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Study of carotid intima media thickness and its correlation with novel risk factors in ischemic stroke

Avishek Saha *MD*, Pradip K Sinha *MD DM*, Rudrajit Paul *MBBS*, Ramtanu Bandyopadhyay *MD*, Koushik Biswas *MBBS*, Amit K Banerjee *MD*

Department of Medicine, Medical College Kolkata, Kolkata, West Bengal, India

Abstract

Carotid intima media thickness (CIMT) is used commonly as a non-invasive test for assessment of degree of atherosclerosis. The present study, in a tertiary care centre of Eastern India was aimed to determine the relation between CIMT and the known and novel risk factors of ischemic stroke. Of the 62 patients studied, 82% were hypertensive and 61% were smokers, 95% were above 50 years of age. Seventy one percent of our patients had high CIMT. CIMT was significantly related to high sensitivity C-reactive protein (hsCRP), fibrinogen, LDL levels and age of the patient. By logistic regression analysis, hsCRP, fibrinogen and age were significantly associated with high CIMT. When the patients have elevation of both hsCRP and fibrinogen, the risk of high CIMT were even higher. Our study highlights the needs for better screening in high risk patients.

INTRODUCTION

Stroke is defined as 'abrupt onset of a neurologic deficit that is attributable to a focal vascular cause'.[1] Approximately 80% cases of stroke are due to cerebral ischemia.[1] Incidence and prevalence of stroke has risen exponentially worldwide in last few decades and incidence of stroke is also rising among Indians.[2,3] Ischemic stroke may be thrombotic or embolic. One of the main causes of cerebral ischemia is atherosclerotic stenosis in carotid arteries that occurs most frequently around the bifurcation of common carotid and proximal internal carotid artery. The carotid artery stenosis may cause brain ischemia by decrease of blood flow to the brain[1], and artery to artery embolism.

Carotid intima media thickness (CIMT) is now used commonly as a non-invasive test for assessment of degree of atherosclerosis in different arteries. In previous studies it has been found to have direct correlation with incidence of stroke.[4] A meta-analysis of 8 studies[5] concluded that CIMT is a strong predictor of future vascular events and its etiology may be more than can be explained by the classic cardiovascular risk factors. Of late, there are intense interests in novel risk factors of atherosclerosis and hematological markers, e.g. high sensitivity C-reactive protein level (hsCRP), fibrinogen level, and serum level of homocysteine, which can predict the morbidity & mortality in cerebrovascular diseases besides the conventional risk factors. Systemic inflammation is now thought to be part of the process for accelerated atherosclerosis. Measurements of the marker of systemic inflammation, i.e. hsCRP, may provide a good indication of the of risk to develop stroke.[6] Other novel markers for atherosclerosis i.e. plasma fibrinogen and homocysteine, may also need to be evaluated, especially in high risk patients.

In this cross-sectional observational study conducted at Medical College, Kolkata, our objectives were to measure the different blood parameters and CIMT in patients of ischemic stroke; and to correlate the blood parameter values with the CIMT.

METHODS

Sixty-two patients with history of cerebrovascular accident (CVA) within past one year, who were attending the Medicine Outpatient Department, were included in the study, after taking the appropriate consent. The study has the approval of the ethics board of the Institution. In all patients, CT-scan or MRI demonstration of cerebral infarct was required. The exclusion criteria were: patients with congestive cardiac failure, renal disease, thyroid disease, hepatic disease, ketosis, HIV infection, any other infections, known coagulation disorders and known vitamin deficiencies. All patients had detailed history and clinical examination. Laboratory investigations performed include estimation of fasting and postprandial

Address correspondence to: Dr. Rudrajit Paul, 15/5, Bose pukur Road, Kolkata 700 039, West Bengal, India. docr89@gmail.com; 91-9433824341

blood sugars, lipid profile (total cholesterol, LDL, HDL, triglyceride), hsCRP level, serum homocysteine and fibrinogen levels. Fibrinogen was estimated using Beckman Coulter Synchron CX5 pro, USA and hsCRP estimated using the immunoturbidimetic method. The normal value of hsCRP was taken as 1 mg/L[7], that of fibrinogen were 200-380 mg/dl (reference range in kit) and that of homocysteine was< 15 μmol/L.[7] Blood glucose and lipid profiles were estimated within 12 hours of the CVA. hsCRP, fibrinogen and homocysteine were done at least two months after the stroke event. This was done to ensure that acute phase reactants like fibrinogen settled down to their pre-event values.

