

Lipoprotein (a) and other Lipid Profile in Patients with Thrombotic Stroke: Is it a Reliable Marker?

Shashidhar K Nagaraj, Pareenta Pai¹, Gopalakrishna Bhat², Hemalatha A³

Department of Biochemistry, Sri Devaraj Urs Medical College, Tamaka, Kolar, ¹Jawaharlal Nehru Medical College, Belgaum, Karnataka, ²Kasturiba Medical College, Manipal, Karnataka, ³Department of Pathology, Sri Devaraj Urs Medical, College, Tamaka, Kolar, Karnataka, India

Address for correspondence: Dr. Shashidhar K N, E-mail: drshashikn1971@yahoo.co.in

ABSTRACT

Background: Cerebrovascular disease (CVD) and coronary heart disease (CHD) cause 40%–50% of deaths in developed countries with CVD causing 10%–12% of deaths. Though increased Lipoprotein (a) is a risk factor in developing CHD, its role is poorly defined in etiopathogenesis of CVD.

Aims: To find the association of lipoprotein (a) and lipid profile in thrombotic stroke patients after acute phase.

Settings and Design: The study was conducted at Kasturba Medical College, Manipal. Twenty one cases of thrombotic stroke and 18 cases of age and sex matched controls were taken for the study. Informed consent was taken from both case and control.

Materials and Methods: Overnight fasting sample was collected from both case and control. Serum was separated and parameters such as total cholesterol, triglycerides, high density lipoproteins-C, low density lipoprotein-C, lipoprotein (a), fasting blood sugars were estimated. Statistical analysis: Data were analyzed by SPSS software, Student's *t*-test, standard deviation (SD), and standard error of mean (SEM), *P*-value <0.05 is considered to be significant.

Results: In this study, we found no statistical significant differences in serum lipid and lipoprotein (a) profile between controls and thrombotic stroke patients.

Conclusions: Highest frequency (38%) of stroke was found in the age group of 70–80 years. There were other associated risk factors such as diabetes in five cases (24%), hypertension in nine cases (43%), and family history of stroke in four cases. However, further studies are required to evaluate the importance of serum Lp(a) estimation in the assessment as a risk factor for thrombotic stroke.

Keywords: Lp(a), lipid profile, thrombotic stroke

INTRODUCTION

Stroke is a syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral infarction with symptoms lasting for more than 24 h or longer or resulting in death with no apparent cause other than vascular origin.^[1–3] Incidence of cerebrovascular disease (CVD) (stroke) increases considerably with age, affecting many people in their “Golden year of life.”^[2] Even though

frequency of stroke varies from place to place, cerebral thrombosis is the most frequent form encountered in clinical studies followed by others.^[3,4] Even though the incidence of stroke is increasing, mortality rate is decreasing in the recent years.^[5]

Coronary heart diseases (CHD) and CVD are responsible for around 40%–50% of all deaths in developed countries and out of this 10%–12% are due to stroke.^[6] Recently, there is a decline in the mortality from stroke which may be due to socioeconomic changes, early diagnosis and treatment of hypertension and other risk factors.^[4] Compared with CHD, studies conducted in stroke patients are inconclusive, and not clearly proved, whether stroke is due to increased lipid profile, hypertension or due to the life style of an individual. Moreover, due to alteration in lipid profile which lipid parameter or the lipoprotein gets altered in the thrombotic stroke

Access this article online	
Quick Response Code: 	Website: www.jlponline.org
	DOI: 10.4103/0974-2727.78560

is not clear. Lp(a) has been observed to be increased in CHD, but very few studies comment on estimation of this parameter in the case of thrombotic stroke, even though the etiopathogenesis is almost similar in both these conditions. Keeping this in mind, we conducted a cross-sectional study to find out association of Lp(a) and thrombotic stroke.

