ASSOCIATION OF ANTI PHOSPHOLIPID ANTIBODIES IN THE PATIENTS WITH ISCHEMIC STROKE UNDER 45 YEARS OF AGE IN TERTIARY LEVEL HOSPITAL: A CASE CONTROL STUDY

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Abstract

Background: Antiphospholipid antibodies have been associated with a clinical syndrome consisting of thrombosis and recurrent, unexplained fetal loss. Stroke may be caused by Antiphospholipid Antibodies (APLA) especially in young persons without other conventional risk factors like Diabetes mellitus, Hypertension, Dyslipidemia, Smoking etc. The aim of this study was to evaluate the association of antiphospholipid antibodies (Anticardiolipin antibody and lupus anticoagulant) in young ischemic stroke patients presenting with sudden neurological deficit. **Materials and methods:** This was a case-control study performed in Medicine and Neurology department of Chittagong Medical College Hospital, Chittagong, a tertiary care government hospital. A total no. of 195 respondents consisting of 95 consecutive young patients (Age less than 45 years) diagnosed as ischemic stroke and 100 age and sex matched healthy controls were included in this study. The clinical details and related investigations of the respondents were reviewed. Anticardiolipin antibodies (IgM, IgG isotypes) were evaluated by an Enzyme-Linked Immunosorbent Assay (ELISA) and lupus anticoagulant by phospholipid dependent coagulation test Activated Partial Thromboplastin Time (APTT). When APTT was found more than 5 sec prolonged than control value, mixing studies using a 50:50 mixture of patient and normal plasma were performed and the result was considered positive if prolongation of APTT was not corrected which were needed to be confirmed after 12 weeks. The tests of Anticardiolipin antibodies (aCL) were repeated after 12 weeks when prior results were abnormal and the tests were considered positive when both results of aCL were found above normal value (IgM > 7.2 MPL/ml, IgG>14.4 GPL/ml). **Results:** Anti-cardiolipin antibodies Anticardiolipin antibodies (aCL) were present in 10.5% of the patients (IgM in 3 cases, IgG in 6 cases, IgM/IgG both in 1 case) and in 1% (IgG type) of control, but none of the study subjects were found LA positive. The odds ratio of aCL was 11.647 (95% Confidence interval= 1.461 – 92.852). Overall, the risk factors profile in cases were, Hypertension (32.6%) Diabetes mellitus (10.5%) Cigarette smoking (26.3%) and Dyslipidaemia (25.3%). Among the 10 aCL positive cases, 3 were Hypertensive, 1 was Diabetic, 2 were Smoker and 2 were Dyslipidemic but anticardiolipin antibody positive group did not differ significantly with respect to distribution of major risk factor frequency from anticardiolipin antibody negative group. **Conclusion:** Anticardiolipin antibodies are associated significantly with ischemic stroke in young patients less than 45 years of age (p= 0.004). Hence screening for anti-cardiolipin antibodies in young patients with stroke is recommended.

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Key words

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Introduction

Stroke is the most important cause of disablement worldwide. Commonly stroke occurs in older age group of people. But 10% of all strokes occur in younger patients less than 50 years of age[1].

Regarding aetiology of ischemic stroke in young, premature atherosclerosis remains an important risk factor (15-25% of stroke in young adult) and cardioembolic stroke is more common among younger patients (15-35% of cases). Other causes include Extracranial artery dissection (2-25% of cases) Migraine, Drug misuse (Up to 5% of cases) Oral contraceptive use (Up to 8% of cases) Thrombophilic disorder, Vasculitis, Antiphospholipid antibody syndrome (5-10% of cases) and Rheumatic valvular heart disease[2,3].

Antiphospholipid Syndrome (APS) is a noninflamatory autoimmune disease and its principal pathogenic process is thrombosis[4]. It is characterized by recurrent arterial or venous thrombosis, recurrent fetal loss and thrombocytopenia in the presence of Antiphospholipid Antibodies (APLA) in serum[4,5].

Antiphospholipid Antibodies (APL) are a heterogeneous spectrum of autoantibody against anionic phospholipid of IgG, and/ or IgM, or less frequently also IgA immunoglobulins[6]. Lupus Anticoagulants (LA) and Anticardiolipin antibodies (aCL) were the first two antiphospholipid antibodies to be described (Mishra MN 2009). Most anticardiolipin antibodies are detected by ELISA using CL (Cardiolopin) as target antigen and are dependent on the co-factor β_2-glycoprotein-I[7]. LA is screened by coagulation baised assay like Activated Partial Thromboplastin Time (APTT) Kaolin Clotting Time (KCL) and dilute Russell viper venom time[8].

