International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Neurological Worsening and Association between LDL, HDL Ratio, Mean Platelet Volume and Platelet Count in Cerebrovascular Ischemic Stroke

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Received: 24/02/2016 *Revised: 21/03/2016* *Accepted: 21/03/2016*

ABSTRACT

A direct and definite relationship between LDL, HDL ratio is more significant rather than individual lipid parameters in cerebrovascular stroke. As Platelets play a major role in the pathogenesis of vascular disease - stroke and mean platelet volume (MPV) being a physiological variable of hemostatic importance this study was undertaken to investigate the role of LDL: HDL ratio, MPV and platelet count in relation to neurological outcome in cerebrovascular ischemic stroke.
The study was conducted by the Department of Biochemistry in collaboration with the Department of Neurology and Pathology at SAIMS, Indore, MP, India between January 2011 and July 2014. 90 cases of cerebrovascular ischemic stroke based on CT Scan/MRI findings were included. Neurological worsening was monitored by NIHS scale and cases were categorized into mild, moderate and severe disability.5 ml venous blood sample was collected from all subjects in a plain serum separator tube and EDTA vial and HDL, LDL values with ratio along with platelet study was estimated.
Our study showed a significant correlation ($p < 0.05$) between LDL, HDL ratio, MPV and platelet count with neurological worsening in Cerebrovascular ischemic stroke. The measurement of MPV, LDL/HDL ratio may add useful prognostic information for clinicians managing patients with a history of cerebrovascular disease.

Key words: National Institute of Health Stroke Scale (NIHSS); Ischemic Stroke (IS); Mean Platelet Volume (MPV).

INTRODUCTION

The cerebrovascular diseases rank first in frequency and importance among all the neurological diseases of adult life. Cerebrovascular stroke spells emergency and clinical intervention at the earliest. Lipids are known to be associated with increased risk of atherogenesis and coronary artery diseases but their role in cerebrovascular stroke has been less consistently observed with varying results. [(1,2)] Some studies have reported a direct association with total cholesterol and ischemic stroke, [(3)] or cerebral infarction, [(4-7)] others showed little or no association with ischemic stroke or cerebral infarction. [(8-10)] The only three available prospective population-based studies on the association between HDL cholesterol and risk of stroke showed a tendency towards a higher risk of

cerebral infarction with a lower HDL level. [11,12] Recent data has shown LDL-Cholesterol/HDL-Cholesterol ratio and even Total-Cholesterol/HDL-Cholesterol ratio, to be accurate predictors of stroke (Helsinki Study).

Platelet activation and aggregation play key roles both in the pathogenesis of atherosclerosis and in the development of acute thrombotic events. Platelet volume is a marker of platelet activation and function, and is measured using Mean Platelet Volume (MPV). Large platelets that contain more dense granules are metabolically and enzymatically more active and have higher thrombotic potential compared to small platelets. [13,14] Patients with very low platelet counts ($< 50 \times 10^9$/L) have higher rates of bleeding, whereas those with very high platelet counts ($> 600 \times 10^9$/L) are more likely to develop thrombosis. [15] In normal individuals platelet count is inversely proportional to MPV but varying results are observed in stroke in various studies. With hyperlipidemia being commonly associated with altered platelet volume indices this study was undertaken to see their association with neurological worsening in cerebrovascular stroke.

MATERIALS AND METHODS

A study on hematological and biochemical parameters among cerebrovascular stroke patients in comparison with normal individuals was conducted by the Department of Biochemistry in collaboration with the Department of Neurology and Pathology at Sri Aurobindo Institute of Medical Sciences (SIAMS), Indore, Madhya Pradesh, between January 2011 and July 2014.This work was approved by the Institute Ethics Committee, selection of cases was done from in-patient wards and ICU from the Department of Neurology and Department of Medicine, SAIMS Medical College, Indore.

Study population included 90 ischemic stroke patients diagnosed by a neurologist as per WHO definition [8] along with CT Scan /MRI suggestive of cerebrovascular stroke and differentiating from hemorrhagic stroke. Age and sex matched 101 apparently healthy individuals not having any history of cerebrovascular stroke was taken as "control group" selected mostly from the patient's attendants and a few from among the hospital staff. All the volunteers were explained the purpose of the experiment, and a written consent was obtained from each of them.

