A CLINICAL STUDY OF ISCHAEMIC STROKE WITH REFERENCE TO LIPOPROTEIN (a), LDL AND HDL CHOLESTEROL AS RISK FACTOR

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ABSTRACT: AIM: Lipoprotein (a) and other lipid sub-fractions are known to be associated in atherosclerosis of the carotid vessels and ischemic stroke. This study was conducted to compare the specificity of variables like lipoprotein (a), HDL and LDL as independent risk factor in determining the severity and outcome of ischemic stroke.**METHODS:** The data for this study was collected from 50 patients of ischemic stroke who presented to KIMS hospital from June 2012 to 2013. All of who underwent clinical assessment by NIHSS score with relevant investigations, including CT scan brain, lipoprotein (a) and lipid profile.**RESULTS:** Male patients had higher incidence than females. Female population had higher BMI. Stroke scoring was done using NIHSS scoring;severe cases of stroke remain equal in both the sexes. The conventional risk factors were studied and were associated with severity of stroke but statistically not significant. Various lipid subfractions were studied with respect to severity of stroke and it was found that TC had p value=0.146, HDL-C p value=0.686, LDL-C p value =0.296, VLDL-C p=0.54, TG p=0.721 and Lp (a) p<0.001.Patient with LDL-C>150mg/dl were 2.79 times more likely to have NIHSS score>15 and patients with HDL-C<40mg/dl were 2.23 times more likely to have NIHSS score >15. Low HDL-C was the only lipid abnormality seen in patients with no conventional risk factors. **CONCLUSION:**Ischemic stroke is associated with lipid abnormalities. Lp (a) which is genetically determined factor and HDL-C were found to be independent risk factors for predicting the outcome of ischemic stroke. Conventional risk factors were found to be less reliable.

INTRODUCTION: The incidence of stroke in Indian population is difficult to estimate due to lack of accurate data. The available statistics are from urban Teaching Hospitals but these are not truly representative of the whole population as 85% of the population reside in the rural areas. According to such studies stroke constitutes about 0.9-4.5% of total hospital admissions. Ischemic strokes constitute about 57.3-82.7% of all strokes[1]. Cardiogenic cerebral embolism forms 15-20% of all ischemic strokes though in the younger population group <45 years it is responsible for 23-36% of strokes. Lacunar infarcts constitute about 15-20% of all ischemic strokes.[2] Once stroke has occurred, the management strategies are limited and the disability it leaves behind is often devastating. With advances in our knowledge of risk factors andprecipitating events the emphasis has now shifted to stroke prevention. Like in coronary artery disease, primary prevention can go a long way in preventing stroke.

The National Institute of Health Stroke Scores (NIHSS) is a 42 point clinical examination system that has become the standard clinical severity scale in most clinical trials.[4]The scale is as follows:

NIHSS Stroke Scale

	Neurological Examination	Score
1.	A. Level of consciousness (alert to coma)	0-3
	B. Month/age	0-2
	C.Commands eye open/closed	0-2
2.	Best gaze	0-2
3.	Visual	0-3
4.	Facial palsy	0-3
5/6.	Best motor arm/leg (right/left)	0-4
7.	Limb ataxia	0-2
8.	Sensory	0-2
9.	Best language	0-3
10.	Dysarthria	0-2
11.	Neglect	0-2

SCORING[5]

Normal	0-1
Minor stroke	1-5
Moderate stroke	-14
Moderate/severe stroke	15-20
Severe stroke	>20

LIPOPROTEIN (a): In 1963, Kare Berg discovered a new antigen lipoprotein (a) while working with rabbits immunized with human low density lipoprotein (LDL). He found out that the level of this protein was genetically determined by an autosomal dominant mode of inheritance.

Lipoprotein (a): The Lp (a) molecule is formed by the assembly of at least two major proteins, a molecule of apo B 100 covalently linked to a molecule of apolipoprotein (a) by a single disulfide bond. It is structurally very similar to low density lipoprotein (LDL) in protein and lipid composition, the essential difference between the two being apolipoprotein (a).[6]Lp (a) in appropriate levels, competes for some physiologic functions of plasminogen in the coagulation and fibrinolytic cascade and may thus be thrombogenic.[6]

There is a marked variation in the serum levels of Lp (a). The blood levels fluctuate from a minimum of <0.1mg/dl, to a maximum of>150mg/dl, the normal levels being 0-30mg/dl.[6]

Low Density Lipoproteins: LDLs are the major cholesterol carrying lipoproteins in the plasma. LDLs are composed of 75% lipid and 25% protein. Apo-B100 is the principal protein in these particles, along with trace amounts of apo-E.

