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Anti-phospholipid antibodies and osteopontin in young adults with cerebrovascular stroke

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Abstract

To estimate the frequency of antiphospholipid syndrome (APS) in young adults with cerebrovascular events (CVE), assess whether aPLA-positive individuals have a higher risk of ischemic stroke than aPLA-negative individuals, and evaluate the correlation between osteopontin (OPN) levels, aPLA, and cerebrovascular stroke (CVS). This prospective randomized controlled study was conducted on 72 patients aged less than 50 years, of both sexes, with cerebrovascular events (CVE) (Group I) and 40 apparently healthy individuals as controls (Group II). There was a significant correlation between the level of osteopontin (OPN) and anticardiolipin immunoglobulin G (ACL IgG) (P= 0.012). OPN, baseline ACL IgM, ACL IgG, lupus anticoagulant (LA1), LA1/LA2 ratio, and anti-β2 glycoprotein (GPI) IgG were significantly higher in the case group than in the control group (P<0.05). ROC curves for diagnosis of CVS in young adults by OPN, at a cut off ≥ 45, sensitivity 72.2%, specificity 68.7% and P<0.001. The study documented the increased incidence of APS as an etiology of CVE in young adults. Osteopontin markers might be sensitive and specific for the diagnosis of CVE in young adults. Early screening for APS and osteopontin levels in young adults presenting with CVE may enable earlier diagnosis, targeted treatment, and potentially improved prevention of recurrent strokes.

Keywords: Anti-phospholipid antibodies, Cerebrovascular stroke, Osteopontin, Young adults.

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1. Introduction

Stroke is classically defined as a neurological deficit resulting from an acute focal injury of the central nervous system (CNS) caused by vascular factors, including cerebral infarction, intracerebral hemorrhage (ICH), and subarachnoid hemorrhage (SAH). It remains a major cause of disability and death worldwide [1].

The burden of stroke is increasing in many low- and middle-income countries [2]. Around 10% of all thrombotic cerebrovascular events (CVEs) occur in the young population, defined as individuals younger than 50 years old [3]. In most of these patients, the cause of the ischemic stroke remains undetermined. [4].

Arterial thrombosis is a major clinical manifestation of APS, an autoimmune condition characterized by thrombotic events and/or pregnancy morbidity with persistently positive aPLA [5].

The pathogenesis of ischemic stroke is complex, and several studies have documented hypercoagulable states as a significant mechanism underlying stroke. This has been hypothesized to result from specific genetic factors, including genetic background and mutations in procoagulant factors [6].

Anti-phospholipid antibodies are a common acquired risk factor for stroke. These antibodies comprise a heterogeneous group of antibodies that react with phospholipids or phospholipid-binding proteins, of which beta-2 glycoprotein-I (β2-GPI) is considered the main antigenic target [7].

The contribution of lupus anticoagulant (LA) to the overall risk of both venous and arterial thrombosis, including ischemic stroke, is now well recognized. While the contribution of aPLA (including LA and anticardiolipin (ACL) antibodies) to thrombosis is well established, their role as independent risk factors in the pathogenesis of ischemic stroke yielded apparently conflicting results [8].

Osteopontin (OPN) was first identified as a protein involved in bone remodelling but was later shown to have important immunological roles. The protein is produced by various cells, including B cells, T cells, dendritic cells, macrophages, neutrophils, bone cells, and neurons, and it is upregulated in response to injury and inflammation [9].

The aim of this work was to estimate the frequency of APS in young adults (less than 50 years old) with CVE, determine whether aPLA-positive young individuals have a greater risk of an episode of ischemic stroke compared with individuals without aPLA, and correlate the levels of OPN and aPLA with CV changes.

2. Material and Methods

This prospective randomized controlled study was conducted on 72 patients aged less than 50 years, of both sexes, with CVE (group I) and 40 apparently healthy individuals as a control group (group II). The study was carried out from December 2018 to February 2021 after approval by the Ethical Committee of the Faculty of Medicine. An informed written consent was obtained from all patients.

Patients with rheumatic heart disease, ventricular arrhythmias, uncompensated heart failure, stroke secondary to atrial fibrillation, hematoma, brain tumors, accidental or iatrogenic stroke, arterial malformation, and recent acute myocardial infarction were excluded from the study.

Subjects were divided into two groups: Group I (n=72): with CVE and Group II (n=40) (control group).

All patients were subjected to complete history taking and neurological examination

The national institutes of health stroke scale (NIHSS) [10] and modified Rankin scale (mRS) [11] were evaluated in patients of this study .

Laboratory investigations [complete blood count (CBC), kidney function test, blood sugar, lipid profile, coagulation profile, osteopontin] for both groups, [anticardiolipin IgM, anticardiolipin IgG, lupus anticoagulant (LA)1, LA2, LA1/LA2 ratio, anti-beta-2 glycoprotein-I IgM, anti-beta-2 glycoprotein-I IgG] were performed at baseline for both groups and repeated after 3 months for group I. Radiological investigations [brain computed tomography (CT), brain magnetic resonance imaging (MRI)] were conducted for group I.

2.1. Anti-Cardiolipin Igg/IgM: (Catalog Number: ORG 515)

It is an enzyme-linked immunoassay (ELISA) test system for the quantitative measurement of IgG and IgM class autoantibodies against cardiolipin in human serum or plasma.

2.2. Anti- β2-GPI IgG/IgM: (Catalog Number: ORG 521)

It is an ELISA test system for the quantitative measurement of IgG and IgM class autoantibodies against β2-GPI in human serum or plasma.

2.3. Lupus Anti-Coagulant Antibodies

Method type: Sysmex CS-2500.

The presence of LA in plasma samples was assessed using diluted Russell's viper venom time (dRVVT) screen and confirm reagents (LA1-screen and LA2-confirm) and the LA1/LA2 ratio.

Human osteopontin: (Catalog number: SG-10445).

Method type: Sandwich ELISA Detection.

Specimen requirements: serum at room temperature for 10-20 minutes, centrifuge at the speed of 3000 rpm for 20 minutes. Remove supernatant. Assay immediately or aliquot and store samples at -80°C.