Lp(a) is a LDL like particle, discovered as a sinking prebeta lipoprotein. This variant lipoprotein fraction contains one molecule of an apolipoprotein B100 and another large protein called apolipoprotein (a) [Apo (a)]. Following its discovery Lp(a) was shown in case control studies to be associated with CHD.^[7-9] It has been demonstrated that there is a structural homology between Apo (a) and plasminogen.^[10] Ninety percent of Lp(a) concentration is under genetic regulation. Despite this genetic regulation, some metabolic abnormalities may have effect on Lp(a) levels in plasma. Notable among them are (1) acute phase response, (2) diabetes, and (3) liver and renal failure.^[7] Studies on general population have shown that Lp(a) levels are skewed, i.e., it ranges from 1 to 200 mg/dl with geometric mean of about 10 mg/dl.^[11] Certain pharmacological agents and disease conditions also influence the circulating Lp(a) levels.^[11,12]

Pathophysiological role of Lp(a) in atherothrombosis

Accumulation of Lp(a) has been demonstrated in the arterial walls of human coronary and cerebral vessels.^[13] Lp(a) particles are susceptible to oxidative modification and this modified Lp(a) is taken up by scavenger receptor macrophages leading to intracellular cholesterol accumulation and foam cell formation, which contributes to atherogenesis.^[11,14-18] Atherogenic lipoproteins include in addition to LDL, almost all classes of lipoproteins that contains Apo-B (VLDL, beta VLDL, IDL, Lp(a), and oxidized LDL). A common feature of these atherogenic lipoproteins is that they contain various amounts of cholesteryl esters and either Apo-B 100 or Apo-B 48. In addition Lp(a) contain Apo (a), a protein that is a disulfide linked to Apo -B and is homologous to plasminogen, Apo (a) may contribute to atherogenesis by mechanisms related to thrombosis.^[19]

Aim

To find the association of lipoprotein (a) and lipid profile in thrombotic stroke patients after acute phase.

MATERIALS AND METHODS

Patients of coastal Karnataka with neurological manifestations confirmed as thrombotic stroke on

computed tomography scan, patients with or without hypertension, diabetes, and those with thromboembolic stroke were included in our study.

We excluded the overnight nonfasting patients, those with hemorrhagic stroke, stroke secondary to congenital heart diseases, arrhythmias, patients presenting within 21 days after occurrence of stroke and with any acute illness. Cases not confirmed as thrombotic stroke, patients with doubtful thrombotic stroke, and those with renal and hepatic disease were also excluded in this study. None of these patients were on any drugs that are known to alter the Lp(a) levels at the time of our study, because certain drugs are known to alter the Lp(a) levels. As we wanted to check the basal Lp(a) levels in the confirmed cases.

Written approval of ethical committee, following the national guidelines was taken before the initiation of study. Blood samples were collected after 21 days of occurrence of stroke.

Overnight fasting blood samples were collected without adding anticoagulant to the tube from 21 cases of thrombotic stroke patients not less than three weeks after the occurrence of stroke, because Lp(a) levels are known to get altered due to acute phase response. Eighteen healthy age and sex-matched control fasting samples were also drawn after explaining to them about the whole procedure. Informed consent was taken from both cases and controls before collection. Serum was separated after centrifuging at 3000 rpm for 15 min. Lipoprotein (a) and lipid parameters were analyzed in Hitachi-912. Fasting blood sugar was estimated using Hitachi-902 using Roche/Hitachi analyzer kits. Samples were analyzed within 2 h after collection. Internal quality controls were run on routine basis.

Serum total cholesterol (TC) was measured by CHOD-PAP methods at 505 nm using Roche/Hitachi analyzer kit (reportable range 3–800 mg/dl) Cat No. 1489232. Serum triglycerides (TG) by GPO-PAP, Roche/Hitachi analyzer kit. Cat. No. 1488872 at wavelength 505 with measuring range 4–1000 mg/dl.

HDL cholesterol (HDL-c) by HDL-c plus 2nd generation HDL-c, no pretreatment, Roche/Hitachi analyzer kit Cat. No. 03030024, measuring range 3–120 mg/dl.