LDLs are the end products of lipase-mediated hydrolysis of VLDLs. As the triglyceride-rich core of the larger VLDL particles are removed, the surface lipids and proteins are remodeled and

excess surface constituents are transferred to HDL, resulting in the formation of a small, cholesterol-rich LDL devoid of all apolipoproteins except apo-B100.

High Density Lipoproteins: HDLs are small particles that float at densities of 1.063 to 1.21 g/ml. They are subdivided into two classes, HDL2 and HDL3. HDLs contain about 50% lipids and 50% protein. Their major proteins are apo-AI, apo-AII, and smaller amounts of C apolipoproteins and apo-E. Apo-E is a minor component of a subclass of HDL referred to as HDL1.

HDLs have an important role in the process of reverse cholesterol transport. HDLs acquire cholesterol from cells and transport it to liver or other cells that require cholesterol. HDL_3 particles are converted to HDL_2 and then to HDL_1. Apo-E, which is found on HDL_1, targets this subclass to cells expressing the LDL receptor. A second pathway involves CETP, which transfers cholesterol ester from HDL_2 to VLDL, IDL, LDL and remnants.

High levels of HDL are associated with a lower incidence of CHD, and vice versa. High HDL levels promote redistribution of cholesterol away from the artery wall.

METHODOLOGY: The data for this study was collected from 50 patients who presented to Kempegowda Institute of Medical Sciences, Bangalore, on inpatient basis who were proven cases of ischemic stroke and who satisfied inclusion and exclusion criteria.

Method of collection of data was by patient evaluation, which was done by taking detailed history, clinical examination and laboratory investigations through a proforma specially designed for this study.

Inclusion criteria: All patients above the age of 18 years who presented to KIMS hospital with acute neurological deficits lasting for more than 24 hours with either a hypodense lesion on CT scan, or a normal CT scan, Patients with previous history of stroke, who presented with sudden worsening of their neurological deficits, or development of new deficits.

Exclusion criteria: People with stroke whose CT scan showed evidence of hemorrhage or cortical venous thrombosis.

Sample size: 50 cases.

Study duration: June 2012 to June 2013.

Parameters be evaluated in this study were stroke severity using National Institute of Health Stroke Scale, Lipoprotein (a) level, LDL level and HDL level.

The Procedural Details for Lipid Profile and Lipoprotein (a) Assay

Lipid Profile and Analysis: This was performed 72 hours after the onset of cerebrovascular accident and a fasting serum sample was collected. Total cholesterol, HDL-C, LDL-C, VLDL-C and Triglyceride were estimated.

Lipoprotein (a) Analysis: End point immunoturbidimetric immunoassay was used for the quantitative determination of the lipoprotein (a) in human serum.

Three different reagents were used:

a) Lipoprotein (a) latex: 5% suspension of polystyrene latex particles sensitized with lipoprotein (a) antibodies contains 0.095% sodium azide.
b) Lipoprotein (a) buffer: 0.9% sodium chloride solution with detergent contains 0.095 % sodium azide.
c) Lipoprotein (a) standard high 68mg/dl. It's defibrinated and depilated human plasma. The plasma is liquid stabilized. Preservative is 0.095% sodium azide.

- Additional regent used -0.9% sodium chloride.
  Normal value ofLp (a) is 0-30mg/dl.

Statistical Methods: Binomial test has been used to find the significance of proportion of risk factors, Lipoprotein (a) in relation to NIHSS and analysis of variance has been used to find significant change of Lipoprotein (a) and Lipid parameters in relation to NIHSS. Pearson correlation has been used to find the relationship of NIHSS with age, BMI, HDL, LDL and Lp (a). Student t test has been used to find the significance of correlation co-efficient. Following tests were used for analysis:

1. Z-test for a proportion (Binomial distribution) [7]
2. Analysis of Variance: F test for K Population means
3. Pearson Correlation Co-efficient and its Significance test[8]

RESULTS: A prospective clinical study consisting of 50 stroke patients was undertaken to investigate the relationship of NIHSS (Stroke Scoring System) with LDL-C, HDL-C, Lipoprotein (a) and risk factors for stroke.

Age and sex distribution:74% of the subjects (n=37) were male and 26% (n=13) were female. The highest incidence 44% of the subjects (n=22) were in the age group of 51-60 years.

