

272 **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

273

274 **Table 3:** Mean±SEM Age, aPL, 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

7

Parameter				
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

Furthermore, the Total White Blood Cell Count (4.80±1.59 X10^3/µL) and CD4 Cell 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 [43], who obtained similar results among 58 HIV-infected patients examined. White blood cell count is use 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

Parameters	On ART (N=63)	Not on ART (N=27)	T-Value	P-Value
aPL (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

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

302 Still, the HB levels of the HIV patients (12.17± 1.97 g/dl) in this study, were not significantly different
303 (P>0.05) from that of the healthy controls (14.67±1.77 g/dl). This is in accordance with the work of
304 Abdollahi and Morteza [43]. The clinical values of measuring haemoglobin concentration include: to
305 screen blood donor prior to blood donation, detect anemia and it severity and to monitor an anaemic
306 patient's response to treatment. It is also useful in monitoring patients with HIV disease receiving drugs
307 such as AZT.

308 The mean PCV value of the HIV positive Patients (38.69±7.01%) were found to be significantly (P<0.001)
309 lower than the healthy controls (46.19± 5.67%). This partly support the results of previous study by Osime
310 *et al.* [49], who reported a non-significant (P>0.05) reduction in the PCV value among HIV positive
311 Patients compared to those of the healthy controls. PCV is used to screen for anaemia when it is not
312 possible to measure haemoglobin concentration accurately. And together with haemoglobin, PCV value is
313 useful in the calculation of MCHC [45]. The red cell indices: MCV (78.35±6.22fl), MCH (24.46±1.92pg)
314 and MCHC (27.95±2.60g/dl) of the HIV positive patients were found to be significantly lower than those of
315 the healthy controls (94.52±13.52fl, 29.77±4.88pg and 31.56±0.90g/dl, respectively). Red cell indices are
316 particularly important for the diagnosis of anemia and since the values obtained for the HIV positive group
317 are less than the reference range (MCV: 80-98fl, MCH: 27-32pg and MCHC: 31.5-36.0g/dl), it could be
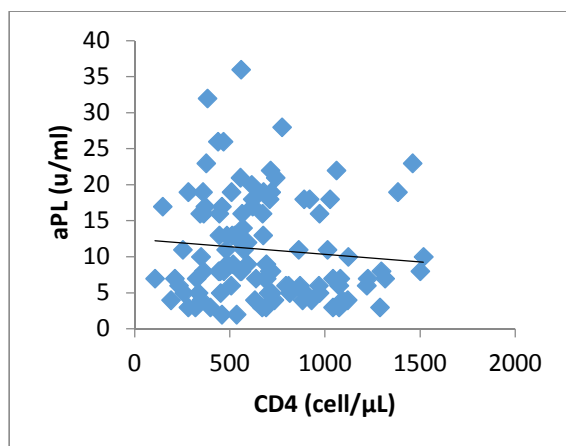318 said that anaemia exist among them, no matter how mild.