As for CIMT, which was used as the marker for extent of atherosclerosis, Doppler study of bilateral carotid arteries was performed using a high-resolution 7.5 MHz phased-array transducer (Philips ™ IU-22 ultrasound scanner). All examinations were carried out in a dark, quiet and temperature-controlled room. With the head in a slightly bent position towards the opposite site of the one being scanned, the ultrasound transducer was placed in an angle of 90° of the vessel wall, to obtain parallel echo lines of the intima and media in both near and far walls. Imaging of both common carotid arteries (CCA) upto their bifurcation, the carotid bulb, as well as proximal 10 mm of internal carotid artery (ICA) of both sides was performed. The best images of far wall of the arteries were taken for measurement, mainly CCA. The depth of the scan was adjusted and the transmit focus zone set at the optimal level to show the best possible image of the far wall of the CCA. The ideal place of measurement of IMT was an area of at least 1 cm devoid of plaques. Mean measured values of CIMT of three sites of a particular side were taken for calculation of CIMT of that side. IMT was finally calculated as the mean IMT of the left and right CCA. The cut off for normal CIMT was taken as 0.8 mm. in different studies, this cut-off has been found to correlate with vascular risks.[8] This cut off is found very appropriate to predict risk also among Indians.[9] The measurements were manual and no software was used due to limited resources. All the measurements were done by a single operator, to avoid inter-observer variation. Standard statistical procedures were used to calculate relation between variables using soft wares like MedCalc and GraphPad. Logistic regression model was used to find relation between variables. P-value <0.05 was taken as significant.

RESULTS

A total of 62 ischemic stroke patients were studied. Figure 1 shows the distribution of patients according to age and gender. As shown, close to half of the patients (30, 48.4%) were in the age group of 50-60 years. About two thirds of the patients were males, (40, 64.5%). However among the 20 diabetic patients, females (12, 60%) outnumbered the males. Thirty eight patients (61.3 %) were smokers and all were males. Past history of diabetes and hyperlipidemia before the stroke was found in 10% (n=6) and 14% (n=9) respectively.

Laboratory studies showed that the patients with stroke had significantly high levels of homocysteine, fibrinogen, hsCRP and LDL compared to standard normal values (Table 1). The patients were divided into two groups: - those with high CIMT (44) and those with normal CIMT (18). (Table 2). Fifty one patients (82 %) were hypertensive (SBP≥ 140 mm of Hg and/or DBP ≥ 90). In those with high CIMT, 95% were hypertensive (Table 2), compared to 50% in those with normal CIMT (p=0.0001; fisher's exact test). In those with high CIMT, 68.1% were smokers, as compared to 44.4% of those with normal CIMT (p=0.0944, chi square test). Expressed in another way, of the smokers, 79% (n=30) had high CIMT; while of non-smokers, 41.6 % (n=10) had high CIMT (Table 2).

Table 3 compares the laboratory values of the groups with high and normal CIMT. As shown, of the ischaemic stroke patients studied, hsCRP, fibrinogen and LDL levels were significantly higher while HDL-C values were significantly lower in patients with high CIMT. The mean age of patients with high CIMT was significantly older than those with normal CIMT.

The average CIMT was elevated in 44 of the 62 patients (71%). Fasting blookd sugar levels were also higher in patients with high CIMT as compared to patients with normal CIMT. But the difference was not significance (p=0.53). The average right CIMT was 0.98±0.19mm in diabetic patients and 0.94±0.27 mm in non-diabetic patients**.** The average left CIMT was 1.01±0.20 mm in the diabetic patients, and 0.87±0.2 mm in nondiabetic patients. The differences were not statistically significance.

By logistic regression model, high hsCRP has significant association with CIMT (OR= 1.78, C.I. 0.54 to 5.9; p=0.0485). High fibrinogen level also has significant association with CIMT (OR=1.03, C.I. 1.01 to 1.067, p=0.0028). For LDL and age,

