



REVIEW ARTICLE

Prevalence of Antiphospholipid Antibodies in HIV-Infected Children in Ile-Ife, South-Western Nigeria

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Abstract

Background: Lupus anticoagulant (LA) and anticardiolipin antibodies (aCL) are acquired autoantibodies referred to as antiphospholipid antibodies (aPL). They can be found in infective and non-infective conditions. Infection with the Human immunodeficiency virus (HIV) can predispose patients to having these antibodies and this has been documented in Nigerian adult patients but not in the pediatric age group. To determine the prevalence of antiphospholipid antibodies in HIV-infected children and its association with the clinical stage of infection and possible hematological derangements were the aim of this study.

Methods: One hundred and sixteen HIV-infected children attending the Pediatric Virology Clinic of a tertiary hospital and 61 healthy children age and sex-matched controls were consecutively selected. Ethical approval and informed consent were obtained from the institution and parents/care-givers respectively. Full blood count, direct Coombs' test (DCT), activated partial thromboplastin time, Kaolin clotting time test and anticardiolipin antibody assay were done to screen for antiphospholipid antibodies.

Results: Thirteen (11.2%) of the subjects and 6 (9.8%) of the controls were positive for antiphospholipid antibodies (aCL and LA). Using aCL as a determinant of aPL, 11 (9.48%) and 6 (9.84%) of the subjects and controls respectively had aPL while with LA as the determinant of aPL 7 (6.03%) of the subjects and 4 (6.56%) of the controls had aPL. Eleven (84.6%) of the subjects positive for aPL had been on highly-active antiretroviral therapy (HAART) for more than 6 months. The effect of antiphospholipid antibodies on PCV, platelet and white cell counts were not significant (p-values; 0.41, 0.82 and 0.66). None had any clinical feature associated with the presence of aPL and DCT was negative in all who had antiphospholipid antibodies.

Conclusion: Antiphospholipid antibodies are higher in children than in adults. The presence of these antibodies not influenced by infection or non-infection with the HIV virus though the infected had higher prevalence rate. Symptoms and signs of antiphospholipid syndrome were absent.

Keywords

Human immunodeficiency virus, Anticardiolipin antibodies, Lupus anticoagulant, Kaolin clotting time

Abbreviations

AIDS: Acquired Immunodeficiency Syndrome; aCL: Anticardiolipin Antibodies; aPL: Antiphospholipid Antibodies; APS: Antiphospholipid Syndrome; aPTT: Activated Partial Thromboplastin Time; DCT: Direct Coombs Test; HAART: Highly-Active Antiretroviral; LA: Lupus Anticoagulant; OI: Opportunistic Infection; PCV: Packed Cell Volume; PT: Prothrombin Time; WBC: White Blood Cell

Introduction

Immunological perturbations are observed phenomena in HIV infection both in children and adults. This combination of autoimmunity and immune dysfunction in patients infected with HIV is an interesting observation [1]. Antiphospholipid antibodies (aPL) are a heterogeneous group of acquired autoantibodies. These could be immunoglobulin G, M, A or a combination of these subtypes. Different combinations of phospholipids, phospholipid-binding proteins or both are recognized by these autoantibodies and they have also been reported in various clinical settings [2].

The three major subtypes that have been described and can be detected by immunoassay and functional coagulation assays are Lupus anticoagulant (LA), anticardiolipin antibodies (aCL) and anti-beta 2 glycoprotein antibodies (anti-β2GPI) [3].

The clinical significance of antiphospholipid antibodies is derived from their association with a syndrome of venous and or arterial thrombosis, recurrent fetal loss and immune thrombocytopenia referred to as Antiphospholipid syndrome (APS) though not all those who have these antibodies develop the syndrome. Other manifestations include stroke, pulmonary embolism and autoimmune haemolytic anaemia [2].

Lupus anticoagulant and anticardiolipin antibodies have been detected in patients with HIV infection [4-6]. Both types of anticardiolipin antibodies (the pathogenic or β_2 GPI-dependent) as well as the non-pathogenic (non- β_2 GPI-dependent) antibodies may be detected and there is diversity not only of the isotypes but also of the aPL. There is also a low prevalence of antibodies directed toward β_2 GPI in HIV-infected patients thus making APS and its manifestation uncommon in HIV infection [1].