Antiphospholipid Antibody (APLA) is found in 10-46% of young patient with stroke and in 10% of stroke patient overall. About 30-50% of patients with SLE will have APLA (pbcers.org 2014). Stroke patient with APLA tends to be younger[9]. The mean age of CNS involvement in APS syndrome is several decade earlier than the general population and it is estimated that the chance of thrombosis in these patients is nearly 20%[4]. These patients also have recurrence rate of 6-30 % / year and a mortality rate of 10%/year (pbcers.org 2014). Certain group of patients appears to be at even high recurrence rate and these would include SLE patients with APLA. However antiphospholipid antibodies have been reported in up to 2% of normal population. Harmless antiphospholipid antibodies can be detected in blood for a brief period occasionally in association with wide variety of conditions, including Bacterial, Viral (Hepatitis, HIV) and parasite (Malaria) infections[9].

In the present study we intented to find out whether there is any association of anti phospholipid antibodies in young ischemic stroke patients in our community. Such a study in our young age group patients relating anti phospholipid antibodies to acute ischemic stroke will shed light on the relative importance on this issue as an independent risk marker of stroke in young and therefore help us to develop necessary therapeutic strategies for secondary prophylaxis in stroke prevention.

Materials and methods

This is a case control study done in the Department of Medicine and Neurology of Chittagong Medical College Hospital (CMCH) during one year study period after approval of protocol. A total of 100 consecutive patients diagnosed as ischemic stroke within 7 days of onset with age under 45 years fulfilling both inclusion and exclusion criteria were selected purposively as case and equal number of age and sex matched healthy people were included as control. In subsequent follow-up at 12 weeks, 2 patients died and 3 patients could not be followed up due to their non-cooperation. These 5 patients were omitted from the study and remaining 95 patients were enrolled as cases in the study. After getting the informed written consent, socio demographic data were collected in case record forms, primary clinical evaluation was done and physical examinations were carried out. Investigations like CBC, FPG & 2HPPG, Fasting lipid profile and Serum creatinine were done for all patient as routine procedures.With all aseptic precaution 10 cc venous blood was taken from study patients by researcher himself from medial

cubital vein and was collected in 2 separate sealed sterile tube with anticoagulant and send for analysis of aCL and LA. After getting written consent 10 cc venous blood was collected from age and sex matched healthy control for comparing. Before use the serum sample was kept at room temperature for 30 minutes and aCL assay was performed using commercially available kit, ELISA Cardiolipin IgM/IgG, REF 212896 (Euro Diagnostica AB, Lundavagen 151, SE-212 24 Maimo, Sweden) by PLATE READER analyzers (Organon teknika microwell system, model- Reader 230 S, Germany) (Appendix-G). Patient and control samples with raised titer of aCL were tested in duplicate at 12 weeks apart and a positive result was considered only when two samples were abnormal.

Reference level of aCL antibody (ELISA)

Normal range	Equivocal range	Positive result
< 10 GPL/ml	10- 14.4 GPL/ml	>14.4 GPL/ml (IgG)
< 5 MPL/ml	5.0 -7.2 MPL/ml	>7.2 MPL/ml (IgM)

According to laboratory criteria for the diagnosis of APS, anticardiolipin antibodies need to be present at medium / high titer. But the reagent kit used in this study considered IgG positive when titer >14.4 GPL/ml and IgM titer > 7.2 MPL/ml in serum samples. For detection of Lupus anticoagulant by APTT, blood specimen was centrifuged at 4000 rpm for 10 minutes at room temperature as soon as possible after collection to obtain platelet poor plasma using commercially available reagent (Dade Actin FSL Activated PTT Reagent) containing liquid purified soya and rabbit brain phosphatides with plasma activator, by fully automated Coagulation analyzer Sysmax CA-500 series (Germany). Result of APTT was expressed in seconds within 8-10 minutes. When initial test was found more than 5 sec prolonged than control value, mixing studies using a 50:50 mixture of patient and normal plasma were performed and the result was considered positive if prolongation of APTT was not corrected which needed to be confirmed after 12 weeks. Data were processed and analyzed by using computer based software SPSS- 20 (Statistical Package for Social Science) (IBM Corp. Armonk NY). Statistical methods used were t-test, Chi- square test, Univariate risk factor analysis and stepwise binary logistic regression analysis of risk factors. Statistical t-test was used to detect significance in mean age between case & control, mean age between aCL positive & aCL negative case group, physical examination & investigation findings between case & control. Significance in age group, sex, risk factors analysis and association of aCL antibody with young ischemic stroke patients were calculated by Chi-square test. Univariate analysis was used to calculate the individual relationship of aCL antibody and other conventional risk factors on ischemic stroke in young. Multiple logistic regression analysis was done to evaluate the relationship of aCL antibody alone or in combination with other major risk factors in young ischemic stroke patients. p value was considered statistically significant when it was less than 0.05.