Inclusion criteria for subjects with stroke:

(1) Diagnosed stroke patients as per WHO definition [16]

The Exclusion criteria for subjects with stroke:

(1) CNS infection
(2) Stroke older than 72 hrs
(3) Peripartum stroke
(4) Diabetes Mellitus
(5) H/O Myocardial infarction and atrial fibrillation

The detailed neurological examination was performed using the National Institute of Health Stroke Scale (NIHSS), a well validated and commonly used stroke impairment scale. [17,18] The disability was assessed by NIHSS at the time of admission (within 72 h from the onset of stroke) and on 7th day after admission. Depending on the disability score, cases were categorized into mild, moderate and severe disability. Early neurological worsening was diagnosed as an increase in National Institute of Health Stroke Score (NIHSS) by two or more points (or stroke-related death) between admission and day 7 and who remained stable or improved in the same period was classified as ''no worsening''.

5 ml venous blood sample was collected from all subjects and divided equally between a plain serum separator tube and EDTA vial. For control subjects, morning fasting blood sample was collected. For patients with stroke, the venous blood samples were drawn within 72 h from the onset of symptoms. The samples were processed as per the laboratory protocol, and the lipid profile parameters, namely

Low density lipoprotein LDL, High Density Lipoprotein (HDL), Mean Platelet Volume (MPV) and platelet count were assayed on the same day, using the Cobas Integra 400 PlusAutoanalyser.

HDL-cholesterol estimation was done using polyethylene glycol (PEG) precipitation followed by cholesterol oxidase method [(19)] with kits from Roche Diagnostics, India. Low density lipoprotein-cholesterol (LDL-C) estimation was done by direct homogenous method [(20)] with kits from Roche Diagnostics, India. LDL/HDL ratio calculated using MS Excel. MPV and platelet count were measured using a Sysmex 8000 autoanalyzer (TOA Medical Electronics UK Ltd) that uses aperture-impedance technology to size platelets Automated full blood counters produce platelet counts with a precision that is much superior to that of manual platelet counts. Manual platelet counting was occasionally done for blood samples with a significant proportion of giant platelets. Manual platelet counts were performed by visual examination of whole blood diluted with 1% aqueous ammonium oxalate, using improved Neubauer's chamber. The samples were run within two to six hours of venipuncture using the analyzer to avoid bias due to excessive platelet swelling [(21)] The quality of test results was validated throughout the study period by regular internal quality control procedures and participation in an External Quality Assessment Scheme.

Data analysis was done by MS Excel for calculating mean and SD. Pearson correlation, chi-square test and student t-test was done using online calculator (OpenEpi) at 95 % confidence interval.

RESULTS

A total of 191 subjects were enrolled for the present study. 90 cases were of ischemic stroke, out of which 60 were males and 30 were females. The patients fell into the age group between 45 to 71 years. Maximum numbers of patients were between 55 to 70 years with a mean age of 59.6 years in ischemic stroke patients. 101 subjects without stroke comprised of control group with a mean age of 56.4 years in control group The study and control groups were found to be age and sex matched statistically(Table 1) Hence, no bias in the results was observed due to age and sex in the results. 52 (57.7%) of 90 cases of ischemic stroke had hypertension and were on antihypertensive treatment. Confounding factors i.e. patients with Type 2 Diabetes Mellitus, Atrial Fibrillation were excluded from our study. Random blood glucose in ischemic stroke cases was 128± 8.5 mg/dl. In controls RBS was 116 ± 14.8 mg/dl.

Mean LDL, LDL/HDL ratio, Platelet volume were found to be higher in cases as compared to controls whereas Mean platelet count and HDL were found to be lower in cases as compared to controls as shown in Table 2 and by using independent sample t-test a significant difference at $p < 0.05$ was observed.