Antiphospholipid antibodies in the pediatric age group bear some uniqueness; in that their presence is more infection-related and clinical manifestations are more severe when present [7-9]. Moreover, antibodies found in this setting do not show anti- β_2 GPI activity and in general not associated with thrombotic events [10].

The mechanism possibly involved in the autoimmune manifestations in HIV infection include the direct effect of HIV on endothelial, synovial and other haemopoietic cells resulting in destruction of CD4+ T-cells, increased cytotoxic cell activity and increased expression of autoantigens. Molecular mimicry is one the proposed mechanisms in the development of autoimmune disease secondary to an infection [1,11].

The pathogenesis of antiphospholipid antibodies in HIV-infected patients is unknown but it has been suggested that it could be due to the stimulation of B cells in response to opportunistic infections or HIV itself, resulting in the production of polyclonal immunoglobulins in a non-specific manner [12]. It could also represent the biological expression of a hyper-reactive immune system, particularly following the commencement of highly-active antiretroviral therapy (HAART) in the HIV-infected [13].

Materials and Methods

Patients sampling and study design

A prospective case-control study was conducted among 116 consecutively selected HIV-infected children on HAART or HAART-naive attending the Pediatric unit of the Virology Research Clinic, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife. Sixty-one healthy HIV-negative children recruited from the community served as controls. The children that served as the control group were apparently healthy children recruited from the communi-

ty; particularly, from places of worship. Subjects and controls were aged 18 months to 14 years. Biodata was collected with the use of a proforma. This was carried out between May 2013 and June 2014. Ethical approval and informed consent were obtained from the institution's research and ethical committee and parents/care-givers respectively.

Materials and methods

Six milliliters of venous blood was aseptically collected from each of the 116 HIV-infected children and the 61 HIV-negative controls.

Full blood count and CD4+ T-Lymphocyte count

One millilitre of venous blood was collected into pediatric-sized vacuum potassium ethylenediaminetetraacetic acid (K_2 EDTA) bottle and mixed well for the assessment of Full blood count and CD4+ cell count.

Full blood count was carried out using the Sysmex KX-21N machine while the CD4+ lymphocyte count was done using the CyFlow Counter 1 machine following both reagent and machine instructions. 2.75 mls was collected into a plain bottle for the direct Coombs' test and anticardiolipin antibody (aCL) assay.

A 1 in 9 dilution of venous blood was prepared by mixing 0.25 ml of 32 g/l Trisodium citrate with 2.25 ml of blood for the assessment of aPTT and KCT. These samples were centrifuged at 4000 revolution per minute for 15 minutes within 2-4 hours of collection to obtain platelet poor plasma which were stored at -80 °C pending analysis. Activated partial thromboplastin time (aPTT) was carried out by using LA-sensitive aPTT reagent (Aniara Diagnostica, USA). Subjects and controls with persistently elevated aPTT values following mixing test were further assayed for antiphospholipid antibodies namely, Lupus anticoagulant and anticardiolipin antibodies (isotypes IgG and M).

Lupus anticoagulant

Lupus anticoagulant was detected using the Kaolin clotting time test in accordance with the recommendations of the Subcommittee on lupus anticoagulant/antiphospholipid antibody of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis.

Anticardiolipin antibody

Anticardiolipin antibody assay was carried out using the ELISA method (Diagnostic Automation Inc/Cortex Diagnostics, USA).

HIV status determination

HIV screening/confirmation was done using both the Determine and Unigold strips according to the serial rapid test algorithm [14].

Direct coombs test

Table 1: Demographic characteristics and laboratory parameters (mean \pm SD) of subjects and controls.