The patients were classified according to anti-phospholipid risk profile according to the following definitions [12]. Low-risk aPLA profile: isolated ACL or antibeta2 glycoprotein I antibodies at low- to medium titers, particularly if transiently positive. Medium-high aPLA titers: ACL antibody of IgG and/or IgM isotype in serum or plasma present in

titers >40 IgG phospholipid (GPL) units or >40 IgM phospholipid (MPL) units, or > the 99th percentile, measured by a standardized ELISA. Anti-beta2 glycoprotein I antibody of IgG and/or IgM isotype in serum or plasma in titer > the 99th percentile, measured by a standardized ELISA. High-risk aPLA profile: the presence (in 2 or more occasions at least 12 weeks apart) of lupus anticoagulant (measured according to International Society on Thrombosis and Haemostasis (ISTH) guidelines), or of double (any combination of lupus anticoagulant, ACL antibodies, or anti-beta2 glycoprotein I antibodies) or triple (all three subtypes) aPLA positivity, or the presence of persistently high aPLA titers.

2.4. Statistical Analysis

Statistical analysis was conducted using SPSS v27 (IBM©, Chicago, IL, USA). The Shapiro-Wilk test and histograms were employed to assess the normality of data distribution. Quantitative parametric data were expressed as mean and standard deviation (SD) and analyzed using ANOVA (F) test with post hoc Tukey's test. Quantitative non-parametric data were presented as median and interquartile range (IQR) and analyzed with the Kruskal-Wallis test, followed by the Mann-Whitney U test for pairwise comparisons. Qualitative variables were summarized as frequencies and percentages (%) and analyzed using the Chi-square test. Correlations between variables were evaluated using Pearson's correlation coefficient. The receiver operating characteristic (ROC) curve was utilized to assess diagnostic performance, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). A two-tailed P value < 0.05 was considered statistically significant.

2.5. Outcomes

According to the results of brain CT and MRI, 84.7% of patients had ischemic stroke, 12.5% had hemorrhagic stroke, and 2.8% had hemorrhagic infarction.

Demographic data, lipid profile, prothrombin time (PT), prothrombin concentration, International Normalization Ratio (INR), activated partial thromboplastin time (aPTT), baseline LA2, and anti-β2 GPI IgM were insignificantly different between the two groups. On the other hand, OPN, baseline ACL IgM, ACL IgG, LA1, LA1/LA2 ratio, and anti-β2 GPI IgG were significantly higher in group I than group II (P<0.05), Table 1.

Table 1.
Comparison between the studied groups regarding demographic and laboratory data.

		Group I (n=72)	**Group II (n=40)**	**P**
Age (years)		40.68±9.89	37.63±12.36	0.307
Sex	Male	33(45.8%)	13(32.5%)	0.287
	Female	39(54.2%)	27(67.5%)	
Laboratory data				
Lipid profile	Triglyceride (mg/dl)	126.0(50.0-474.0)	120(44–150)	0.486
	HDL (mg/dl)	44.0(22.0-89.0)	40(13-60)	0.357
	LDL (mg/dl)	112.5(40.0-250.0)	80(33–90)	0.053
	Cholesterol (mg/dl)	181.5(65.0-450.0)	120(55–180)	0.051
PT (sec)		12.5(9.3-29.7)	9.5(8.2-13)	0.05
Prothrombin concentration (sec)		99.0(42.5-130.0)	85(70-120)	0.137
INR		1.0(0.80-2.3)	1.0(0.9–1.1)	0.054
aPTT		29.0(19.0-40.0)	30(25-35)	0.247
OPN (ng/Ml)		52(23.56-92.33)	41.3(28.1-48)	0.001**
Baseline ACL IgM (U/Ml)		3.97(0.8-25.1)	1.68(1.24-2.49)	<0.001***
Baseline ACL IgG (U/Ml)		2.74(0.75-120.0)	1.76(1.21-5.91)	0.013*
After 3 months, ACL IgM (U/Ml)		4.48(0.98-37.0)	---	---
After 3 months, ACL IgG (U/Ml)		2.88(0.48-180.0)	---	---
Baseline LA1 (sec)		42.1(30.9-124.6)	37.4(31.7-43.9)	0.004**
Baseline LA2 (sec)		35.2(28.0-83.8)	35.8(32.5-38)	0.596
Baseline LA Ratio		1.21(0.91-2.41)	1.04(0.95-1.19)	<0.001***
After 3 months, LA1 (sec)		39.8(30.9-144.0)	---	---
After 3 months, LA2 (sec)		35.0(28.0-66.0)	---	---
After 3 months LA ratio		1.16(0.99-2.40)	---	---
Baseline anti-B-2 GPI IgG (U/Ml)		3.4(0.59-100.0)	2.4(1.8–4.9)	0.049*
Baseline anti-B-2 GPI IgM (U/Ml)		3.7(1.19-26.7)	3.8(1.41–4.72)	0.689
After 3 months anti-B-2 GPI IgG (U/Ml)		3.5(0.98-220.0)	---	---
After 3 months anti-B-2 GPI IgM (U/Ml)		3.70(0.45-56.0)	---	---

Note: Data are presented as mean ± SD or frequency (%) or median (range). * Significant P value <0.05. ** means moderately significant P value. ***means highly significant P value. HDL: high-density lipoproteins, LDL: low-density lipoprotein, OPN: osteopontin, PT: prothrombin time, INR: international normalized ratio, aPTT: activated partial thromboplastin time, ACL: anticardiolipin, LA: lupus anticoagulant, Ig: immunoglobulin, GPI: glycoprotein-I.

Obstetric history of female patients with CVE (n=39), clinical data, medications, neurological evaluation (assessed by NIHSS and mRS), positive aPLA at baseline and repeated after 3 months, and aPL profile risk were enumerated in Table 2.

Table 2.
Obstetric history of female CVS patients, clinical data, medications, neurological evaluation (assessed by NIHSS and mRS), positive aPL at baseline and repeated after 3 months and aPL profile risk of the studied CVS cases group.

		N=39
Obstetric history	History of abortion	20(51.3%)
	Number of abortions	2(1–5)
	History of still birth	9(23.1%)
	Number of still births	1(1–4)
	History of infant death	5(12.8%)
	Number of infant deaths	1(1–5)
		N=72
Clinical data	Stroke duration (months)	3.5(0.25-336.0)
	Recurrence of stroke	15(20.8%)
	Other thromboembolic events in the form of DVT and PE	2(2.8%)
	DM	15(20.8%)
	HTN	23(31.9%)
	Stroke duration (months)	3.5(0.25-336.0)
Medications	Anti-platelets	42(58.3%)
	Warfarin	11(15.3%)
	Statins	14(19.4%)
	Neurotonics	45(62.5%)
Neurological evaluation		
NIHS		5(0–26)
Minor stroke		35(48.7%)
Moderate stroke		31(43.1%)
Moderate to severe stroke		3(4.2%)
Severe stroke		3(4.2%)
mRS		3(0–5)
Slight disability		8(11.1%)
Moderate disability		17(23.6%)
Moderate to severe disability		18(25.0%)
Severe disability		10(13.9%)
aPL	Anti-cardiolipin IgM	17(23.6%)
	Anti-cardiolipin IgG	6(8.3%)
	LA1	18(25.0%)
	LA2	17(23.6%)
	LA1/LA2 ratio	18(25.0%)
	Anti-B-2 GPI IgM	10(13.9%)
	Anti- B-2 GPI IgG	10(13.9%)
	Anti-phospholipid (total)	37(51.4%)
aPL profile risk	Low-risk aPL profile	7 (9.7%)
	Medium-high aPL profile	10 (13.9%)
	High-risk aPL profile	25 (34.7%)