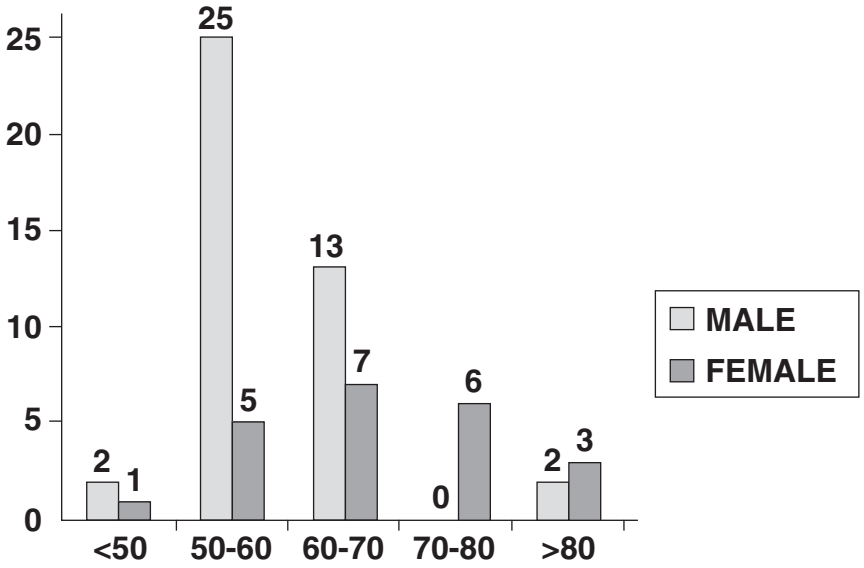

Figure 1. Figure showing the age and sex distribution of the patients (in years); the numbers in figure indicate the number of patients

the OR s were 1.05 (C.I. 0.98-1.11) and 1.11(C. I. 0.96-1.28) respectively.

Table 4 shows that when both hsCRP and fibrinogen are elevated, the odds of high CIMT rise. This is also shown in Figure 2, where patients with high hsCRP and fibrinogen has manifold chance of high CIMT ($p<0.0001$; chi square test).

DISCUSSION

In this study, we found 71% prevalence of high CIMT in patients with ischemic stroke. This high CIMT was significantly related to vascular risk factors such as age, HDL, LDL, serum hsCRP and fibrinogen levels. Of these, by logistic regression analysis, hsCRP, fibrinogen and age were significantly association with high CIMT. When the patients have elevation of both hsCRP and fibrinogen, the risk of high CIMT were even higher. Hypertension was also significantly associated with high CIMT. There was also association of smoking and diabetes with high CIMT, but these were not statistically significant.

There were not many studies of risk factors for stroke from India. The study by Gupta *et al*[10] found

Table 1: Table showing the study parameters in our patients (n=62)

Parameter	Mean ± SD
FBS (mg/dL)	122.63± 54.9
PPBS (mg/dL)	188.7± 86.03
LDL (mg/dL)	105.68± 29.54
HDL (mg/dL)	41.36± 8.9
HSCRP (mg/L)	1.84± 0.68
Fibrinogen (mg/dL)	445.66 ± 94.16
Homocysteine (µmol/L)	18.05± 8.93

HDL: high density lipoprotein; LDL: low density lipoprotein; FBS: fasting blood glucose; PPBS: post prandial blood glucose; hsCRP: high sensitivity C - reactive protein; S.D: standard deviation

Table 2: Table showing distribution of carotid Intima media thickness (CIMT) and the relation with other parameters

CIMT	Diabetic		Hypertensive		Smoking		Gender		Age>55 in female and >45 in male	
	Yes	No	Yes	No	Yes	No	Male	Female	Yes	No
Normal (n = 18)	7	11	9	9	8	10	11	7	14	4
High (n = 44)	13	31	42	2	30	14	29	15	43	1

tobacco use, obesity with high waist: hip ratio, high blood pressure, high LDL cholesterol, low HDL cholesterol, abnormal apolipoprotein A-1: B ratio, diabetes, low consumption of fruits and vegetables, sedentary lifestyles and psychosocial stress to correlate with vascular events. There were also Indian studies showing that newer risk factors such as homocysteine and fibrinogen contribute to stroke.[11,12] In our study we found that our patients of ischemic stroke had high levels of fibrinogen, hsCRP, homocysteine and LDL (Table 1). On homocysteine, it is interesting to note that Modi *et al* from Chandigarh, while reporting raised homocysteine among their Indian stroke patients, also reported no significant difference in the mean homocysteine levels in their patients with large vessel disease (thromboembolic strokes) as compared to small vessel disease (lacunar strokes).[11]

Atherosclerosis, especially carotid atherosclerotic plaques is a known cause of stroke.[4] The Rotterdam study showed that increasing CIMT is a risk factor for stroke; analogous to coronary plaques in acute myocardial infarction.[13] These associations were independent of age, sex, and history of myocardial infarction or stroke.