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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).
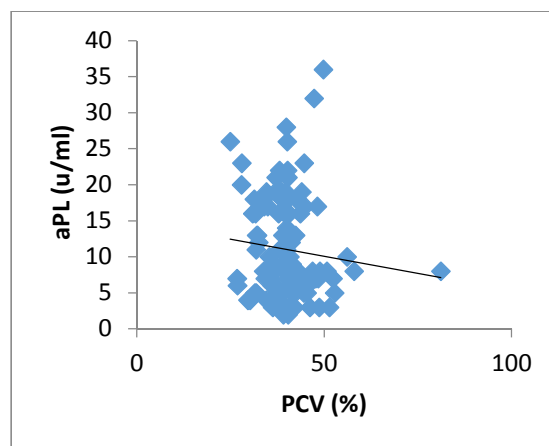
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Figure 2: Scatter Plot showing relationship between aPL and PCV. There is a negative correlation between the levels of aPl and PCV (r= -0.099).
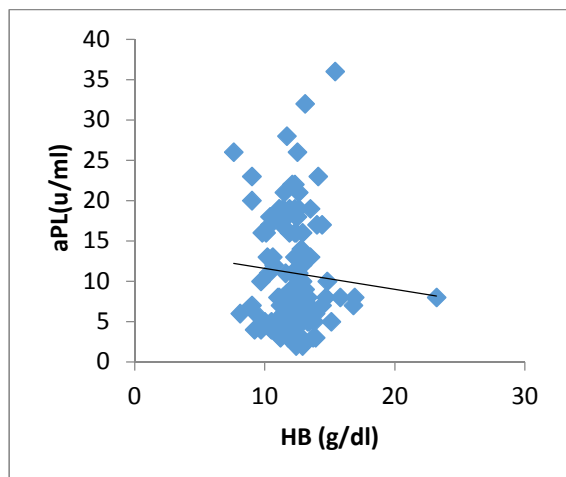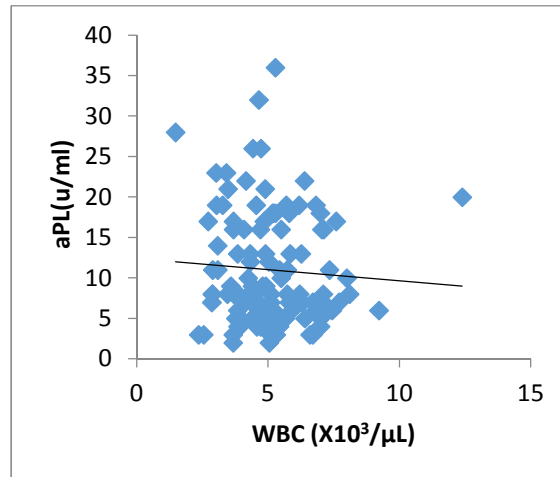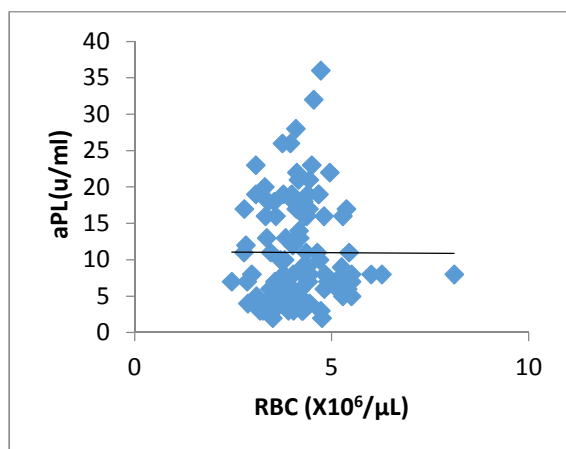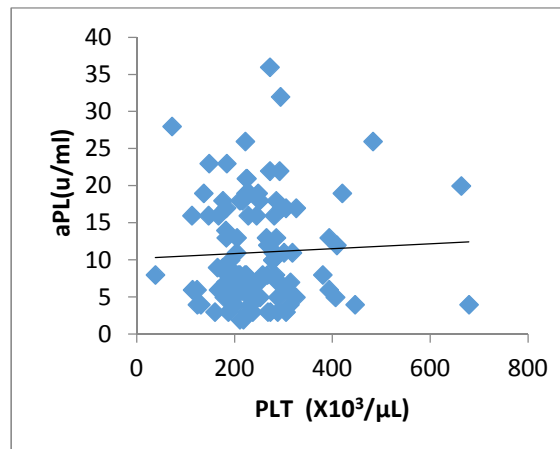
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Figure 3: Scatter Plot showing relationship between aPL and HB. There is a negative correlation between the levels of aPl and HB (r= -0.097).

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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).