Note: Data are presented as frequency (%) or median (range). DVT: deep venous thrombosis, PE: pulmonary embolism, DM: diabetes mellitus, HTN: hypertension, NIHS: national institutes of health stroke scale, mRS: modified Rankin scale, aPL: anti-phospholipid antibodies, Ig: immunoglobulin, LA: lupus anticoagulant, GPI: glycoprotein-I.

Figure 1 showed ROC curve for diagnosis of CVS in young adults by OPN, at a cut-off of ≥ 45, AUC=0.797, sensitivity 72.2%, specificity 68.7%, and $P < 0.001$.

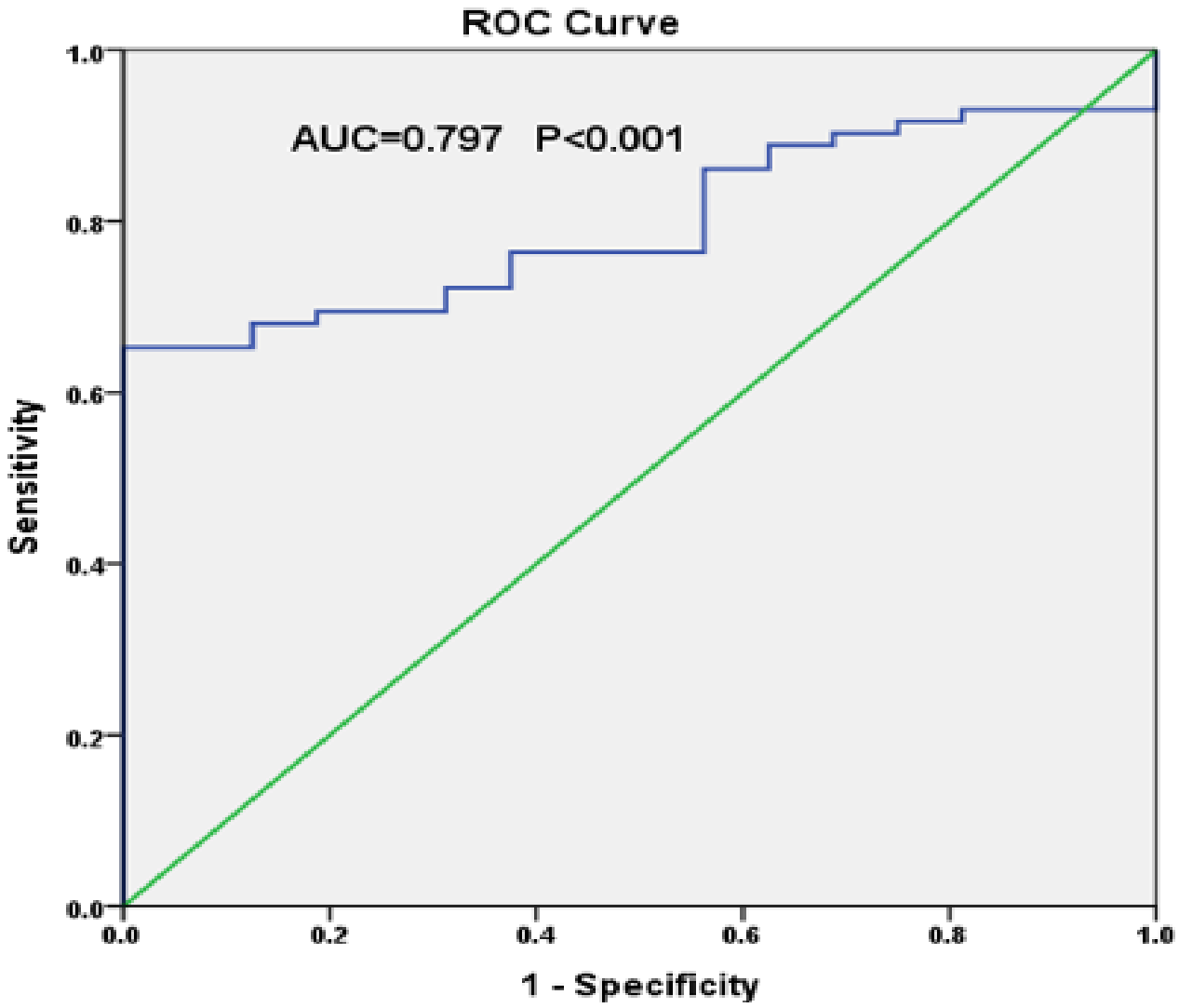

Figure 1.
ROC curves for the diagnosis of cerebrovascular stroke in young adults by Osteopontin. Osteopontin (blue) and reference line (green).

Patients were divided into 2 groups: group with APS negative (n=35) and group with APS positive (n=37).

Age, obstetric history, clinical data, antiplatelets, statins, neurotonics, neurological evaluation, lipid profile, PT, and OPN were insignificantly different between the two groups. Male, warfarin intake, and INR were significantly higher in the APS-positive group than in the APS-negative group (P<0.05). Female, smoking, prothrombin concentration and aPTT were significantly different in APS negative group than APS positive group (P<0.05), Table 3.

Table 3.
Comparison between cases with and without APS regarding demographic data, obstetric history, clinical data, medications, neurological evaluation by NIHS, mRS, and laboratory data.