Parameters	Subjects (n = 116)	Controls (n = 61)	p value
Gender	Male = 63 (54.3%) Female = 53 (45.7%)	Male = 26 (42.6%) Female = 35 (57.4%)	0.0001
Age (years)	7.2 \pm 3.3 (median = 7)	8.8 \pm 3.1 (median = 9)	0.0002
CD4+ (cells/ μ l)	878 \pm 541	1,178 \pm 327	
PCV (%)	33.6 \pm 3.7	37.5 \pm 3.5	< 0.0001
WBC ($\times 10^9$ /L)	5.6 \pm 2.9	4.9 \pm 3.2	0.2
Platelet Count ($\times 10^9$ /L)	268 \pm 104	219 \pm 79	0.002
APTT (sec)	39.4 \pm 4.2	40.1 \pm 4.0	0.3

Legends: CD4+: CD4+ lymphocyte count; PCV: Packed cell volume; WBC: White blood cell count; PLT: Platelet count; APTT: Activated partial thromboplastin time.

Table 2: Haematological profile of subjects and controls positive for antiphospholipid antibodies.

Parameters	Subjects (n = 13)	Controls (n = 6)	t value	p value
PCV (%)	32.9 \pm 3.2	37.3 \pm 3.3	3.3	0.004
WBC (10^9 /L)	5.7 \pm 2.5	4.1 \pm 713	-2.1	0.05
PLT (10^9 /L)	302 \pm 109	235 \pm 119	-1.3	0.1
CD4+ (cells/ μ l)	964 \pm 817	1,200 \pm 284	1.2	0.2
APTT (sec)	43.4 \pm 5.7	45.6 \pm 5.3	0.86	0.39

Legends: PCV: Packed cell volume; PLT: Platelet count; CD4+: CD4+ lymphocyte count; APTT: Activated partial thromboplastin time.

The Direct Coombs' test (DCT) was performed as described by Laffan et al. in Dacie and Lewis Practical Haematology, 10th Edition.

Statistical analysis

SPSS version 16 was used to analyse data collected through a proforma. Percentages were used to describe frequency analysis of categorical variables. Numerical variables were summarized as means and standard deviations where appropriate. Pearson's chi-square was used to determine associations between categorical variables. Means of numerical variables were compared with categorical variables using 2-sample independent t-test. A p-value of < 0.05 defined the level of statistical significance.

Results

A total of 116 HIV-infected children (subjects) and 61 HIV-negative controls were investigated. Subjects were aged between 18 months and 14 years with a mean age of 7.2 \pm 3.3 years. Controls were of similar ages and their mean age was 8.7 \pm 3.3 years. Male and female subjects were 63 (54.3%) and 53 (45.7%) respectively while controls were made up of 26 (42.6%) males and 35 (57.4%) females. One hundred and four (89.7%) of the subjects were on HAART and 12 (10.3%) were HAART-naïve (Table 1).

Full blood count and CD4+ T-Lymphocyte count

The mean values of the packed cell volume (PCV), platelet and CD4+ lymphocyte count of the subjects

were 33.6 \pm 3.7%, 268 \pm 104 $\times 10^9$ /L and 878 \pm 541 cells/ μ l respectively while those of the controls were 37.5 \pm 3.5%, 219 \pm 79 $\times 10^9$ /L and 1,178 \pm 327 cells/ μ l. When these values were compared, the differences were statistically significant (PCV: p < 0.0001; platelet count: p = 0.002; CD4+: p = 0.002). However, the WBC count and the aPTT were not statistically significant (WBC: p = 0.2; aPTT: p = 0.3) (Table 2).

Antiphospholipid antibodies

Thirteen (11.2%) of the 116 subjects were positive for aPLs. Using aCL as a determinant of aPL, only 11 (9.48%) of the 116 subjects had antiphospholipid antibodies. When lupus anticoagulant as determined by KCT was used as a determinant of aPL, only 7 (6.03%) of these subjects had aPL. Of the 13 subjects with aPL, 8 were males (i.e 8 of 63 males were aPL-positive) and 5 were females (i.e 5 of 53 females were aPL positive). The difference using the Pearson's chi square was not statistically significant ($\chi^2 = 0.321$, p = 0.57).

Six (9.8%) of the controls were positive for aPLs. Similarly, many of the positive results were by aCL compared to LA (9.8% vs. 6.6%). There were 3 of the 35 females and also 3 of the 26 males that were positive for aPLs. This was not statistically significant ($\chi^2 = 0.15$, p = 0.7). IgG and IgM isotypes of anticardiolipin antibody were assayed. Eleven of the subjects were positive for both isotypes and six of the controls showed positivity for both aCL IgG and IgM (Table 3).