Age in years	Male	Female	Total
≤ 40	-	1 (7.7%)	1 (2.0%)
41-50	7 (18.9%)	5 (38.5%)	12 (24.0%)
51-60	17 (45.9%)	5 (38.5%)	22 (44.0%)
61-70	10 (27.0%)	2 (15.4%)	12 (24.0%)
>70	3 (8.1%)	-	3 (6.0%)
Total	37 (100.0%)	13 (100.0%)	50

Table 1: Age distribution

Anthropometry: The mean age of male subjects (59.2 yrs.) was higher when compared to that of female subjects (53.8) yrs. The BMI was high in females (25.31) compared to males.

Anthropometry parameters (Mean ± SD)	Male (n=37)	Female (n=13)	Overall (n=50)
Age in years	59.24 ± 8.95	53.85 ± 7.60	57.84 ± 8.87
Weight in kg	63.86 ± 9.67	62.00 ± 8.91	63.38 ± 9.43

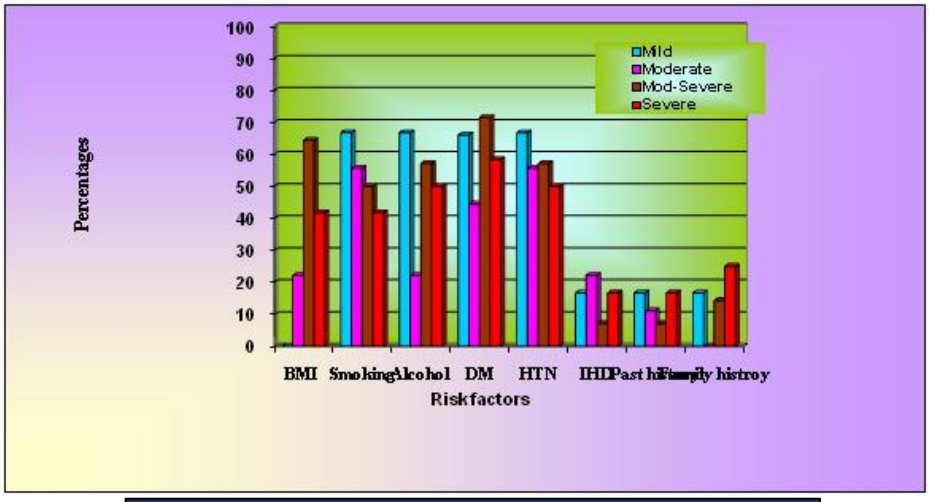
Height in meters	1.66 ± 0.08	1.56 ± 0.06	1.63 ± 0.08
BMI kg/m^2	23.38 ± 3.26	25.31 ± 2.59	23.88 ± 3.19

Table 2: Mean Pattern of Anthropometry parameters

Risk factors and NIHSS: The association of various risk factors of stroke and NIHSS score was done, such as BMI, smoking, alcohol, DM, HTN, IHD, past history and family history. No significant relation was seen with the risk factors and NIHSS score.

Risk factors	NIHSS				Total(n=50)
	Mild(n=6)	**Moderate (n=18)**	**Mod- severe (n=14)**	**Severe (n=12)**	
BMI >24 kg/m^2	-	4(22.2%)	9(64.3%)	5(41.7%)	18(36.0%)
Smoking +	4 (66.7%)	10(55.6%)	7(50.0%)	5(41.7%)	26(52.0%)
Alcohol	4 (66.7%)	4(22.2%)	8(57.1%)	6(50.0%)	22(44.0%)
DM +	4(66. %)	8(44.4%)	10(71.4%)	7(58.3%)	29(58.0%)
HTN +	4 (66.7%)	10(55.6%)	8(57.1%)	6(50.0%)	28(56.0%)
IHD +	1(16.7%)	4(22.2%)	1(7.1%)	2(16.7%)	8(16.0%)
Past History	1(16.7%)	2(11.1%)	1(7.1%)	2(16.7%)	6(12.0%)
Family History	1(16.7%)	-	2(14.3%)	3(25.0%)	6(12.0%)
Inference	Risk factors are not statistically associated with the NIHSS as per the normal Z test for proportion.				

Table 3: Association of NIHSS with Risk factors



Graph 3a: Association of NIHSS with Risk factors

NIHS: The severity of stroke was assessed with the NIHSS. According to scale a score of 1-5 was mild, 6-14 was moderate, 15-20 was moderate-severe and more that 20 was severe. In the study it was found that 18 subjects (36%) had moderate score.