		APS negative (n=35)	**APS positive (n=37)**	**P**
Age (years)		41.86±9.50	39.57±10.24	0.330
Sex	Male	22(62.9%)	11(29.7%)	0.005**
	Female	13(37.1%)	26(70.3%)	
Smoking		14(40.0%)	4(10.8%)	0.004*
		N=13	N=26	
Obstetric history	History of abortion	5(38.5%)	15(57.7%)	0.257
	Number of abortions	1(1–4)	2(1–5)	0.612
	History of still birth	4(30.8%)	5(19.2%)	0.447
	Number of still births	1(1–4)	1(1–1)	0.556
	History of infant death	2(15.4%)	3(11.5%)	1
	Number of infant deaths	2(1–2)	1(1–5)	1
Clinical data	Stroke duration (months)	4.0(0.25-36.0)	3.0(0.25-336.0)	0.607
	Recurrence of stroke	5(14.3%)	10(27.0%)	0.183
	Other thromboembolic events in the form of DVT and PE	0(0.0%)	2(5.4%)	0.493
	DM	9(25.7%)	6(16.2%)	0.321
	HTN	13(37.1%)	10(27.0%)	0.358
Medications	Anti-platelets	21(60.0%)	21(56.7%)	0.780
	Warfarin	2(5.7%)	9(24.3%)	0.028*
	Statins	7(20.0%)	7(18.9%)	0.908
	Neurotonics	22(62.8%)	23(62.1%)	0.951
Neurological evaluation				
NIHS		5(0–26)	5(0–25)	0.781
Minor stroke Moderate stroke Moderate to severe stroke Severe stroke		17(48.6%) 15(42.9%) 2(5.7%) 1(2.9%)	18(48.6%) 16(43.2%) 1(2.7%) 2(5.4%)	0.831
mRS		3(0–5)	3(0–5)	0.334
Slight disability Moderate disability Moderate to severe disability Severe disability		5(14.3%) 11(31.4%) 6(17.1%) 7(20.0%)	3(8.1%) 6(16.2%) 12(32.4%) 3(8.1%)	0.144
Laboratory data				
Lipid profile	Triglyceride (mg/dl)	160.0(62.0-474.0)	126.0(50.0-396.0)	0.376
	HDL (mg/dl)	45.0(27.0-89.0)	43.0(22.0-80.0)	0.197
	LDL (mg/dl)	112.0(49.0-230.0)	113.0(40.0-250.0)	0.769
	Cholesterol (mg/dl)	190.0(80.0-450.0)	180.0(65.0-420.0)	0.407
PT (sec)		12.5 (9.3–23.6)	12.5(10.3-29.7)	0.324
Prothrombin concentration (sec)		100.0(44.3-130.0)	97.8(42.5-130.0)	0.008**
INR		1.0(0.80–1.7)	1.0(0.90-2.3)	0.001**
aPTT		30.0(20.0-35.0)	28.0(19.0-40.0)	0.005**
OPN (ng/Ml)		51.8(23.7–85.6)	52.4(23.6-92.3)	0.830

Note: Data are presented as mean ± SD or frequency (%) or median (range). * Significant P value <0.05. ** means moderately significant P value. ***means highly significant P value. DVT: deep venous thrombosis, PE: pulmonary embolism, DM: diabetes mellitus, HTN: hypertension, NIHSS: National Institutes of Health Stroke Scale, mRS: modified Rankin scale, HDL: high-density lipoproteins, LDL: low-density lipoprotein, PT: prothrombin time, INR: international normalized ratio, aPTT: activated partial thromboplastin time, OPN: osteopontin.

There was a significant correlation between the level of OPN and ACL IgG (P= 0.012). Table 4.

Table 4.
Correlation between the level of OPN and APL antibodies.

		OPN
ACL IgM	R	0.056
	P	0.638
ACL IgG	R	0.293
	P	0.012*
LA1	R	0.110
	P	0.358
LA2	R	0.066
	P	0.583
Ratio LA1/LA2	R	0.128
	P	0.285
Anti-B2GP IgG	R	0.145
	P	0.225
Anti-B2 GP IgM	R	0.074
	P	0.537

Note: r: correlation coefficient. * Significant P value <0.05. OPN: osteopontin, ACL: anticardiolipin, Ig: immunoglobulin, LA: lupus anticoagulant, GP: glycoprotein.

Table 5 showed the comparison between cases with and without APS (ACL IgM and ACL IgG positive and negative groups), (anti-beta 2 GPI IgG and anti-beta 2 GPI IgM positive and negative groups) regarding demographic data, obstetric history, clinical data, medications, neurological evaluation, and laboratory data.

Table 5.
Comparison between cases with and without APS (ACL IgM and ACL IgG positive and negative groups), (anti-beta 2 GPI IgG and anti-beta 2 GPI IgM positive and negative groups) regarding demographic data, obstetric history, clinical data, medications, neurological evaluation and laboratory data.

		ACL IgM negative (n=55)	ACL IgM positive (n=17)	P	ACL IgG negative (n=66)	ACL IgG positive (n=6)	P
Age (years)		40.38±9.85	41.65±10.25	0.648	40.76±9.88	45.33±9.61	0.098
Sex	Male	29(52.7%)	4(23.5%)	0.035*	31(47.0%)	2(33.3%)	0.681
	Female	26(47.3%)	13(76.5%)		35(53.0%)	4(66.7%)	
Clinical data	Stroke duration (months)	6.0(0.25-336.0)	2.0(0.25 - 18.0)	0.069	4.0(0.25-336.0)	2.5(0.25-12.0)	0.517
	Recurrence of stroke	12(21.8%)	3(17.6%)	1	13(19.7%)	2(33.3%)	0.598
	Other thromboembolic events in the form of DVT and PE	1(1.8%)	1(5.9%)	0.419	1(1.5%)	1(16.7%)	0.161
Medications	Anti-platelets	33(60.0%)	9(52.9%)	0.606	38(57.6%)	4(66.7%)	1
	Warfarin	9(16.4%)	2(11.8%)	1	10(15.2%)	1(16.7%)	1
	Statins	11(20.0%)	3(17.6%)	1	12(18.2%)	2(33.3%)	0.330
	Neurotonics	34(61.8%)	11(64.7%)	0.830	41(62.1%)	4(66.7%)	1
Neurological evaluation							
NIHS		5(0–26)	5(0–25)	0.479	5(0–26)	5.5(0–11)	0.640
Minor stroke		25(45.4%)	10(58.8%)	0.581	32 (48.5%)	3(50.0%)	1
Moderate stroke		25(45.5%)	6(35.3%)		28(42.4%)	3(50.0%)	
Moderate to severe stroke		3(5.5%)	0(0.0%)		3(4.5%)	0(0.0%)	
Severe stroke		2(3.6%)	1(5.9%)		3(4.5%)	0 (0.0%)	
mRS		3(0–5)	2(0–5)	0.123	3(0–5)	2.5(0–4)	0.584
Slight disability		6(10.9%)	2(11.8%)	0.092	7(10.6%)	1(16.7%)	0.733
Moderate disability		16(29.1%)	1(5.9%)		16(24.2%)	1(16.7%)	
Moderate to severe disability		13(23.6%)	5(29.4%)		16(24.2%)	2(33.3%)	
Severe disability		9(23.6%)	1(5.9%)		10(15.2%)	0 (0.0%)	
Laboratory data							
Lipid profile	Triglyceride (mg/dl)	126.0(50.0-474.0)	126.0(68.0-396.0)	0.796	147.0 50.0-474.0)	91.0(80.0-265.0)	0.248
	HDL (mg/dl)	44.0(22.0-89.0)	43.0(22.0-67.0)	0.262	44.5(22.0-89.0)	43.0(22.0-67.0)	0.293
	LDL (mg/dl)	113.0(40.0-250.0)	110.0(44.0-190.0)	0.868	113.0(40.0-250.0)	85.0(45.0-141.0)	0.154
	Cholesterol (mg/dl)	179.5(66.0-450.0)	190.0(65.0-290.0)	0.776	186.5(65.0-450.0)	120.0(90.0-300.0)	0.232
PT (sec)		12.5(9.3–23.6)	12.8(11.1-29.7)	0.128	12.5(9.3–29.7)	12.9(11.0–15.3)	0.479
Prothrombin concentration (sec)		100.0(44.0-130.0)	92.3(42.5-130.0)	0.083	99.0(42.5-130.0)	92.2(75.5-100.0)	0.216
INR		1.0(0.80–2.3)	1.01(1.0-2.08)	0.046*	1.0(0.80–2.3)	1.02(1.0–1.32)	0.283
aPTT		29.0(20.0-35.0)	28.0(19.0-40.0)	0.535	28.5(20.0-40.0)	31.5(19.0-34.0)	0.454
OPN (ng/Ml)		51.9(23.7–89.1)	52.4(23.6-92.3)	0.958	51.9(23.6–89.1)	52.3(46.1-92.3)	0.363
		N=26	N=13		N=35	N=4	
Obstetric history	History of abortion	12(46.2%)	8(61.5%)	0.365	17(48.6%)	3(75.0%)	0.605