Results

In this study 95 consecutive patients with Ischemic stroke were selected purposively as cases and 100 age and sex matched healthy controls were included for comparison.

Table I : Distribution of the age among the study groups (n= 195)

AGE IN GROUPS	STUDY GROUPS				Total	
	Case (95)		Control (100)			
	n	%	n	%	n	%
≤ 20 Years	5	5.3	5	5.0	10	5.1
21 – 30 Years	19	20.0	20	20.0	39	20.0
31 – 40 Years	53	55.8	55	55.0	108	55.4
> 40 Years	18	18.9	20	20.0	38	19.5
Total	95	100.0	100	100.0	195	100.0

χ^2 value =0.040. p = 0.998

Table II : Distribution of mean age among the study groups (n= 195)

	Study Groups	n	Mean	± SD	Median	Range	Sign.*
Age	Case	95	35.01	7.20	35.00	16 – 44	t = 0.117
(Years)	Control	100	35.13	7.05	36.00	16 – 44	p = 0.907
	Total	195	35.07	7.09	36.00	16 – 44	NS

* Independent samples t – test.

Table III : Distribution of sex among the study groups (n=195)

SEX	Study Groups				Total	
	Case		Control			
	n	%	n	%	n	%
Male	44	46.3	46	46.0	90	46.2
Female	51	53.7	54	54.0	105	53.8
Total	95	100.0	100	100.0	195	100.0

* χ^2 value = 0.002. p = 0.965.

Table IV : Distribution of anti-cardiolipin antibody among the study groups (n= 195)

Anti-cardiolipin Antibody (aCL)	Study Groups				Total (195)	
	Case (95)		Control (100)			
	n	%	n	%	n	%
Positive	10	10.5	1	1.0	11	5.6
Negative	85	89.5	99	99.0	184	94.4
Total	95	100.0	100	100.0	195	100.0

* χ^2 value = 8.306. p = 0.004

Table V : Distribution of anti-cardiolipin antibody IgM (Day 0 & after 12 weeks) in case and control (n=195)

aCL antibody (IgM)		Study Groups	
		Case (95)	Control (100)
Ig M (Day-0) (n = 195)	Raised	8	0
	Not raised	87	100
Ig M (After 12 weeks) (n = 8)	IgM Positive	3	0
	IgG seroconversion	2	0
	IgM, IgG Positive	1	0
	Seronegative	2	0

Table VI : Distribution of anti-cardiolipin antibody IgG (Day 0 & after 12 weeks) in case and control (195)

aCL antibody (IgG)		Study Groups	
		Case (95)	Control (100)
Ig G (Day-0) (n = 195)	Raised	4	1
	Not raised	91	99
Ig G (After 12 weeks) (n = 5)			
	IgG Positive	4	1

Table VII : Distribution of risk factors according to anti-cardiolipin antibody status among the cases (n = 95)

Risk Factors		Anti-Cardiolipin Antibody Status				Total (n = 95)		χ^2 Test Significance
		Positive (n = 10)		Negative (n = 85)				
		n	%	n	%	n	%	
Hypertension	Present	3	30.0	28	32.9	31	32.6	$\chi^2 = 0.035$ $p = 0.851^{NS}$
	Absent	7	70.0	57	67.1	64	67.4	
Diabetes Mellitus	Present	1	10.0	9	10.6	10	10.5	$\chi^2 = 0.003$ $p = 0.954^{NS}$
	Absent	9	90.0	76	89.4	85	89.5	
Cigarette Smoking	Present	2	20.0	23	27.1	25	26.3	$\chi^2 = 0.230$ $p = 0.632^{NS}$
	Absent	8	80.0	62	72.9	70	73.7	
Dyslipidemia	Present	2	20.0	22	25.9	24	25.3	$\chi^2 = 0.224$ $p = 0.610^{NS}$
	Absent	8	80.0	63	74.1	71	74.7	

Table VIII : Univariate risk factor analysis for control versus case (n = 195)

Risk Factors of Stroke	Control vs Case Odd'S Ratio (95 % Confidence Interval)
Anti-Cardiolipin Antibody(Positive / Negative)	11.647 (1.461 – 92.852)
Dyslipidemia (Present / Absent)	3.418 (1.405 – 7.811)
Hypertension (Present / Absent)	2.975 (1.464 – 6.047)
Diabetes Mellitus (Present / Absent)	2.824 (0.854 – 9.334)
Cigarette Smoking (Present / Absent)	1.523 (0.774 – 2.996)

Table I and II show that mean age of case (Ischemic stroke) was 35.01 and that of control was 35.13 having no statistically significant difference between them (p > 0.05). Maximum subjects were in 31-40 years age group (53% vs. 55%) in both case & control.