Lipoprotein (a) [Lp(a)] by Roche/Hitachi analyzer kit Cat. No. 1660390. Immunoturbidometric assay for the quantitative *in vitro* determination of Lp(a) in human serum and plasma on automated clinical chemistry analyzer.

Lp(a) antigen + Anti Lp(a) antibody → antigen/antibody complex → turbidimetric measurement after agglutination.

R2 anti lipoprotein (a) antibody

Anti human lipoprotein (a) antibodies (rabbit): dependent on titre; NaCl: 100 mmol/l stabilizer, preservative.

Three calibrator: human lipoprotein (a).

Sample volume: 12 µl; measured at wavelength 340 nm, reportable range: 6–160 mg/dl. Sample with higher and lower concentrations was determined via the rerun function.

Low density lipoprotein-cholesterol (LDL-c) was calculated using Friedwald formula after considering its limitations.

$LDLc = TC - (HDLc + TG/5)$.

RESULTS

Our study includes 21 cases of thrombotic stroke patient's confirmed by CT scan and clinical findings. The age of patients ranged from 35 years to 82 years. There were 12 males and 9 females. Some of the patients had associated diseases like diabetes ($n = 8$), hypertension ($n = 9$), both diabetes and hypertension ($n = 5$), and few had family history of stroke ($n = 5$). Eighteen controls were included in our study ($n = 18$) (males 11 and females 7); of these three were diabetic and none were known to be hypertensive. Data was analyzed by SPSS software program, Students *t*-test was used for group statistics to find mean, standard deviation (SD), and standard error of mean (SEM). Independent samples test was used to find the *P*-value, and *P*-value <0.05 is considered to be significant. These statistical data were used to find the association of serum lipoprotein (a) [Lp(a)], total cholesterol (TC), HDL-cholesterol (HDL-c), triglycerides (TG), LDL-cholesterol (LDL-c), in thrombotic stroke patients compared to normal subjects.

Lipid profiles and Lp(a) levels in thrombotic stroke cases versus controls [Table 1].

Mean TC values of the controls (198 mg/dl) versus cases (191 mg/dl) did not show significant differences. However, we were surprised to note that mean TC values in cases are lesser than controls. HDLc (39.66 mg/dl) were lower in cases when compared to controls (44.22). TG values were slightly elevated in cases (controls 118.11 mg/dl versus cases 146.09 mg/dl), but statistically not significant.

Table 1: Lipid profiles and Lp(a) levels in cases vs controls

Parameter concentration (mg/dl)	Cases ($n = 21$), range mean \pm SEM (\pm SD)	Controls ($n = 18$) range Mean \pm SEM (\pm SD)	<i>P</i> value
TC	132–271 191.19 \pm 7.89 (\pm 36.17)	142–256 198.16 \pm 7.39 (\pm 31.36)	NS
TG	84–293 146.09 \pm 12.29 (\pm 56.35)	41–215 118.11 \pm 11.78 (\pm 50.00)	NS
HDLc	26–57 39.66 \pm 12.29 (\pm 8.84)	25–67 44.22 \pm 11.78 (\pm 10.89)	NS
LDLc	72–170 122.19 \pm 5.70 (\pm 26.30)	77–178 130.22 \pm 7.16 (\pm 30.41)	NS
TC/HC	4.94 \pm 0.20 (\pm 0.91)	4.78 \pm 0.36 (\pm 1.52)	NS
Lp(a)	0–151 44.23 \pm 9.65 (\pm 44.23)	3–137 40.44 \pm 9.80 (\pm 41.43)	NS
Lp(a) geometric mean	27.43 \pm 1.32 (3.26)	22.59 \pm 1.31 (3.39)	

NS: Not significant; TC, Total cholesterol; TG, Triglycerides; HDLc, HDL cholesterol; LDLc, LDL cholesterol

Serum Lp(a) values of controls (3–137 mg/dl) and stroke cases (0–151 mg/dl) varies over a wide range as seen in most of the studies. In our study 45%–50% of stroke cases had Lp(a) levels in the range 0–20 mg/dl. The values we have obtained may represent their basal lp(a) levels and, moreover, we have collected the blood samples of the patients, 21 days after the incidence of stroke. We observed a positive skewness.