	Number of abortions	2(1-4)	2(1-5)	0.427	2(1-5)	1(1-2)	0.356
	History of still birth	6(23.1%)	3(23.1%)	1	9(25.7%)	0(0.0%)	0.556
	Number of still births	1(1-4)	1(1-1)	0.714	1(1-4)	0(0.0%)	0.988
	History of infant death	3(11.5%)	2(15.4%)	1	5(14.3%)	0(0.0%)	1
	Number of infant deaths	1(1-2)	3(1-5)	0.800	1(1-5)	0(0.0%)	0.789
		Anti-beta 2 GPI IgG negative (n=62)	Anti-beta 2 GPI IgG positive (n=10)	P	Anti-beta 2 GPI IgM negative (n=62)	Anti-beta 2 GPI IgM positive (n=10)	P
Age (years)		40.00±9.81	44.90±9.78	0.147	40.79±9.70	40.00±11.52	0.816
Sex	Male	29(46.8%)	4(40.0%)	0.745	30(48.4%)	3(30.0%)	0.326
	Female	33(53.2%)	6(60.0%)		32(51.6%)	7(70.0%)	
Clinical data	Stroke duration (months)	5.5(0.25-336.0)	2.0(0.25-5.0)	0.044*	4.5(0.25-336.0)	2.5(0.25-12.0)	0.307
	Recurrence	14(22.6%)	1(10.0%)	0.676	14(22.6%)	1(10.0%)	0.676
	Other thromboembolic events in the form of DVT and PE	1(1.6%)	1(10.0%)	0.260	1(1.6%)	1(10.0%)	0.260
Medications	Anti-platelets	35(56.5%)	7(70.0%)	0.506	35(56.5%)	7(70.0%)	0.506
	Warfarin	10(16.1%)	1(10.0%)	1	10(16.1%)	1(10.0%)	1
	Statins	10(16.1%)	4(40.0%)	0.095	12(19.4%)	2(20.0%)	1
	Neurotonics	36(58.1%)	9(90.0%)	0.079	37(59.7%)	8(80.0%)	0.302
Neurological evaluation							
NIHS		5(0–26)	5.0(0–13)	0.577	4.0(0–26)	5.5(0–25)	0.123
Minor stroke		30(48.4.0%)	5(50.0%)	0.928	32(51.6%)	3(30.0%)	0.316
Moderate stroke		26(41.9%)	5(50.0%)		25(40.3%)	6(60.0%)	
Moderate to severe stroke		3(4.8%)	0(0.0%)		3(4.8%)	0(0.0%)	
Severe stroke		3(4.8%)	0(0.0%)		2(3.2%)	1(10.0%)	
mRS		3(0–5)	3.5 (0–4)	0.708	3(0–5)	3(1–5)	0.496
Slight disability		7(11.3%)	1(10.0%)	0.393	8(12.9%)	0(0.0%)	0.137
Moderate disability		15(24.2%)	2(20.0%)		13(21.0%)	4(40.0%)	
Moderate to severe disability		13(21.0%)	5(50.0%)		15(24.2%)	3(30.0%)	
Severe disability		10(16.1%)	0(0.0%)		9(14.5%)	1(10.0%)	
Laboratory data							
Lipid profile	Triglyceride (mg/dl)	147.0(50.0-474.0)	105.9(80.0-360.0)	0.813	155.0(50.0-474.0)	94.0(63.0-260.0)	0.062
	HDL (mg/dl)	44.0(22.0-89.0)	43.5(22.0-80.0)	0.660	45.63(22.0-89.0)	31.0(22.0-55.0)	0.001**
	LDL (mg/dl)	112.5(40.0-230.0)	105.0(45.0-250.0)	0.738	113.0(40.0-250.0)	93.5(44.0-162.0)	0.118
	Cholesterol (mg/dl)	186.5(65.0-450.0)	144.0(90.0-420.0)	0.282	192.5(65.0-450.0)	115.5(67.0-256.0)	0.027*
PT (sec)		12.5(9.3–23.6)	13.1(10.3–29.7)	0.215	12.5(9.3–23.6)	12.3(10.3–29.7)	0.896
Prothrombin concentration (sec)		99.0(42.5-130.0)	92.2(75.5-130.0)	0.513	99.0(42.5-130.0)	98.5(77.9–130.0)	0.980
INR		1.0(0.80–2.30)	1.02(0.94–1.32)	0.279	1.0(0.80–2.30)	1.0(0.90–1.15)	0.980
aPTT		28.5(20.0-40.0)	29.0(19.0-33.0)	0.838	29.0(20.0-40.0)	27.5(19.0-33.0)	0.140