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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).
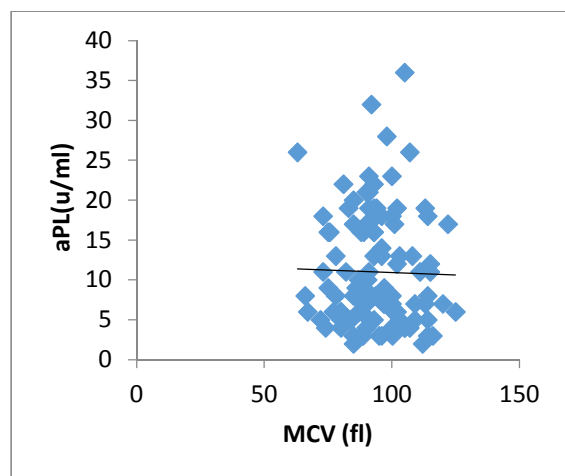
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Figure 7: Scatter Plot showing relationship between aPL and MCV. There is a weak negative correlation between the levels of aPl and MCV (r= -0.023).
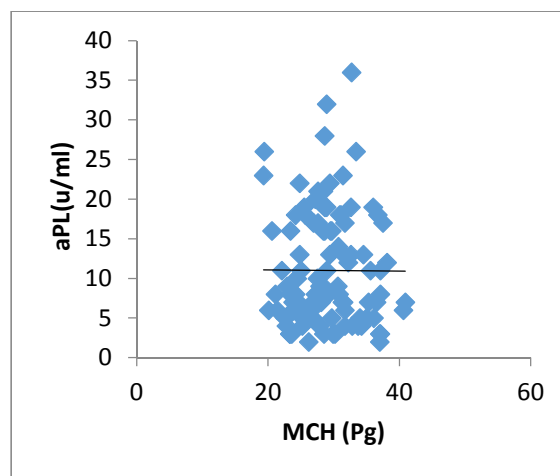
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Figure 8: Scatter Plot showing relationship between aPLand MCH. There is a very weak negative correlation between the levels of aPl and MCH (r= -0.005).
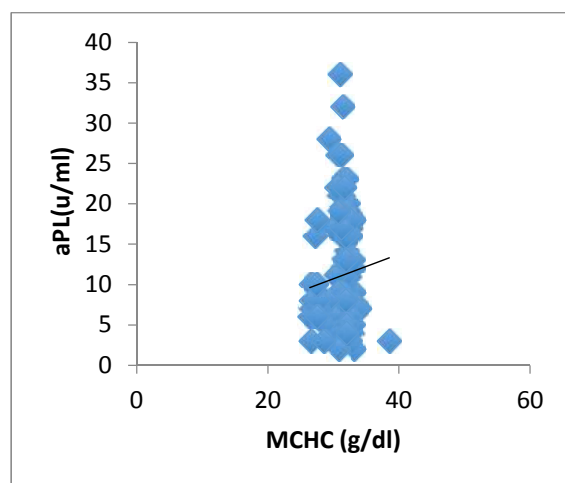
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Figure 9: Scatter Plot showing relationship between aPL and MCHC. There is a positive correlation between the levels of aPl and MCHC (r= 0.084).
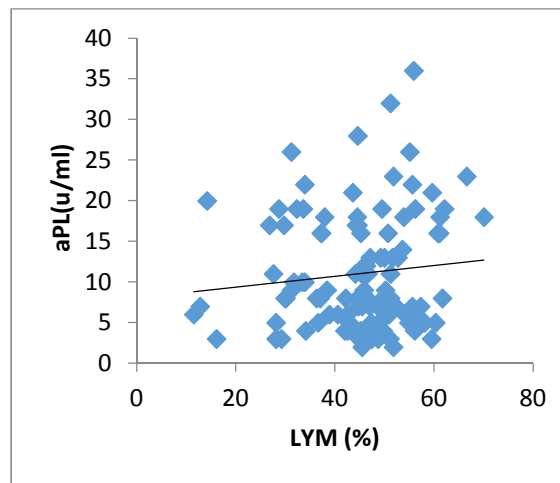
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Figure 10: Scatter Plot showing relationship between aPL and LYM. There is a positive correlation between the levels of aPl and LYM (r= 0.105).
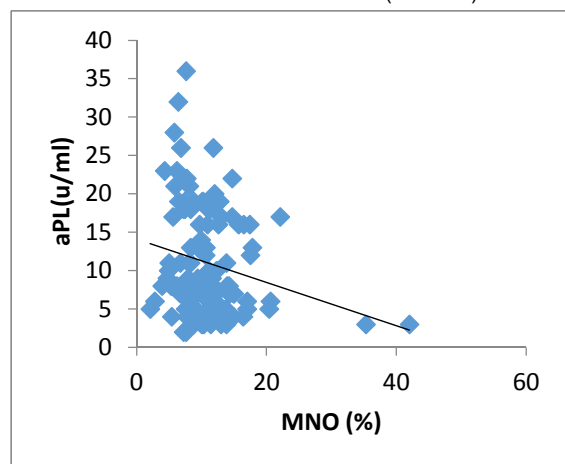
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Figure 11: Scatter Plot showing relationship between aPL and MID. There is a negative correlation between the levels of aPl and MNO (r= -0.213).
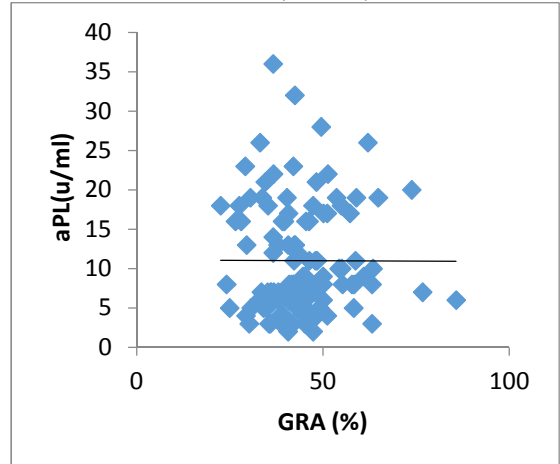
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Figure 12: Scatter Plot showing relationship between aPL and GRA. There is a weak negative correlation between the levels of aPl and GRA (r= -0.003).