Table 3: Comparison of the investigatory findings of ischaemic stroke patients with high carotid Intima media thickness (CIMT) with normal CIMT

Parameter	High CIMT (mean ± S.D)	Normal CIMT (mean ± S.D)	p-value (t test)	Correlation coefficient with high CIMT
hsCRP (mg/L)	2.27 ± 1.77	0.795± 0.34	0.0012	r=0.408; p=0.0012
Fibrinogen(mg/dl)	481 ± 85.5	357 ± 44.9	<0.0001	r=0.605; p<0.001
Homocysteine(µmol/L)	17.6 ± 8.23	19.2 ± 10.7	0.53	NS
HDL(mg/dl)	37.9 ± 7.59	42.9 ± 7.64	0.0024	NS
LDL (mg/dl)	114 ± 28.7	98.7 ± 28.4	0.043	r=0.15; p=0.25
FBS (mg/dl)	125 ± 48	117± 35	0.53	NS
PPBS (mg/dl)	180 ± 71.5	185± 66.2	0.79	NS
Age (yrs)	64.3 ± 9.43	58.4 ± 11.3	0.039	r=0.263; p=0.039
Triglyceride (mg/dl)	138.7 ± 14.98	132.4 ± 21.6	0.517	NS

HDL: high density lipoprotein; LDL: low density lipoprotein; FBS: fasting blood glucose; PPBS: post prandial blood glucose; hsCRP: high sensitivity C - reactive protein; S.D: standard deviation; NS: not significant

Table 4: Table showing the distribution of patients according to hsCRP and fibrinogen levels

Increased carotid intima media thickness (>0.8 mm) (N =44)		Fibrinogen (mg/dl)	
		≤ 380	>380
hsCRP	(≤ 1mg/L)	2	8
	(> 1 mg/L)	1	33
Normal carotid intima media thickness		Fibrinogen (mg/dl)	
		≤ 380	>380
hsCRP	≤ 1	12	2
	> 1	3	1

The noninvasive assessment of common carotid intima-media thickness may thus be a promising method to assess the degree of atherosclerosis, in populations at large. A study by Cao *et al* showed that C-reactive protein level is an independent risk factor for ischemic stroke, and it has even higher risk in the presence of high CIMT.[14] In our study, we found high hsCRP had an odds ratio of 3.02 for high CIMT. In the group with high CIMT, the hsCRP level was significantly higher as compared to the group with normal CIMT (2.27 ± 1.77 g/L vs. 0.795± 0.34; p=0.0012). C-reactive protein is not just a risk marker. It has been shown to play an active role in atherogenesis.[15] C-reactive protein is chemotactic for freshly isolated human blood monocytes. A specific C-reactive protein receptor is demonstrated on monocytes in vitro as well as in vivo, and blockage by use of a monoclonal anti-receptor antibody completely abolishes CRP-induced chemotaxis. CRP may thus play a major role in the recruitment of monocytes during atherogenesis.[15]

Serum Homocysteine is also now considered a risk factor for stroke. The study by Tanne *et al* found that high levels of serum homocysteine were associated with increased incidence of cerebral ischemia.[16] It is well recognized that ischemic stroke is pathologically and etiologically heterogeneous and risk factors for one etiologic subtype may not be risk factors for other subtypes of stroke. Recent studies have shown that acute hyperhomocysteinemia causes endothelial dysfunction, which might affect cerebrovascular reactivity and promote atheroma development.[17] However, a meta-analysis by Durga *et al* found that observational studies generally failed to demonstrate a relationship between homocysteine and CIMT in homocystinuric, uremic, hypercholesterolemic or non-insulin-dependent diabetes mellitus patients or in subjects with insulin insensitivity.[18] Weak associations, only in certain sub-populations were found in vascular disease patients and in population-based studies. In our study, although the average homocysteine level was high in the stroke patients, we did not find any association between raised homocysteine level and high CIMT (Table 3). This may be due to the small number