OPN (ng/Ml)		52.0(23.7–89.1)	51.9(23.6-92.3)	0.660	52.2(23.7–89.1)	43.4(23.6-92.3)	0.366
		N=32	N=7		N=32	N=7	
Obstetric history	History of abortion	17(51.5%)	3(50.0%)	1	17(53.1%)	3(42.9%)	0.695
	Number of abortions	2(1-5)	1(1-2)	0.358	2(1-5)	2(1-3)	0.842
	History of still birth	9(27.3%)	0(0.0%)	0.305	8(25.0%)	1(14.3%)	1
	Number of still births	1(1-4)	0(0.0%)	0.89	1(1-4)	1(1-1)	0.889
	History of infant death	5(15.2%)	0(0.0%)	0.574	5(15.6%)	0(0.0%)	0.563
	Number of infant deaths	1(1-5)	0(0.0%)	0.79	1(1-5)	0(0.0%)	0.98

Note: Data are presented as mean ± SD or frequency (%) or median (range). * Significant P value <0.05. ** means moderate significant P value. ***means highly significant P value. DVT: deep venous thrombosis, PE: pulmonary embolism, DM: diabetes mellitus, HTN: hypertension, NIHSS: national institutes of health stroke scale, mRS: modified Rankin scale, HDL: high-density lipoproteins, LDL: low-density lipoprotein, PT: prothrombin time, INR: international normalized ratio, OPN: osteopontin, aPTT: activated partial thromboplastin time.

Table 6.
Comparison between cases with and without APS (LA1, LA2 and LA1/LA2 ratio) regarding demographic data, obstetric history, clinical data, medications, neurological evaluation and laboratory data.

		LA1negative (n=54)	**LA1positive (n=18)**	**P**	**LA2 negative (n=55)**	**LA2 positive (n=17)**	**P**	**LA1/LA2 negative (n=54)**	**LA1/LA2 positive (n=18)**	**P**
Age (years)		41.52±9.80	38.17±9.99	0.215	41.53±9.74	37.94±10.16	0.193	41.41±9.97	38.50±9.59	0.283
Sex	Male	28(51.9%)	5(27.8%)	0.076	29(52.7%)	4(23.5%)	0.035*	29(53.7%)	4(22.2%)	0.020*
	Female	26(48.1%)	13(72.2%)		26(47.3%)	13(76.5%)		25(46.3%)	14(77.8%)	
Clinical data	Stroke duration (months)	3.5(0.25-240.0)	3.5(0.25-336.0)	0.906	3.0(0.25-336.0)	8.0(0.25-204.0)	0.203	3.0(0.25-204.0)	6.5(0.25-336.0)	0.347
	Recurrence of stroke	11(20.4%)	4(22.2%)	1	11(20.0%)	4(23.5%)	0.742	11(20.4%)	4(22.2%)	1
	Other thromboembolic events in the form of DVT and PE	0(0.0%)	2(11.1%)	0.060	1(1.8%)	1(5.9%)	0.419	0(0.0%)	2(11.1%)	0.060
Medications	Anti-platelets	31(57.4%)	11(61.1%)	0.783	33(60.0%)	9(52.9%)	0.606	34(63.0%)	8(44.4%)	0.168
	Warfarin	4(7.4%)	7(38.9%)	0.004**	4(7.3%)	7(41.2%)	0.002**	5(9.3%)	6(33.3%)	0.023*
	Statins	11(20.4%)	3(16.7%)	1	10(18.2%)	4(23.5%)	0.728	13(24.1%)	1(5.6%)	0.165
	Neurotonics	32(59.3%)	13(72.2%)	0.325	34(61.8%)	11(64.7%)	0.830	35(64.8%)	10(55.6%)	0.482
Neurological evaluation										
NIHS		4(0–26)	5.0(0–25)	0.181	4(0–26)	5.0(0–25)	0.273	4.5(0–26)	5.0(0–25)	0.517
Minor stroke Moderate stroke Moderate to severe stroke Severe stroke		28(51.9%) 22(40.7%) 3(5.6%) 1(1.9%)	7(38.9%) 9(50.0%) 0(0.0%) 2(11.1%)	0.378	28(50.9%) 24(43.6%) 2(3.6%) 1(1.8%)	7(41.1%) 7(41.2%) 1(5.9%) 2(11.8%)	0.442	27(50.0%) 23(42.6%) 3(5.6%) 1(1.9%)	8(44.5%) 8(44.5%) 0(0.0%) 2(11.1%)	0.530
mRS		3(0–5)	3.5(0 –5)	0.577	3(0–5)	3.5(0–5)	0.903	3(0–5)	3.5(0–5)	0.577
Slight disability Moderate disability Moderate to severe disability		6(11.1%) 15(27.8%) 12(22.2%)	2(11.1%) 2(11.1%) 6(33.3%)	0.647	7(12.7%) 15(27.3%) 13(23.6%)	1(5.9%) 2(11.8%) 5(29.4%)	0.636	6(11.1%) 15(27.8%) 12(22.2%)	2(11.1%) 2(11.1%) 6(33.3%)	0.647