NIHSS	Number	%
Mild (1-5)	6	12.0
Moderate (6-14)	18	36.0
Mod-Severe (15-20)	14	28.0
Severe (>20)	12	24.0
Total	50	100.0

Table 4: Frequency distribution of NIHSS[5]

In the study it was found that has the Lp (a) value increases the stroke score was high with a statistically significant p value (<0.001). No such significant correlation was seen with other lipid parameters.

Study Parameters Mean ± SD	NIHSS				p value
	Mild (1-5)	Moderate (6-14)	Mod-Severe (15-20)	Severe (>20)	
Lipoprotein (a)	14.73±5.50	27.81±10.87	45.33±9.23	60.17±23.51	p<0.001**
Total Cholesterol	178.50±48.47	204.06±27.69	215.14±31.06	213.42±37.05	0.146
HDL Cholesterol	31.67±8.17	28.83±2.83	29.43±5.91	28.83±5.37	0.686
LDL Cholesterol	112.17±45.35	124.22±40.13	145.00±30.02	137.00±47.09	0.296
VLDL	34.67±10.73	48.39±32.51	40.54±6.12	42.83±15.61	0.544
Triglycerides	174.17±53.28	192.50±81.56	207.57±37.84	195.33±45.99	0.721

Table 5: Mean pattern of Lp (a) and lipids in relation to NIHSS

p value obtained using ANOVA

Lipid parameters and NIHSS: A sub group analysis of various elevated lipid parameters with NIHSS score was done such as Total cholesterol, LDL, HDL and Triglycerides. It was found that subjects with LDL>150mg/dl are 2.79 times more likely to have NIHSS score>15 with a p value of 0.104 and subjects with HDL <40mg/dl are 2.23 times more likely to have NIHSS score>15with a p value of 0.164.

Lipid parameters	NIHSS				Total (n=50)
	Mild (n=6)	Moderate (n=18)	Mod-Severe (n=14)	Severe (n=12)	
Total cholesterol (>200 mg/dl)	2(6.9%)	11(37.9%)	8(27.6%)	8(27.6%)	29(100.0%)
HDL (<40 mg/dl)	2(7.1%)	9(32.1%)	8(28.6%)	9(32.1%)	28(100.0%)

LDL (>150 mg/dl)	1(6.3%)	4(25.0%)	7(43.8%)	4(25.0%)	16(100.0%)
Triglycerides (>160 mg/dl)	4(10.5%)	13(34.2%)	12(31.6%)	9(23.7%)	38(100.0%)
Inference	Patients presented with LDL >150mg/dl are 2.79 times more likely to have NIHSS score >15 with p=0.104 and patients presented with HDL <40 mg/dl are 2.23 times more likely to have NIHS score >15 with p=0.164.				

Table 6: Association NIHSS with Lipid Parameters

Anthropometry and lipid parameters correlation with NIHSS

NIHSS score was correlated with Age, BMI, HDL, LDL and Lipoprotein (a) using Pearson correlation co-efficient. It was found that BMI and Lipoprotein (a) significantly correlated with the p value of 0.002 and <0.001 respectively.

Correlation of NIHSS with	Pearson correlation co-efficient	p value
Age in years	0.243	0.089
BMI kg/m^2	0.422	0.002**
HDL cholesterol	-0.137	0.342
LDL cholesterol	0.287	0.061
Lipoprotein (a)	0.810	p<0.001**

Table 7: Correlation of NIHSS with anthropometry and Lipid parameters

DISCUSSION: The present study was a prospective clinical study consisting of 50 consecutive patients admitted to Department of Medicine, Kempegowda Institute of Medical Sciences, Bangalore, from to June 2012 to June 2013, who were diagnosed to have Ischemic stroke confirmed on CT scan.

The majority of the study population 74% (n=37) consisted of male patients and 26 %(n-13) were females. This is in correlation with similar studies done both within country and outside.[9]The highest incidence was found between the age group of51-60 years consisting of 22 (44%) patients with male dominance in same ratio. Females in the reproductive age group are generally considered to be protected from coronary and cerebrovascular events with menopause rendering them to be at the same risk as their male counter parts. However, in the present study the male dominance persisted in the older age groups also. One patient below 40 year was found to be a female who had moderately elevated Lp (a) levels and serum triglycerides with no other lipid abnormalities or conventional risk factors.