The effect of antiphospholipid antibodies on PCV,

platelet and white cell counts were not significant (Table 4).

Eleven (84.6%) of the subjects positive for antiphospholipid antibodies had been on HAART for more than 6 months. Nine were in World Health Organization (WHO) clinical stage 1 and two were in stages 2 and 3. None of the subjects presented with any feature associated with the presence of aPLs and DCT was negative in all of them.

Comparing prevalence of aPLs in HAART-naïve patients (2 of 12) and patients on ARV treatment (11 of 104), there was no association established ($\chi^2 = 0.4$, $p = 0.52$). Furthermore, HIV infection did not influence the development of antiphospholipid antibodies as we document 13 of 116 infected to have aPL and 6 of 61 HIV-negative patients to have aPL ($\chi^2 = 0.49$, $p = 0.78$).

Table 3: Subjects and controls with positivity for aCL and titres of Ig G and Ig M.

Subjects	Ig G	Ig M
6	High Positive	High Positive
7	Low Positive	Low Positive
13	Low Positive	Low Positive
17	Low Positive	Moderate Positive
18	Low Positive	Low Positive
19	Low Positive	Negative
27	Moderate Positive	Low Positive
80	Low Positive	Low Positive
104	Low Positive	Negative
113	Negative	Low Positive
117	Low Positive	Low Positive
Controls	Ig G	Ig M
C6	Low Positive	Low Positive
C15	Low Positive	Low Positive
C35	Negative	Low Positive
C43	Negative	Low Positive
C53	Negative	Moderate Positive
C58	Low Positive	Moderate Positive

Legends: Ig: Immunoglobulin; aCL: Anticardiolipin antibody.

Table 4: Comparison of the effect of antiphospholipid antibody positivity and negativity on haematological variables.

Subjects					Controls				
PCV (%)	aPL POS	aPL NEG	t-test	p-value		aPL POS	aPL NEG	t-test	p-value
Mean \pm SD	32.7 \pm 4.1	33.7 \pm 3.6	0.84	0.41	Mean \pm SD	37.4 \pm 3.3	37.5 \pm 3.5	0.04	0.97
WBC ($\times 10^9/L$)	5,845 \pm 2,541	5,635 \pm 3,026	-0.23	0.82		4,171 \pm 713	5,099 \pm 3,475	0.69	0.49
PLT ($\times 10^9/L$)	280,333 \pm 85,970	265,913 \pm 107,423	-0.44	0.66		235,000 \pm 119,983	216,880 \pm 73,442	-0.56	0.58
CD4 (μl)	812 \pm 557	885 \pm 542	0.43	0.67		1,248 \pm 284	1,168 \pm 333	-0.61	0.55
APTT (secs)	46.6 \pm 3.9	38.4 \pm 3.2	-8.45	0.0000		45.6 \pm 5.3	39.3 \pm 3.2	-4.48	0.0000

aPL: Antiphospholipid antibodies; POS: Positive; NEG: Negative; PCV: Packed cell volume; WBC: White cell count; PLT: Platelet; CD4: Cluster of differentiation 4; APTT: Activated partial thromboplastin time.

Discussion

Infection and autoimmune disorders have been implicated in the development of antiphospholipid antibodies in children. Viral infections are the commonest triggers but others could be bacterial, parasitic and spirochaetal. Antiphospholipid antibodies triggered by infections have been described as being transient and non-pathogenic [15].

Concerning the prevalence of aPLs in HIV-infected children, the literature is sparse, most reports being incidental findings and case reports [16].

A higher prevalence rate of lupus anticoagulant was observed in this study compared to similar studies amongst adult HIV-infected patients [4,5]. This could be due to the methods used in this study. The use of two various methods increases the sensitivity and specificity of results.