Severe disability		7(13.0%)	3(16.7%)		7(12.7%)	3(17.6%)		7(13.0%)	3(16.7%)	
Laboratory data										
Lipid profile	Triglyceride (mg/dl)	123.0(62.0-474.0)	147.0(50.0-300.0)	0.405	160.0(62.0-474.0)	100.0(50.0-270.0)	0.050	132.0(62.0-474.0)	126.0(50.0-300.0)	0.198
	HDL (mg/dl)	44.0(22.0-89.0)	46.5(22.0-70.0)	0.855	44.0(23.0-89.0)	44.0(22.0-70.0)	0.396	44.5(22.0-89.0)	42.8(22.0-70.0)	0.443
	LDL (mg/dl)	113.0(40.0-250.0)	109.7(40.0-190.0)	0.740	120.0(44.0-250.0)	97.0(40.0-180.0)	0.205	112.5(40.0-250.0)	111.7(40.0-190.0)	0.953
	Cholesterol (mg/dl)	179.8(65.0-450.0)	186.5(66.0-300.0)	0.470	198.0(65.0-450.0)	162.0(66.0-300.0)	0.055	178.8(65.0-450.0)	186.5(66.0-300.0)	0.301
PT (sec)		12.5(9.3–29.7)	12.7(10.8–23.5)	0.252	12.5(9.3–29.7)	12.5(11.1–23.5)	0.243	12.5(9.3–29.7)	12.9(10.8–23.5)	0.216
Prothrombin concentration (sec)		99.0(42.5-130.0)	92.0(43.0-110.7)	0.035*	99.0(42.5-130.0)	99.0(43.0-110.7)	0.173	99.5(42.5-130.0)	92.0(43.0-110.7)	0.046*
INR		1.0(0.80–2.08)	1.03(0.96–2.3)	0.007**	1.0(0.80–2.3)	1.03(0.96–2.15)	0.005**	1.0(0.80–2.08)	1.03(0.90–2.30)	0.010*
aPTT		29.0(20.0-40.0)	27.8(19.0-33.0)	0.021*	29.0(20.0-40.0)	27.0(19.0-33.0)	0.009**	29.0(20.0-40.0)	27.8(19.0-33.0)	0.005**
OPN (ng/Ml)		51.7(23.6–85.6)	53.5(23.7-92.3)	0.391	51.9(23.6–85.6)	52.4(26.9-92.3)	0.667	52.2(23.6–85.6)	51.7(23.7-92.3)	0.979
		N=26	N=13		N=27	N=12		N=26	N=13	
Obstetric history	History of abortion	16(61.5%)	4(30.8%)	0.07	14(53.8%)	6(46.2%)	0.651	15(60.0%)	5(35.7 %)	0.146
	Number of abortions	2(1-5)	1(1-2)	0.554	2(1-5)	2(1-3)	0.602	2(1-5)	2(1-2)	0.672
	History of still birth	8(30.8%)	1(7.7%)	0.225	8(30.8 %)	1(7.7%)	0.225	8(32.0%)	1(7.1 %)	0.119
	Number of still births	1(1-4)	1(1-1)	0.889	1(1-4)	1(1-1)	0.889	1(1-4)	1(1-1)	0.889
	History of infant death	4(15.4%)	1(7.7%)	0.648	4(15.4%)	1(7.7%)	0.648	4(16.0%)	1(7.1%)	0.636
	Number of infant deaths	2(1-5)	1(1-1)	0.800	2(1-5)	1(1-1)	0.800	2(1-5)	1(1-1)	0.800

Note: Data are presented as mean ± SD or frequency (%) or median (range). * Significant P value <0.05. ** means moderate significant P value. DVT: deep venous thrombosis, PE: pulmonary embolism, DM: diabetes mellitus, HTN: hypertension, NIHS: national institutes of health stroke scale, mRS: modified Rankin scale, HDL: high-density lipoproteins, LDL: low-density lipoprotein, PT: prothrombin time, INR: international normalized ratio, aPTT: activated partial thromboplastin time, OPN: osteopontin.

Age, obstetric history, clinical data, medications, neurological evaluation, lipid profile, PT, prothrombin concentration, aPTT, and OPN were insignificantly different between ACL IgM, IgG negative, and positive groups. Sex and INR were significantly different between ACL IgM negative and ACL IgM positive groups ($P<0.05$). Age, sex, obstetric history, recurrence, other thromboembolic events in the form of deep venous thrombosis (DVT), pulmonary embolism (PE), medications, neurological evaluation, triglyceride, low-density lipoprotein (LDL), PT, prothrombin concentration, INR, aPTT, and OPN were insignificantly different between anti-beta 2 GPI IgG, IgM negative and positive groups. Stroke duration was significantly different between anti-beta 2 GPI IgG negative and positive groups ($P<0.05$). High-density lipoprotein (HDL) and cholesterol levels were significantly different between Anti-beta 2 GPI IgM negative and positive groups ($P<0.05$).

Table 6 showed the comparison between cases with and without APS (LA1, LA2, and LA1/LA2 ratio) regarding demographic data, obstetric history, clinical data, medications, neurological evaluation, and laboratory data.

Age, obstetric history, clinical data, drug intake in the form of (anti-platelets, statins, and neurotonics), neurological evaluation, lipid profile, PT, and OPN were insignificantly different between all groups. Sex was significantly different between LA2 and LA1/LA2 ratio-negative and positive groups ($P<0.05$). Warfarin intake, INR, and aPTT were significantly different between all groups ($P<0.05$). Prothrombin concentration was significantly different between the LA1 negative and positive groups and between the LA1/LA2 ratio negative and positive groups ($P<0.05$).

3. Discussion

CVE is one of the leading causes of mortality, with a reported annual 6 million fatal events worldwide [13]. While stroke mainly affects elderly people, approximately 10% occur in patients aged 50 or younger. Despite these alarming figures, limited data exist on the frequency of other non-conventional risk factors in the young population affected by CVE [14].

An international multi-center study evaluated young stroke patients from prospective databases of North America, Europe, and Asia and described differences in hospitalization, functional outcomes, and mortality [15]. This study found that Asians with an NIHSS score of eight had significantly higher stroke severity at admission than Blacks and Whites, and this agrees with our results, as we found a median NIHSS score of three that was significantly associated with higher stroke severity.

As regards the neurological evaluation of CVS cases, the median initial NIHSS score was three, and the mean discharge NIHSS was one. The mean discharge mRS was also one, as shown in the study by Yang et al. [16] which agrees with the results of our study, as the median NIHSS score was five and the mean mRS was three at the time of case evaluation in the study.

In agreement with our result about studied groups regarding neurological evaluation and laboratory data. A study by Christian-Albrechts [17] found a marked increase in OPN level among stroke cases after 7 days of CVE in comparison to baseline measurement, and a significant difference was detected between the follow-up OPN values of the patients and the control group. This agrees with our results, as all our cases were subjected to OPN level evaluation after 1 week of CVE. Brey et al. [18] found that there was a highly significant difference between cases and control groups regarding baseline ACL IgM and baseline LA1/LA2 ratio, and a moderately significant difference between cases and control groups regarding baseline LA1, as well as a significant difference between cases and control groups regarding baseline ACL IgG. Finazzi et al. [19] found that main independent factors influencing the risk of vascular complications in ACL-positive patients were found to be previous thrombosis and a high ACL IgG titer. Previous studies have identified a high ACL titer as an independent risk factor for atherosclerotic vascular diseases, mainly myocardial infarction, stroke, and peripheral occlusion. Nencini et al. [20] found a higher frequency ranging from 15% for anti-β2GPI to 22% for ACL, with a strong association between aPLA and CVE in patients aged < 50 years. The mean age was 38.6 years, with a range of 18-49. Sciascia et al. [7] found a higher frequency ranging from 15% for anti-β2GPI to 22% for ACL and a strong association between aPLA and CVE. Kahles et al. [21] suggested a role for anti-β2GPI in stroke pathogenesis, evidenced by the increased prevalence of anti-β2GPI antibodies in stroke patients (20.8%) compared with normal controls (3.6%).