Table III shows that male and female ratio in case was 1: 1.5 (44% vs 51%) and in control was 1: 1.7 (46% vs. 54%) with no statistically significant difference between male and female (p > 0.05).

Table IV showing Anti cardiolipin antibody (aCL) was found in 10.5% of case and 1% of control indicating that anti-cardiolipin antibody was significantly associated with stroke patients (p= 0.004).

Table V shows that 95 patients and 100 control subjects were tested for aCL antibody (IgM) at day 0, and of them 8 patients had raised IgM. After 12 weeks, aCL antibody was repeated for those 8 patients and of them 3 patients remained IgM positive, 2 patients converted to IgG seropositivity, 1 patient had both IgM & IgG positive and 2 patients became seronegative. None of the control subjects were found to have raised titer of IgM.

Table VI shows that 95 patients and 100 control subjects were tested for aCL antibody (IgG) at day 0, and of them 4 patients had raised IgG. Tests of these 4 patients were repeated after 12 weeks and remained IgG positive. In control only 1 study subject was IgG positive at day 0 and after 12 weeks.

Table VII shows that there was no statistically significant difference for any of the risk factors like Hypertension (30% vs 32.9%) Diabetes (10% vs 10.6%) Cigarette smoking (20% vs 27.1%) and Dyslipidemia (20% vs 25.9%) between aCL positive and aCL negative stroke patients (p > 0.05).

Table VIII shows univariate risk factor analysis for control versus case, where anticardiolipin antibody was found highly associated with ischemic stroke among study subjects (OR= 11.647).

Discussion

This is a hospital based case-control study carried out in medicine and neurology ward of CMCH from June 2014 to July 2015 to evaluate the association of anti-phospholipid antibodies with ischemic stroke in young below 45 years of age. A total of 95 ischemic stroke patients were compared with 100 age and sex matched healthy control in this study.

In the present study anticardiolipin antibodies were found in 10.5% of young ischemic stroke patients and 1% of healthy control but none of the study subjects were found LA positive. Among them, 3 had Immunoglobulin M (IgM) antibody, 6 had Immunoglobulin G (IgG) antibody and 1 had both IgM and IgG antibodies. Odds ratio for aCL was 11.647 (95% CI= 1.461 – 92.852). The study patients had one clinical criterion (Stroke) and one laboratory criteria (aCL antibody) and therefore can be considered to have APS. But according to most recent guidelines to classify APS, the study patients belong to uncertain thrombotic and/or obstetric antiphospholipid syndrome group due to presence of single positivity (Only aCL positive) and one clinical critria[10,11]. The study patients are lowest-risk group APS and treatment should be considered by clinical event only not by the presence of single positivity APS. But it can be said that anticardiolipin antibody is significantly associated with ischemic stroke in young (p= 0.004). In the present study aCL was detected by ELISA and LA was screened by APTT (Activated Partial Thromboplastin Time). However, APTT reagents are generally not sensitive to all types of LA, and because of the variety and heterogeneity of plasma anti-phospholipid antibodies, APTT cannot be used alone for the diagnosis of LA[12]. Moreover the most recent recommendations for LA detection require the use of two different phospholipid dependent clotting assays, based on different methodologies. DRVVT is the most common screening & confitmatory test for LA in the laboratory but the combination of Silica Clotting Time (SCT) and dRVVT have the highest sensitivity in the detection of LA in APS patients[12]. These more sensitive tests could not be used due to lack of availability in this context and the failure to find LA positivity may be due to this methodological difference.

Anticardiolipin antibodies and lupus anticoagulant were found in young ischemic stroke patients in several previous studies. Van Goor et al found aCL in 10.15% of study patients and used APTT & diluted prothrombin time for 10.9% LA detection[13]. Blohorn A et al detected aCL antibody in 13.6% of young stroke patients and LA was 17.3% by using APTT and Tisue Thromboplastin Inhibitors assay (TTI)[14]. In a previous study by Nenacini P. et al aCL was found 10.9% and LA by diluted tissue thromboplastin inhibition test was 14.5%[15]. In a population based case-control study by Robin LB et al aCL was

26.9% and LA by dRVVT was 20.9%[8]. In two previous studies by Montalban J et al antiphospholipid antibodies were found in 6.8% (n=146, age range 27-59 years) and 1.04% (n=481, age range 16-90 years) of study subjects[16,17]. It seems that antiphospholipid antibody associated ischemic stroke is common in younger age group.

