ORIGINAL RESEARCH PAPER

Biochemistry

LIPOPROTEIN(A) LEVELS IN STROKE CASES OF TRIPURA, NORTHEAST INDIA.

KEY WORDS: Lipoprotein (a), stroke, risk factor.

Tapan Debnath	MBBS, MD, Associate Professor, Department of Biochemistry, Tripura Medical College and Dr. BRAM Teaching Hospital, Agartala, Tripura.
Arpita Das	MBBS, MD, Assistant Professor, Department of Biochemistry, Tripura Medical College and Dr. BRAM Teaching Hospital, Agartala, Tripura.
Elvia Jamatia*	MBBS, MD, Senior Resident, Department of Biochemistry, Tripura Medical College and Dr. BRAM Teaching Hospital, Agartala, Tripura. *Corresponding Author
Avik Chakraborty	MBBS, MD, Professor, Department of Medicine, Tripura Medical College and Dr. BRAM Teaching Hospital, Agartala, Tripura.
Sankar Roy	MBBS, MD, Professor, Department of Biochemistry, Tripura Medical College and Dr. BRAM Teaching Hospital, Agartala, Tripura.

ABSTRACT

Owing to its structural similarity with plasminogen, lipoprotein (a) is a thrombogenic and atherogenic molecule. Several case-control and prospective studies done on Indian subjects have shown lipoprotein (a) as an independent risk factor for stroke. Due to lack of data from the north eastern part of the country, this case control study was done with the aim of finding an association and also to estimate the serum levels of lipoprotein (a) in patients with acute stroke. Lp(a) was quantified by immunoturbidiometric method. The serum lipoprotein (a) levels were significantly elevated ($p< 0.0001$) in cases (57.01+5.99 mg/dl) as compared to controls(17.75 + 6.61mg/dl). Elevated Lp(a) is a risk factor for stroke and might play an important role in the genesis of cerebrovascular thrombosis. It may be appropriate to assess lipoprotein(a) levels in Indians along with routine lipid profile testing in subjects with other risk factors.

INTRODUCTION:

Lipoprotein (a) [Lp(a)], first described by Berg in 1963, is a cholesterol– rich lipoprotein particle composed of a low density lipoprotein (LDL) particle and a large glycoprotein-apolipoprotein(a). Elevated Lp(a) level is considered to be an independent risk factor for cardiovascular diseases (CVD) shown by multiple clinical trials and meta-analysis done during last two decades. [1]

The pathogenicity of Lp(a) is owing to its structure, which is similar to plasminogen and tPA (tissue plasminogen activator) and it competes with plasminogen for its binding site, leading to reduced fibrinolysis.[2] Lp(a) also carries cholesterol and thus contributes to atherosclerosis.In addition, Lp(a) transports the more atherogenic proinflammatory oxidized phospolipids which attract inflammatory cells to vessel walls and leads to smooth muscle cell proliferation. [3] It has been shown that Lp(a) is ten-times more atherogenic than LDL-C [4], thus predisposing to early atherosclerosis and thrombosis.

Hence, elevated Lp(a) levels (≥50 mg/dl according to the 2018 National Heart, Lung, and Blood Institute (NHLBI) report [5], also known as hyperlipoproteinemia(a), is a highly prevalent genetic risk factor for CVD. Indians have a high prevalence of approximately 25%, which surpasses the prevalence of diabetes (8.5% national prevalence) by 2–3 times. [6] It has been suggested that these high levels of Lp(a) has an important and independent genetic propensity for atherogenetic and thrombotic events as compared to people of European descent. [7]

The association of high plasma levels of Lp(a) with increased risk of stroke has been a focus of research for the past few years. It has been found that elevated Lp(a) is an independent risk factor for stroke, especially among young stroke patients. [8] This association of high Lp(a) levels with stroke in have been found by several Indian case control studies. [9,10,11] However, there is no such study that have shown the association of Lp (a) with stroke in the North East Indian population.

With this background, this hospital based, case control study was designed to a) determine the association of Lp(a) and b) estimate the serum levels of Lp(a) level among the patients of acute stroke in Agartala, Tripura.

MATERIALS & METHODS:

Selection of subjects and controls:

The study was conducted in the Department of Medicine and the Department of Biochemistry, Tripura Medical College and Dr.BRAM Teaching Hospital, Agartala. The study was approved by the Institutional Ethics Committee vide no. F.3 (P0-75)/IEC/SFTMC/ 2010 – 11/16805 - 16822. Forty nine cases of acute stroke were selected from the patients admitted in the medicine ward from April 2018 to March 2020. Complete history about the course of disease, family history, laboratory findings and clinical data were collected from the patients, after informed consent. The absence of a history of smoking, previous cerebro-vascular disease and age over 35 years old were the inclusion criteria. Patients below the age of 35 years and those with subarachnoid hemorrhage, transient ischemic attacks and those on statin group of drugs or any other medication known to modify lipoproteins were excluded from the study. Forty nine age and sex matched healthy controls were selected from the healthy relatives of the patients admitted in the medical wards and also the willing medical, paramedical staff of the hospital.