Since frequency distribution is positively skewed; the mean value differ markedly from the geometric mean. Geometric mean of Lp(a) (22.59 mg/dl in controls versus 27.43 mg/dl in cases) was 21% higher in the cases of stroke patients. This difference between stroke cases versus controls is not statistically significant.

When compared to earlier studies where incidence of stroke was more frequent in elderly age group in western population, we have also got similar findings in our study. Among this group of stroke victims, only 9.5% were aged less than 50 years. Highest were found in 72–80 years age group.

In this study, we found no significant statistical differences in serum lipid and lipoprotein (a) profile between controls and thrombotic stroke patients.

DISCUSSION

Stroke is the manifestation of infarction of the brain, and is the third leading cause of death in both developed and developing countries, affecting the people in their “Golden years of life.”^[2]

Stroke is classified into hemorrhagic type and thrombotic type. Among these types, thrombotic stroke is more frequent

(around 80%). Prevalence of stroke in Indian population is around 0.5 per 1000 population as reported by Dallal.^[19]

Various risk factors known to cause coronary artery diseases are also known to cause stroke. Among them, dyslipidemia is a well-established risk factor for atherosclerotic coronary artery disease but its relationship to ischemic cerebrovascular disease has remained unclear, perhaps due to heterogeneous nature of stroke etiology. Studies done before the availability of CT scan were likely to include patients with cerebral hemorrhage as well as infarction together. However, now even with the availability of CT scan facility it is often difficult to differentiate between cardioembolic and atherothrombotic stroke due to heterogeneous nature of stroke. Even though the risk factor for cardioembolic stroke may resemble those for atherothrombotic stroke, the two processes are not identical.^[20-22]

Prevention of cerebrovascular disease should be the goal because, there is no treatment available to reverse the damages occurring after attack. This may be effectively accomplished by identifying and modifying any risk factors present that are known to be associated with atherosclerosis; thereby we can decrease the stroke rates.

Dyslipoproteinemia is well established as being associated with the genesis of ischemic heart disease, but it is not been conclusively demonstrated to be associated with the pathogenesis of atherothrombotic stroke or transient ischemic attack (TIA). The failure to establish an association appears to have been the result of methodological flaws and a shift in the principal cause of stroke from hemorrhage to thrombotic with improved detection and treatment of hypertension.^[21]

Some of the earlier studies have found TC and TG levels to be elevated in patients with stroke.^[16] Our study could not find any significant differences between cases and controls except HDLc and TG. HDLc was slightly lower in cases compared to controls and TG levels were slightly elevated; 30% higher (with P -value = 0.1) in the stroke patients.^[20] However, to our surprise, the TC values were slightly lower in case compared to control group, but to what extent hemiplegic condition of patients as well as their reduced food intake and lifestyle modification have affected the lipid profile could not be assessed.

Studies indicate lipoprotein (a) is associated with the development of ischemic heart disease and cerebrovascular disease. But studies by Hachinski *et al.*, suggested that cerebrovascular disease is associated with higher Lp(a) levels in patients.^[20] However, the difference was not significant

because of the wide variability and skewness in the general population. Because of this skewness, geometric mean was considered, and we observed the geometric mean of Lp(a) in cases was slightly higher in cases than in controls (27.43 mg/dl in cases versus 22.59 mg/dl in controls with the median value of 22 in case versus 20 in controls), but for the arithmetic mean we could not find significant differences between cases and controls. Several observations on Lp(a) indicated that it may have antithrombotic activity and an elevated level of Lp(a) may contribute to thromboembolic conditions. Apolipoprotein (a); having sequence homology with plasminogen, may compete with plasminogen in binding to fibrin and consequently slowdown fibrinolysis.