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337 It was also observed in this study that the Platelet count (245.49±98.88 X10^3/μL) of the HIV groups were
338 lower than the healthy controls (253.75±71.76 X10^3/μL), although not statistically significant (P>0.05).
339 This is in accordance with the reports of Osime et al. [49] and Raman et al. [50]. Thrombocytopenia in
340 HIV infection, is due to increased platelet destruction by deposition of circulating immune complexes on
341 platelets. Presence of specific antiplatelet antibodies and direct infection of megakaryocytes by HIV are
342 also being hypothesized [51].

344 Furthermore, there was no significant difference (P>0.05) between the anti-phospholipid antibodies of HIV
345 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
346 haematological parameters like PLT, PCV, HB, RBC and red cell indices, of the HIV group on ART did
347 not differ significantly (P>0.05) from those not on ART. This is in agreement with the work of Raman et al.
348 [52], who reported no significant differences among HIV patients on ART and those not on ART with
349 regard to these parameters. Howbeit, the CD4 Cell Count (638.89±119.56 cell/μL), WBC
350 (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
351 present study were significant higher than those not on ART (465.30±145.92 cell/μL, 4.55±1.57X10^3/μL,
352 42.23±10.96% and 39.10±7.81%, respectively). This does not agree with the work of Raman et al. [52],
353 who reported otherwise.

355 ART is an important intervention in leucopenic HIV patients, as it alleviates the cytokine disturbances. An
356 increase in CD4 cell count ranging from 154cellmm^{-3} to 262 cellmm^{-3} following exposure to ART have
357 been reported by Akele et al. [53]. No doubt, elevated CD4 Cell Count, have been suggested to be a
358 strong indicator of a promising life expectancy as the immune cells which are the major target of the virus
359 appreciated in number. However, if patients develop a new leucopenia while taking ART, then a drug-
360 induced leucopenia must be considered and a change of ART regimen may be necessary [48].

362 The use of ART therapy is giving rise to a substantial change in the clinical spectrum of HIV infection, in
363 which an increasing frequency of associated autoimmune and lymphomatous processes is being
364 described. The persistent stimulation of the immune system triggered by HIV infection may favor the
365 development of autoimmune manifestations [54].

367 **4.0 CONCLUSION**

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

377 **CONSENT**

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

382 **ETHICAL APPROVAL**

384 Ethical approval for the study was obtained from the Babcock University Health Research Ethics
385 Committee (BUHREC), Babcock University, Ilishan-Remo, Ogun State, Nigeria.

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