The only study done among children in Nigeria was by Olayemi, et al. in sickle cell disease patients who found a prevalence of 1.8% for LA [17]. Although sickle cell disease is also considered an immunosuppressive disorder, the degree of immunosuppression is not comparable to that obtained in HIV infection; in which patients are susceptible to bacterial, viral, fungal and parasitic infections that may trigger the formation of antiphospholipid antibodies.

Of the two subtypes of antiphospholipid antibodies (LA and aCL) tested for in this study, anticardiolipin antibodies (aCL) were found to be more prevalent than lupus anticoagulant.

9.8% of the HIV-negative controls were also positive for aPLs; supporting the fact that there are other triggers for the presence of these antibodies in children apart from HIV infection [18]. Anticardiolipin antibodies among healthy children have been reported to be between 2% and 82% [19-21]. Frequent infections by varying etiological agents characterize childhood, hence putting the immune system under immense pressure and in effect making children produce more antiphospholipid antibodies than adults.

The IgG isotype was higher in the subjects in this

study and similar finding was reported by Stimmler, et al. [22] in 26 adult HIV-infected patients in whom a higher mean of IgG aCL was observed. However, these had AIDS and opportunistic infections (OIs) but interestingly, none of the subjects positive for anticardiolipin antibodies in this study had AIDS or OIs.

Anticardiolipin antibodies were similarly found at a higher frequency among controls in this study with aCL IgM being significantly higher than IgG isotype (100% vs. 42.9%). The finding of a higher aCL positivity among this healthy controls in this study is similar to findings in other studies [19-21]. Studies by Rapizzi, et al. [20] and Galrao, et al. [23] revealed a higher IgG isotype and this may be considered as the epiphenomenon of vaccinations, nutrition and infections in the pediatric population [24].

A study by Ankri, et al. [25] among 90 HIV-infected patients found a lower CD4+ lymphocyte count in patients with antiphospholipid syndrome (APS) compared to those without APS and that gender, risk factors and stage of disease between patients with HIV and IgG aCL and HIV-infected patients without antibody was not significant.

Generally, antibodies induced by infections are transient, of low titre, non-pathogenic and most often of the IgM subtype however, several reports have shown otherwise [15,26,27]. Low-positive titres of aPL found in this study agrees with the description of aPL induced by infective organisms and this might explain the absence of any clinical feature related to the presence of aPLs.

Clinical manifestations due to the presence of these antibodies vary among studies and systematic studies are sparse because of the relatively low prevalence of APS and its heterogeneity among children [6]. Known clinical features associated with the presence of these antibodies were not seen in any of those positive for aPL.

A “two-hit” hypothesis has been proposed to explain the presence of high aPL titres and the absence of symptoms. This hypothesis states that endothelial dysfunction is induced by the presence of aPLs representing the first-hit and then the second-hit which may be pregnancy, infection, smoking, hypertension, atherosclerosis, obesity or vascular injury triggers thrombosis [2,27]. However, in children most of these are not common risk factors.

The absence of clinical features can also be explained by the concept of developmental haemostasis which states that there are age-dependent differences in the coagulation and fibrinolytic system [28]. Several studies have reported varying findings relating to thrombosis. Hunt, et al. [29] observed that the incidence of thrombosis is lower in children than in the adults because of increased levels of physiologic anticoagulants. A case of thrombosis and recurrent

thrombosis in children was reported by Shah, et al. [16] with reduced CD4+ lymphocyte counts (< 200 cells/ μ l), presence of opportunistic infections, neoplasms, or autoimmune disorder such as autoimmune haemolytic anaemia as events predisposing to thrombosis.

A strong relationship between the presence of LA and thrombotic events was found by Berube, et al. [30] who observed that having both LA and aCL did not strengthen the relationship between patients with thrombotic complications and aPLs. This was also observed in this study as none of those who were positive for both LA and aCL presented with or had history suggestive of thrombosis.

The dysfunctional state of the immune system in HIV infection and/ or immature nature of the immune system in children could explain the negative DCT in all subjects and which is similar to the findings of Ndakotsu, et al. in adult patients [4].

Conclusion

The presence of these antibodies were not influenced by infection or non-infection with the HIV. Sex, clinical stage of disease and HAART does not influence its presence and antiphospholipid syndrome may not be present.

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