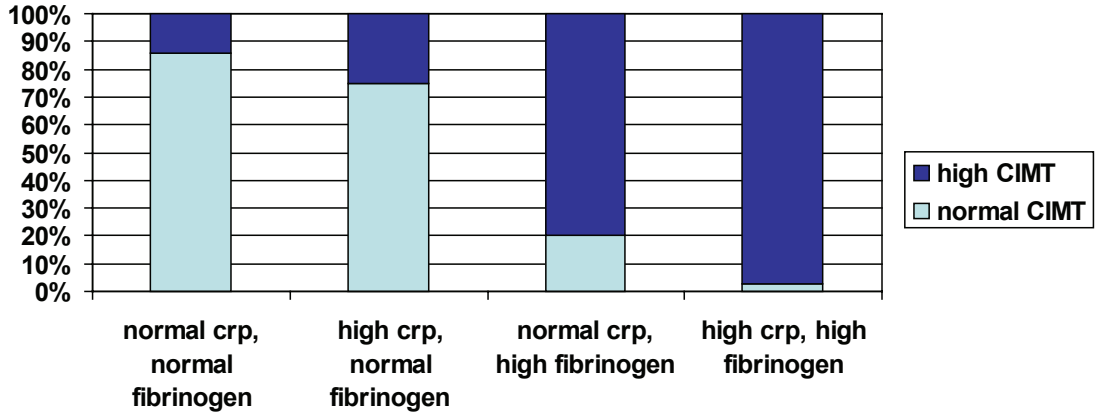

Figure 2. Figure showing the distribution of the two groups according to hsCRP and fibrinogen

of patients. Also, the high level of homocysteine in Indian population is sometimes attributed to the deficiency of cobalamin.[19] This is especially so in the vegetarian population, who may thus be at risk for vasculopathy.[20]

Fibrinogen is a novel risk factor for vascular events.[21] The CARDIA study showed that in young adults, elevated serum fibrinogen levels were independently associated with cardiovascular events and this also correlated with CIMT.[21] Approximately 30% of CIMT variability was attributable to genetic factors. Associations between CIMT and polymorphisms in the apolipoprotein CIII, cholesteryl ester transfer protein, methylene tetrahydrofolate reductase and fibrinogen genes were observed and explained approximately 20% of CIMT variation.[22] Thus patients with certain fibrinogen genes may be predisposed to high CIMT. In our study we found significantly high fibrinogen levels in patients with high CIMT (481 ± 85.5 mg/dl vs. 357 ± 44.9; $p<0.0001$; Table 3). The odds ratio for fibrinogen was 1.02, but when associated with hsCRP, the odds ratio was significantly higher (Table 4). This relation between hsCRP and fibrinogen is also supported by the study of Sabeti *et al* from Austria.[23] They have shown that fibrinogen was significantly associated with progression of atherosclerotic lesions in the carotid arteries with a consistent temporal correlation between progressive disease and elevation of fibrinogen at baseline and follow-up. However, adjusting for other inflammatory parameters such as hs-CRP or serum amyloid A substantially diminished these associations, suggesting that fibrinogen is mainly an indicator of the level of inflammatory activity rather than exerting specific properties in promoting progression of the disease.

We also found significant association between serum HDL, LDL levels and CIMT (Table 3). These risk factors of atherosclerosis are well established. A Finnish study has shown association between LDL and CIMT.[24] In the Muscatine study[25], a risk factor load model showed relation between HDL and CIMT. This was especially true for males. Since most of our study patients were males, this may partly contribute to the association of low HDL and high CIMT in our study. However, although these new risk factors have significant associations with atherosclerosis and hence the risk of developing ischemic stroke, it may not mean that these should be screened in all in the general populations.[26] Some working groups have proposed that only patients classified as 'high' or at least 'intermediate' risk be screened with these markers to find the additional risk burden.[27]

This study has limitation of low number of patients. It is also a cross sectional study which can give the odds ratio only. To better determine the association between these variables, we need a prospective study with serial follow ups. The studies such as by Cao *et al*[14] and Sabeti *et al*[23] give better idea of the true risk. Also, the manual measurement techniques used in this study was inferior to the automated software based methods of IMT measurement, which can simultaneously take multiple measurements.

In conclusion, this small study has shown a significant association between CIMT and the atherogenic variables like age, hsCRP, fibrinogen, HDL and LDL, with hsCRP and fibrinogen as strong risk markers for high CIMT. This may indicate the need to screen patients for these risk factors. Also, in places where CIMT measurement is not available, these blood tests can be used as surrogate markers to define the patient population at risk of ischemic stroke. Conversely, in patients with ischemic stroke, these markers can be used to monitor and prevent further events by appropriate interventions.

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