Out of 75 cases presenting with hypertension (Systolic BP >140mm Hg and Diastolic BP >90 mmHg) 85% were on anti-hypertensives while 15 % of cases were diagnosed to be having hypertension at the time of admission.95% of hypertensive controls were on anti-hypertensives. Among ischemic stroke cases no significant difference was observed at $p<0.05$ (95%CI) in HDL, LDL and platelet count in Hypertensive and Non-hypertensive cases.LDL/HDL ratio and MPV was found to be significantly ($p<0.05$) higher in Hypertensive than in non-hypertensive ischemic stroke cases. (Table 3)

TABLE 1: Demographic Profile of Cases and Controls

Demographic Variables		Subjects (n= 191)	
		Cases IS(90)	Controls n=101
Age (Mean ±SD ; years)		59.7±12.6	59.3 ± 13.3
Sex	M (%)	66.6	61.5
	F (%)	33.3	38.5
Hypertension (%)		83.3	25.7
Smokers (%)		31.1	21.7
Alcohol (%)		15.5	24.5

TABLE 2 Mean HDL, LDL, LDL/HDL, MPV, Platelet Count in cases and controls

PARAMETERS	GROUP	No. of patients	Mean ± SD	p-value
HDL (mg/dl)	IS	90	36.7± 6.7	p <0.001
	CONTROLS	101	42.6± 8.2	
LDL(mg/dl)	IS	90	178.5± 32.4	p <0.001
	CONTROLS	101	106.4 ± 9.2	
LDL/HDL	IS	90	5.1 ± 1.2	p <0.001
	CONTROLS	101	2.6 ± 0.3	
MPV (fl)	IS	90	10 ± 2.2	p <0.001
	CONTROLS	101	8.2 ± 0.4	
Platelet Count (10^9/L)	IS	90	200 ± 6.5	p <0.001
	CONTROLS	101	296.4 ± 5.6	

TABLE 3: Mean HDL, LDL, LDL/HDL, MPV, Platelet Count in subjects with Hypertensive (HT) and non-Hypertensive (Non-HT) ischemic stroke

PARAMETERS	ISCHEMIC STROKE		p value (95% CI)
	HT	NON HT	
HDL(mg/dl)	34.5±6.5	37.5±7.6	0.38
LDL(mg/dl)	180±34.2	176±30.3	0.65
LDL/HDL	5.8±1.4	4.6±0.8	0.02
MPV(fl)	10.1±1.3	9.9±2.3	0.001
Platelet count(10^9/L)	201±7.7	200±6.7	0.57

A significant positive Pearson correlation (r=0.6313,p<0.0001) was observed between LDL/HDL Ratio and MPV and significant negative Pearson correlation (r= - 0.3538, p<0.001) was observed between LDL/HDL Ratio and platelet count in ischemic stroke patients with significance at p <0.05. A highly significant correlation was observed (p<0.05) between neurological disability and LDL/HDL ratio MPV in ischemic stroke (Table 4). 50.6% of hypertensives with Systolic BP > 170mm Hg and Diastolic BP >110 mmHg had moderate disability and 22.6% had severe disability. 20% of non-hypertensive was found to have severe disability as observed from their NIHSS scoring.

Overall significant difference at p <0.05 was observed between mean HDL, LDL/HDL ratio, MPV and Platelet count in cases with neurological deterioration as compared to cases with no neurological deterioration in ischemic stroke (Table 5).Higher LDL/HDL ratio, MPV and lower HDL and Platelet count were seen in patients with neurological deterioration65% of hypertensives with severe disability had neurological deterioration.

TABLE 4: HDL, LDL, LDL/HDL Ratio, MPV, Platelet Count and Neurological Disability in Ischemic Stroke

Variables	Disability			p value (95% CI)
	Mild	Moderate	Severe	
	IS(28)	IS(42)	IS(20)	
HDL	38.2±5.3	37.8±6.5	33.2±10.1	0.005
LDL	146.0±32.5	182.9±15.8	201±4.4	<0.01
LDL/HDL	3.8±0.7	4.9±1.0	6.7±2.2	<0.01
MPV	9.7±1.1	10.2±1.5	11.3±0.9	0.02
Platelet Count	201.4±7.9	200.8±7.6	199.1±8.2	0.92