Fasting venous blood samples were collected for Lp(a) level, lipid profile and routine investigations. Blood was centrifuged at 3000 rpm for 10 minutes and the serum stored at -20°c until analysis.

Lipoprotein (a) estimation: Turbidimetric immunoassay for estimation of Lp(a) in human serum by Quantia-Lp(a) kit provided by Coral Clinical Systems (Goa, India) that is based on the principle of agglutination reaction was used. The normal reference range of Lp(a) was taken as ≤ 30 mg/dl.

Lipid Profile: Serum total cholesterol, triglyceride , high density lipoprotein cholesterol (HDL-c) and were determined by enzymatic methods adapted for Beckman Coultier , USA.

LDL-c was calculated using Friedewald formula.

Statistical Analysis: All statistical analysis was done using SPSS statistical software. Values are expressed as Mean ± Standard deviation. Chi square test was used to see if there is any difference in the gender distribution in the two groups. Student's t test (for parametric data) and Mann Whitney U test (for non-parametric data) was used to analyze the differences between the cases and control groups. A p-value < 0.05 were considered significant.

RESULTS:

Demographic Profile: The demographic profile of stroke patients and control subjects have been presented in Table 1. The mean age of the stroke patients was 61 years with the youngest case of 40 years and the oldest patient of 70 years. 46.94% of the cases and 44.89 % of the controls were women. There was no difference between the age and gender distribution between the two groups (p value >0.05). Out of the cases, 20% had type 2 Diabetes mellitus and 14.28 % had hypertension as comorbidities.

Serum Lp(a) levels: Serum Lp(a) levels were 17.75 ± 6.61mg/dl in the control group and 57.01±5.99 mg/dl in the cases group. There is significant difference between the two groups. (p< 0.0001). Elevated lipoprotein (a) levels were found in 85.71% cases and in only one control. Among the cases, mean levels of serum Lp(a) in females were 61.76 ± 15.93 mg/ dL and in males were 52.26 ± 12.05 mg/ dL, which is statistically significant (p value<0.05)

Serum Lipid profile: The serum cholesterol, serum triglyceride and LDL-c levels were higher and HDL-c levels were lower in cases as compared to control group, which is a statistically significant. We did not observe a significant correlation between Lp(a) with high LDL-c and cholesterol levels in the cases.

Table.1 Demographic data of stroke patients and controls

	Cases	Control
No.	49	49
Age (Mean)	61	59
Male/Female	26/23	27/22
T2DM	10	-
Hypertension	07	-

Table. 2 Lipoprotein (a) and lipid profile in stroke patients and controls.

	Cases	Controls.	P value
LP(a) (mg/ dL)	57.01 ± 15.99	17.75±11.61	< 0.0001
Total Cholesterol (mg/ dL)	214.7±50.02	165±35.29	< 0.0001
LDL-Cholesterol (mg/ dL)	141.77±39.96	103.9±31.59	< 0.0001
HDL-Cholesterol (mg/ dL)	41.82±6.76	52.92±7.72	< 0.0001
Triglyceride (mg/ dL)	222.67±125	114.45±17.6	< 0.0001

DISCUSSION:

Lp(a) is an independent genetic risk factor in patients of stroke. South Asian population have comparatively higher incidence of stroke compared to white population.[6] Studies conducted to show the association of Lp(a) with stroke, collectively emphasize that Lp(a) is an important but overlooked source of premature stroke and advanced atherosclerosis. [9,11,12] In our study also Lp(a) levels were significantly elevated in stroke patients as compared to healthy controls. The elevelated Lp(a) levels might have been the cause of acute stroke in the patients.

In other Indian studies quoted here, the Lp(a) levels were elevated and seen as a risk factor in young patients of stroke, whereas in our study we found that our stroke patients were of the mean age of 61 years .This might be because of ethnic and geographical variations of the north eastern population that lesser young people suffer from stroke in this region of the country.

We also found that Lp(a) levels were significantly higher in female cases as compared to their male counterparts. This finding is peculiar in our study, as it has been reported that incidence of athero-thrombotic cerebrovascular events are about 30% higher in men as compared to women. [13]. Also in a study by Abraham A. Ariyo, it was shown that elevated Lp (a) is an independent predictor of stroke, death from vascular disease and death from any cause among older men in United states but not in women [14].