It is found that Lp(a) levels are constant over time, appear to be genetically determined and associated with the presence of atherosclerotic disease this has led many investigators to consider Lp(a) as an independent risk factor for occlusive vascular disease.^[21] Slunga *et al.*, have observed that Lp(a) levels are transiently elevated after an acute myocardial infarction and return to base line within 30 days and, therefore, reflects long term Lp(a) levels.^[21,22] To overcome this acute phase response, we collected the blood sample from patients with a minimum gap of 3 weeks to 6 months after the occurrence of stroke. Therefore, the serum Lp(a) levels estimated in our study reflect the basal Lp(a) levels in both patients and controls and whatever the difference observed could be attributed to their base line level.

It has been suggested that Lp(a) may only be a significant marker for risk of coronary disease among hyperlipidemic subjects.^[20] However, unlike in myocardial infarction, lipid markers such as LDLc and HDLc have proven far less useful in identifying subjects at risk for stroke.^[22]

Lipoprotein (a) has attracted the attention as a possible risk factor of stroke due to its association with the atherosclerosis incidence. Several but not all cross-sectional and retrospective studies have reported association of Lp(a) with atherosclerosis. However, retrospective studies are susceptible to selection and observation bias and many exclude the possibility that the presence of disease may influence levels, an important consideration as Lp(a) concentrations have been reported to change following acute ischemia and cerebral infarction. Moreover, Lp(a) concentrations may have different predictive values in different populations.^[21]

We believe that Lp(a) levels estimated in the current study reflect the baseline levels and is not subjected to any variation due to the storage of samples. The current

findings suggest that Lp(a) is unlikely to be an important risk factor for stroke. This conclusion should not be construed to imply that no association between haemostatic and thrombotic markers and the risk of future stroke.

Moreover some of the prospective studies demonstrate Lp(a) assessment has a very low predictive value in terms of screening for underlying atherosclerotic disease.^[22] This observation is consistent with our findings.

Because the distribution of Lp(a) is markedly skewed in most populations and any postulated association between Lp(a) and vascular risk appears to be related to individuals with very high levels and great majority of vascular occlusive events will occur among subjects with normal Lp(a) levels. Thus, from the perspective of clinicians considering whether or not to screen, the totality of current evidence suggests that any true increase in risk of future thrombosis associated with Lp(a) is likely to be of small absolute magnitude. Further, the cost of estimation is quite high and currently there are no proper prophylactic measures to modify Lp(a) levels. These considerations weigh against the use of Lp(a) as a risk factor for stroke.

In this study the serum Lp(a) and other lipid parameters did not show any significant difference between thrombotic stroke cases and controls except HDLc, which was slightly lower in cases compared to controls and TG levels were slightly elevated; 30% higher in cases. About 45%–50% of the cases had Lp(a) levels in the range 0–20 mg/dl and 9.5% of cases were aged less than 50 years.