The mean age of male subjects was 59.2 years, higher when compared to females 53.8 years. The body mass index was found to be higher in females (25.31±2.59) as compared to the males (23.38±3.26). The severity of the stroke as assessed by NIHSS showed that 2/3rd of the study population were in the moderate to moderate-severe class frequency. 9 out of the 13 female patients (79%) were found to be in moderate-severe to severe class frequency, while 17 out of 37 (45.9%) among the males belong to moderate to severe category. Severity of clinical stroke as assessed by NIHSS was found to be narrowing down the difference in incidence between the two sexes.

Conventional risk factors implicated in the etiopathogenesis of Ischemic stroke was assessed in the present study. The analysis of which showed that 11 patients had none of the conventional

risk factors, 8 had one risk factor, 10 had two risk factors, 11 had 3 risk factors and 10 had more than three risk factors. Among the patient with no risk factors the incidence was found to be equal between males and females. Male dominance was seen in association with the conventional risk factors. In the no risk factor category 5 patients had normal levels of Lp (a), while the another 5 had mildly elevated Lp (a) levels with only one showing severely elevated levels. Masao Nagayama in 1993 in their study on Ischemic stroke, found that atherothrombotic stroke was associated with none of the conventional risk factors but with high serum Lp (a) levels.[10] Such correlation could not be found in the present study. But it was seen that patients with no risk factor had only low HDL-C as their lipid abnormality.

In the present study and several other studies no apparent correlation between the incidence of any conventional risk factor and serum Lp (a) was found.[9] 50% 0f the subjects with no conventional risk factor had only mild elevated Lp (a) levels as their abnormality.

Blood was drawn for estimation of Lp (a) and Lipid subfractions from the anterior cubital vein on a fasting state earliest at 72 hrs. from the onset of the stroke. This was done to overcome the abnormalities that could occur during the acute event and also later when therapeutic intervention and diet modification could alter the values. It was found that Lp (a), total cholesterol, low density cholesterol, very low density cholesterol and triglycerides linearly correlated with the severity of stroke as assessed by NIHSS. While HDL-C inversely correlated with severity of stroke. However, only Lp (a) correlation was statistically significant. An inverse linear correlation was found between HDL-C and severity of stroke, which was not found with other lipid parameters.

Lp (a) value in the present study was found to be mildly elevated (30-60mg/dl) in 29 subjects (58%).

Various studies done showed that serum Lp (a) could be considered as an independent risk factor of ischemic stroke. Masao Nagayama in 1993 found that Lp (a) is an independent risk factor for Ischemic stroke. Higher levels of Lp (a) were found as age advanced in the above study even in absence of conventional risk factors. Such results were not reduplicated in the present study.**[10]**M Bewn and Durrington in 1990 suggested that Lp (a) would become critical when other factors, particularly LDL, had led to the development of significant atheroma.[11] Pedro-Botet et al 1992 recently reported that increased serum Lp (a) levels and IDL abnormalities together with decreased HDL levels are major factors for stroke.[12]

Masao Nagayama in 1993 in their study found that patients with Lacunar stroke had only slightly elevated serum Lp (a) levels than the control group, but still significant.[10] Lacunes are thought to results from occlusion of cerebral penetrating arteries that is caused by several distinct but related etiopathogenesis implying less important role of Lp (a) in the pathogenesis of penetrating artery occlusion. In the present study it was found that elevated levels of Lp (a) were found in the classical Ischemic infarcts, involving the right and left MCA territory. Lacunar infarct cases (n=3) showed normal levels of Lp (a).

Lp (a) levels were also found to correlate with angiographic studies of carotid artery narrowing in some studies. Gerald Zenker in 1985 showed the correlation of vascular lesion of the carotid system and Lp (a) and it was best seen in patients with age group 40-65 years.[13]

Using Pearson correlation co-efficient it was found that body mass index andLp (a) statistically significant and correlated with severity of ischemic stroke.

CONCLUSION: This study was done on 50 patients of ischemic stroke to study the clinical pattern and effect of Lipoprotein (a), HDL-C and LDL-C as risk factors. Males had higher incidence of Ischemic stroke. Severity of the stroke was assessed by NIHSS score and majority of the patients were in the moderate to moderate-severe group. Severe cases of ischemic stroke had equal incidence in both the sexes. Conventional risk factors did not have effect on severity of stroke nor did it have influence on Lp (a) levels. The Lp (a) levels linearly correlated with the severity of stroke and HDL-C inversely correlated with the severity of stroke. Prevention of Ischemic stroke and its resulting disability may be possible by using Lp (a) and HDL-C levels as biochemical markers, Since both have been found consistently to be independent risk factors for Ischemic stroke.

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