However, the limitation of small sample size should be taken into account. More prospective studies in wider population need to be done to confirm the validity of the findings.

CONCLUSION:

An elevated Lp(a) level along with South Asian type of dyslipidemia has an added detrimental effect on Indians, predisposing them to thrombotic cerebro-vascular occlusion and the occurrence of stroke. The price of Lp(a) test is almost same as that of lipid profile testing. Hence it may be logical to measure Lp(a) levels in Indians along with routine lipid profile testing in subjects with other risk factors.

REFERENCES:

1) Nordestgaard, B. G., Chapman, M. J., Ray, K., Borén, J., Andreotti, F., Watts, G. F., ... & Tybjærg-Hansen, A. (2010). Lipoprotein (a) as a cardiovascular risk factor: current status. *European heart journal*, *31*(23), 2844-2853.
2) Stein JH, Rosenson RS. Lipoprotein Lp(a) excess and coronary heart disease. Arch Intern Med 1997; 157: 1170-6.
3) Jenner JL, Ordovas I, Lamon-Fava S, et al. Effects of age, sex and menopausal status on plasma lipoprotein(a) levels: The Framingham Offspring Study. Circulation 1993; 87: 1135-41.
4) Lawn RM. Lipoprotein (a) in heart disease. Sci Am 1992; 266 (6): 54-60.
5) Tsimikas, S., Fazio, S., Ferdinand, K. C., Ginsberg, H. N., Koschinsky, M. L., Marcovina, S. M., Moriarty, P. M., Rader, D. J., Remaley, A. T., Reyes-Soffer, G., Santos, R. D., Thanassoulis, G., Witztum, J. L., Danthi, S., Olive, M., & Liu, L. (2018). NHLBI Working Group Recommendations to Reduce Lipoprotein(a)-Mediated Risk of Cardiovascular Disease and Aortic Stenosis. *Journal of the American College of Cardiology*, *71*(2), 177–192. https://doi.org/10.1016/j.jacc.2017.11.014
6) Enas, E. A., Varkey, B., Dharmarajan, T. S., Pare, G., & Bahl, V. K. (2019). Lipoprotein(a): An underrecognized genetic risk factor for malignant coronary artery disease in young Indians. *Indian heart journal*, *71*(3), 184–198. https://doi.org/10.1016/j.ihj.2019.04.007
7) Bhatnagar D, Anand I, Durington PN et al. Coronary risk factors in people from Indian subcontinent in West London and their siblings in India. Lancet 1995; 345: 405-9.
8) Nave, A. H., Lange, K. S., Leonards, C. O., Siegerink, B., Doehner, W., Landmesser, U., ... & Ebinger, M. (2015). Lipoprotein (a) as a risk factor for ischemic stroke: a meta-analysis. *Atherosclerosis*, *242*(2), 496-503.
9) Christopher, R., Kailasanatha, K. M., Nagaraja, D., & Tripathi, M. (1996). Case-control study of serum lipoprotein (a) and apolipoproteins A-I and B in stroke in the young. *Acta neurologica scandinavica*, *94*(2), 127-130.
10) Sharobeem, K. M., Patel, J. V., Ritch, A. E. S., Lip, G. Y. H., Gill, P. S., & Hughes, E. A. (2007). Elevated lipoprotein (a) and apolipoprotein B to AI ratio in South Asian patients with ischaemic stroke. *International journal of clinical practice*, *61*(11), 1824-1828.
11) Dhamija, R. K., Arora, S., Gaba, P., & Bhattacharjee, J. (2007). Role of Lipoprotein (a)-a genetic risk factor in patients of acute Ischaemic stroke: a case-control study in North Indian Urban population. *Case Rep Clin Pract Rev*, *8*, 112-117.
12) Chakraborty, B., Vishnoi, G., Goswami, B., Gowda, S. H., Chowdhury, D., & Agarwal, S. (2013). Lipoprotein (a), ferritin, and albumin in acute phase reaction predicts severity and mortality of acute ischemic stroke in North Indian patients. *Journal of Stroke and Cerebrovascular Diseases*, *22*(7), e159-e167.
13) Kurtzke, J. F., & Kurland, L. T. (1980). Epidemiology of Cerebrovascular Disease, Cerebrovascular Survey Report. *1985*, 1-34.
14) Ariyo, A. A., Thach, C., & Tracy, R. (2003). Lp (a) lipoprotein, vascular disease, and mortality in the elderly. *New England Journal of Medicine*, *349*(22), 2108-2115.