REFERENCES

1. Aho K, Harmsen P, Hatano S, Marquardsen J, Smirnov VE, Strasser T. Cerebro vascular disease in the community: Results of a WHO collaborative study. *Bull World Health Organ* 1980;58:113-30.
2. Smith WS, Hauser SL, Easton JD. Ischemic Stroke. In: Braunwald E, editor. *Harrison's Principles of internal medicine*. 15th ed. New York: Mc Graw Hill Medical publishing division; 2001. p. 2369.
3. Fieschi C, Argentino C, Rasura M. Italian study of reversible ischemic attacks. Report of a meeting in Rome, Oct. 14-16, 1980. *Stroke* 1981;12:293-5.
4. Von Arbin M, Britton M, De Faire U, Tisell A. Circulatory Manifestations and Risk Factors in Patients with Acute Cerebrovascular Disease and in Matched Controls. *Acta Med Scand* 1985;218:373-80.
5. Pessah-Rasmussen H, Engstrom G, Jerntorp I, Janzon L. Increasing stroke incidence and decreasing case fatality, 1989- 1998: A study from the stroke register in Malmo, Sweden. *Stroke* 2003;34:913-8.
6. Community prevention and control of cardiovascular diseases. Report of a WHO Expert Committee. *World Health Organ Tech Rep Ser* 1986;732:1-62.
7. Lippi G, Guidi G. Lipoprotein (a): From ancestral benefit to modern pathogen? *QJM* 2000;93:75-84.
8. Trommsdorff M, Köchl S, Lingenhel A, Kronenberg F, Delport R, Vermaak H, *et al.* A pentanucleotide repeat polymorphism in the 5' control region of the apolipoprotein (a) gene is associated with lipoprotein (a) plasma concentrations in Caucasians. *J Clin Invest* 1995;96:150-7.
9. Lippi G, Guidi G. Standardization and clinical management of lipoprotein (a) measurements. *Clin Chem Lab Med* 1998;36:5-16.
10. Mclean JW, Tomlinson JE, Kuang WJ, Eaton DL, Chen EY, Fless GM, *et al.* cDNA sequence of human apolipoprotein (a) is homologous to plasminogen. *Nature* 1987;330:132-7.
11. Milionis HJ, Winder AF, Mikhailidis DP. Lipoprotein (a) and stroke. *J Clin Pathol* 2000;53:487-96.
12. Utermann G, Hoppichler F, Dieplinger H, Seed M, Thompson G, Boerwinkle E. Defects in the low density lipoprotein receptor gene affect lipoprotein (a) levels: Multiplicative interaction of two gene loci associated with premature atherosclerosis. *Proc Natl Acad Sci U S A* 1989;86:4171-4.
13. Rath M, Niendorf A, Reblin T, Dietel M, Krebber HJ, Belslegel U. Detection and quantification of lipoprotein (a) in the arterial wall of 107 coronary bypass patients. *Arterioscler Thromb Vasc Biol* 1989;9:579-92.
14. Aznar J, Estellés A, Bretó M, España F. Euglobin clot lysis induced by tissue type plasminogen activator in subjects with increased levels and different isoforms of lipoprotein (a). *Thromb Res* 1993;72:459-65.
15. Edelberg JM, Pizzo SV. The inhibition of tissue type plasminogen activator inhibitor- 1. The effects of Fibrinogen, Heparin, Vitronectin and lipoprotein (a). *J Biol Chem* 1991;266:7488- 93.
16. Buechler C, Ullrich H, Ritter M, Porsch-Oezcuereomez M, Lackner KJ, Barlage S, *et al.* Lipoprotein (a) up- regulates the expression of the plasminogen activator inhibitor 2 in human blood monocytes. *Blood* 2001;97:981-6.
17. Caplice NM, Panetta C, Peterson TE, Kleppe LS, Mueske CS, Kostner GM. Lipoprotein (a) binds and inactivates tissue factor pathway inhibitor: A novel link between lipoproteins and thrombosis. *Blood* 2001;98:2980-7.
18. Gaigner DJ, Kirchenlohr HL, Metcalfe JC, Weissberg PI, Wade DP, Lawn RM. Proliferation of human smooth muscle cells promoted by lipoprotein (a). *Science* 1993;260:1655-8.
19. Dalal P. Strokes (CVD) in India: Session 1- 9: The first Asian pacific symposium on Stroke: Session I, Panel discussion present status of stroke problems in each country. *Jpn Circ J* 1982;46:621-4.
20. Hachinski V, Graffagnino C, Beaudry M, Bernier G, Buck C, Donner A, *et al.* Lipids and stroke: A paradox resolved. *Arch Neurol* 1996;53:303-8.
21. Slunga L, Johnson O, Dahlen GH, Eriksson S. Lipoprotein (a) and acute-phase proteins in acute myocardial infarction. *Scand J Clin Lab Invest* 1992;52:95-101.
22. Ridker PM, Stampfer MJ, Hennekens CH. Plasma concentration of lipoprotein (a) and the risk of future stroke. *JAMA* 1995;273:1269-73.

How to cite this article: Nagaraj SK, Pai P, Bhat G, Hemalatha A. Lipoprotein (a) and other lipid profile in patients with thrombotic stroke: Is it a reliable marker?. *J Lab Physicians* 2011;3:28-32.

Source of Support: Grant from ICMR Project number – 5/4/1-5/98 NCD - 11. **Conflict of Interest:** None declared.