TABLE 5: HDL, LDL, LDL/HDL Ratio, MPV, Platelet Count and Neurological Worsening In Ischemic Stroke

VARIABLES	Neurological Deterioration ISCHEMIC STROKE		p value 95%CI
	ND(14)	NO ND(76)	
HDL	25.3±4.5	39±5.5	<0.0001
LDL	200.7±21.2	170.8±32.1	0.001
LDL/HDL	8.1±1.5	4.4±0.8	<0.0001
MPV	11.9±0.8	9.95±1.18	<0.0001
Platelet Count	193.7±5.0	201.9±7.6	0.0002

DISCUSSION

In the present study HDL and platelet count were found to be lower while LDL, LDL/HDL ratio and MPV were found to be higher in cerebrovascular ischemic stroke. Shahar et al [(22)] and Bowman et al [(23)] reported the lack of association between HDL and stroke which they proposed may be due to inability to differentiate between hemorrhagic and ischemic stroke. Our findings were in accordance with studies [(24-26)] which showed an inverse relation between HDL stroke. A higher LDL/HDL ratio in cerebral ischemic stroke points to the more atherogenic serum lipids profile than the healthy control group. Soyama et al, in a 10-year follow-up study of 4,989 individuals aged 35-79 years, found

significant association between low HDL levels and increased risk of stroke. [27,28]

Increased MPV in stroke in present study is in accordance with previous studies [29-31] with the known greater reactivity of larger platelets [32,33] the pathophysiological role of platelets in the occurrence of ischemic stroke, [34] Increased MPV and lower platelet count might lead to development of stroke rather than associated with stroke.

In steady state Inverse but nonlinear relationship between MPV and platelet count (PC) [35,36] has been observed. Though the increase in platelet size is in conjunction with the elevated PC thus increasing the thrombotic potential we observed decreased platelet count in ischemic stroke. This may be due to increased platelet consumption in thrombus formation in ischemic stroke as seen in few other studies. [37-39]

A positive correlation between LDL/HDL ratio and MPV and platelet count in present study may account for atherogenic potential of platelets. Larger platelets are considered to be metabolically, enzymatically and functionally more active than the smaller platelets. They contain more dense granules and hence are more potent and thrombogenic and this might be a cause for their direct association with thrombogenic states like a high LDL/HDL ratio. Chang et al [40] found an unadjusted, inverse relationship between MPV and LDL-cholesterol.

Increased LDL/HDL ratio, MPV and lower platelet count was observed with neurological worsening in Ischemic stroke. In the Helsinki Study, the LDL-C/ HDL-C ratio had more prognostic value than LDL-C or HDL-C alone. [41-43] Factors such as vascular endothelial growth factors released from activated platelets may interact with thrombin to increase vascular permeability and contribute to the development of edema that is significantly correlated with low PC in stroke and poor prognosis. [44] To avoid bias samples were collected in EDTA bulbs and run between 24-48 hrs after venipuncture (when platelet swelling would have ceased).Hypertensive patients on ACE Inhibitors and Angiotensin Receptor Blocker may have affected subsequent platelet indices though some studies have shown little effect on MPV. [45]

CONCLUSION

Increased LDL/HDL, MPV and low Platelet count may be an early prognostic marker for cerebral ischemic stroke. Our study agrees with the clinical importance of MPV in ischemic stroke and of LDL/HDL ratio to be accurate predictors of stroke risk rather than isolated lipid parameters showing their unique ability to reflect the bidirectional cholesterol traffic (in and outward) through the arterial intima in a way that the individual LDL and HDL-Cholesterol levels cannot reach. The estimation of these indices thus can be considered as an early and rapid procedure for identification of clinical outcome in cerebrovascular ischemic stroke patients.

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How to cite this article: Bharosay A, Bharosay VV, Bandyopadhyay D et al. Neurological worsening and association between LDL, HDL ratio, mean platelet volume and platelet count in cerebrovascular ischemic stroke. Int J Health Sci Res. 2016; 6(4):209-215.

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