In the current study, 32.6 % of case and 14% of control had hypertension and the difference was highly significant (p = 0.002). Dyslipidemia was found significantly high in case than control (25.3 % vs 9%. and p= 0.007) but not diabetes (10.5 % vs 4% and p = 0.078) or Cigarette smoking (26.3 % vs 19% and p = 0.222). In a study by Deepa D et al hypertension was found 44.5%, Diabetes was 13.9% and Dyslipidemia was 26.1% in young ischemic stroke patients of 18-45 years[18].

Among aCL positive (n = 10) and aCL negative (n = 85) stroke patients group hypertension was 30% and 32.9%, diabetes was 10% and 10.6%, dyslipidemia was 20% and 25.9% and cigarette smoking was 20% and 27.1% respectively. Statistically significant difference was not found between aCL positive and aCL negative patients group for any of the risk factors like Hypertension (p= 0.851) Diabetes (p= 0.954) Smoking (p= 0.632) and Dyslipidaemia (p= 0.610). Similar observation was noticed by Nagaraja D et al 1997 and Nenacini P et al in their studies[10,15]. Among 10 aCL antibody positive patients, 2 patients had multiple risk factors, 3 had single risk factor and 5 cases had none. It was observed that 5 (50%) aCL positive young stroke patients and 23 (27.1%) aCL negative young stroke patients had no major risk factors for stroke other than aCL antibody, but the difference between them was not found statistically significant (p = 0.213). Comparable result was found by Mishra MN et al where analysis of 28 young ischemic stroke patients showed that 11 patients had a combination of risk factors with APL, 10 had only one risk factor and 7 patients had neither[6].

In the present study Univariate risk factors analysis for control versus case reports that anticardiolipin antibody is highly associated (OR= 11.647, 95% CI= 1.461 – 92.852) with stroke in young when compared with other risk factors like Dyslipidemia (OR= 3.418, 95% CI= 1.405 – 7.811) Hypertention (OR= 2.975, 95% CI= 1.464 – 6.047) Diabetes (OR= 2.824, 95% CI= 0.854 – 9.334) and Cigarette smoking (OR= 1.523, 95% CI= 0.774 – 2.996). Stepwise binary logistic regression analysis of risk factors showed that anticardiolipin antibody is significantly associated with ischemic stroke ($p < 0.05$) and the risk of developing stroke in aCL positive patients is progressively increased with consecutive addition of other conventional risk factors. It is suggested that anticardiolipin antibody may be an independent risk factor for ischemic stroke in young age group along with other modifyable risk factors. Similar observation was also made by Robin LB et al[8].

In the present study mean age were 35.01 years and 35.13 years in case and control respectively. Majority of the study subjects were in 31-40 years age group (55.8 % in case and 55% in control, age range 16 – 44 years). In a study by Razzaq AA et al the mean age of study subjects (n= 118, age range 15-45 years) was 38 years and about three quarters of the patients were in the 35-44 years age group[19]. The mean age of aCL positive stroke patients (n = 10) was 29.50 years and that of aCL negative stroke patients (n = 85) was 35.66 years and the difference between them was found statistically significant (p= 0.01). It means that aCL antibody is associated with stroke in younger age group. In a previous study by Nenecini P et al similar observation was noticed[15].

Several studies indicate that anti-cardiolipin antibodies are associated with recurrent thrombo-embolic events[15]. In a cohort study, Levine SR et al reported that 58% of study (n=81) patients suffered from recurrent stroke, TIA, Deep vein thrombosis, Pulmonary embolism or Myocardial infarction with a median time to event was 0.62 years (Mean, 1.14 yr)[20]. None of the aCL positive stroke patients in this study suffered from previous TIA or Cerebral ischemia.

Thus in this study the asociation between anticardiolipin antibody and ischemic stroke in young is more obvious.The aCL positive stroke patients belonged to lowest-risk APS group and warrant treatment for clinical event not for APS.

Limitations

The study sample is small and not randomized. Further studies from multiple centers are required on this important issue which might represent the nation-wide picture.

Conclusion

The study established a positive association between anticardiolipin antibody and ischemic stroke in young and anti-cardiolipin antibodies may be an independent risk factor for stroke in this age group.

Disclosure

All the authors declared no competing interest

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