Regarding our study, only 7 patients had a low-risk aPLA profile, 10 patients had medium-high aPLA titers, and 25 patients had a high-risk aPLA profile. This agrees with Brey et al. [18] who found that ACL IgG and LA are associated with a higher stroke risk than other ACL isotypes. The highest risk for stroke was observed when any ACL isotype or LA was present, underscoring the importance of evaluating for LA alone.

In agreement with our results regarding cases with and without APS concerning obstetric history, clinical data, medications, neurological evaluation by NIHS, and mRS. Heilmann et al. [22] found that 57.7% of female cases who were APS positive had a history of abortion; the mean number of abortions was 2 (range 1-5). Pezzini et al. [23] calculated a cumulative risk of 14% for brain ischemia at 10 years. This agrees with our results, as 27.0% of APS cases had a history of recurrent stroke. Yang et al. [16] study in which only 19.6% of APS patients with CVS were on anti-platelets and 5.4% of APS patients with CVS were on anti-coagulants.

Although a significant increase in the sub-acute phase of ischemia was detected, the magnitude of the OPN increase was not significantly correlated with disease severity. This is also in line with Ozaki et al. [24] who did not find a correlation between OPN levels and infarct size, concomitant diseases, blood pressure, symptom duration, or NIHSS scores.

In our study we found associations regarding OPN and a positive ACL IgG. In contrast to Quaglia et al. [25] who did not find any APS associations. And, in contrast to Wirestam et al. [26] who identified associations regarding OPN and a positive lupus anticoagulant test, as well as with the occurrence of IgM ACL antibodies.

In our study, the number of ACL IgM-positive patients who had a history of abortion was 8 (61.5%), the number of ACL IgM-positive patients with a history of stillbirth was 3 (23.1%), and the number of ACL IgM-positive patients with a history of infant death was 2 (15.4%). The number of ACL IgG-positive patients with a history of abortion was 3 (75.0%), with no history of stillbirth or infant death among ACL IgG-positive patients. This obstetric history of our studied female cases agrees with Andreoli et al. [27] who found that ACL IgM/IgG positive patients who had a history of abortion were 79%, anti-β2GPI IgG/IgM positive patients who had a history of abortion were 21%, LA positive patients who had a history of abortion were 73.7%.

In our study, most stroke patients showed a significant difference between LA2 and LA1/LA2 ratio in relation to sex, with a male predominance. This disagrees with Gebhart et al. [28] who found female predominance of LA positive patients about 62.5%.

Regarding our results on cases with and without APS (antiphospholipid syndrome), specifically anti-β2 GPI IgG and anti-β2 GPI IgM positive and negative groups, in relation to obstetric history, Bouvier et al. [29] study on 1313 women with unexplained pregnancy loss were included; recurrent unexplained embryonic loss was the most frequent inclusion criterion for the APS groups. ACL IgG positive cases were 47.2%, ACL IgM positive cases were 71.9%, and LA positive cases were 61.7%. Anti-β_2 glycoprotein I IgG positive cases were 22.1%, and anti-β_2 glycoprotein I IgM positive cases were 40.6%. This agrees with our results, as the percentage of ACL IgM positive patients who had a history of abortion was 61.5%, the percentage of ACL IgM positive patients who had a history of stillbirth was 23.1%, and the percentage of ACL IgM positive patients who had a history of infant death was 15.4%. The percentage of ACL IgG positive patients who had a history of abortion was 75.0%. Faden et al. [30] study in which 500 healthy women who were prospectively screened for aPLA in early pregnancy, 4% were found to have anti-β2 GPI without other aPLA. Pre-eclampsia and eclampsia occurred significantly more frequently among these women compared with aPLA-negative women, raising a potentially important association with this obstetric complication. This disagrees with our results.

Variability in test reproducibility and cut-off definitions represent important methodological limitations for current diagnostic testing for aPLA. Both ACL and anti-β2 GPI assays are widely heterogeneous regarding the reporting of the cut-off for 'aPLA positivity.' Approximately 60% of the papers related to ACL used a low cut-off value (<20 units) for the definition of positive results, such a cut-off does not allow stratifying those medium- to high-titre patients who would fulfill laboratory criteria according to the international consensus [31].

Limitations of the study included that the sample size was relatively small. We adjusted for several conventional stroke risk factors; we cannot exclude the possible contribution of other unmeasured risk factors. Large, independent, prospective, population-based studies with different ethnicities are needed to confirm the contribution of these and other aPLA to the risk of stroke.

4. Recommendations

1-The results of this study will require further evaluation and validation but appear promising. The ability of blood aPLA to produce thrombosis in young adults needs further evaluation and serial follow-up of their levels.

2. In view of the limited sample size in this study, our findings should be considered preliminary, and future studies are needed to evaluate the frequency of aPLA antibodies in young adults with CVE or other thrombotic manifestations.

Prospective longitudinal studies are needed to assess the role of monitoring thrombotic risk and to determine whether patients are low, medium-high, or high risk for an aPLA profile because different treatment lines and durations of treatment are recommended for each group.

4- Variability in test reproducibility and cut-off definitions represents important methodological limitations for current diagnostic testing for aPLA. Both ACL and anti-β2 GPI assays were widely heterogeneous with respect to reporting the cut-off for 'aPLA positivity.' Approximately 60% of the papers related to ACL used a low cut-off value (<20 units) for the definition of positive results; such a cut-off does not allow stratifying those medium- to high-titre patients who would fulfill laboratory criteria according to the international consensus.

5- Assessment of concomitant thrombotic risk factors is currently recognized as critical in the evaluation of aPLA-associated events. The stratification of traditional cardiovascular disease risk factors is crucial, especially in older patients who are more likely to have thrombotic events not fully attributable to aPL. Furthermore, the presence of other environmental or genetic thrombotic risk factors was rarely reported in the analyzed studies, impeding the judgment about a potential direct causal relationship between aPL and clinical outcomes.

6- Extra-criteria aPLA, especially IgA anti-B2GPI and IgG/IgM anti-phosphatidylserine/prothrombin antibodies (aPS/PT), have been strongly associated with thrombosis in young adults in multiple studies and should be investigated before exclusion of APS.

7- Serial follow up of serum OPN level for cases of stroke is highly recommended as it has prognostic value to predict recovery.

5. Conclusions

The study documented an increase in the incidence of antiphospholipid syndrome (APS) as an etiology of cerebrovascular events (CVE) in young adults. Osteopontin markers may be sensitive and specific for diagnosing CVE in this population, providing valuable insights for early detection and targeted treatment. Further research is necessary to validate these findings and explore the potential of osteopontin as a reliable biomarker in clinical practice.

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