Original Research Article

Study of lipid profile and oxidative stress in patients of ischemic and hemorrhagic stroke

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Abstract

Background: Stroke is the second leading cause of death worldwide and one of the main causes of long term disability. The association between cholesterol and stroke may not be as straight forward as for coronary heart disease .MDA and SOD are the markers of oxidative stress. **Study design:** An observational prospective comparative study. **Methodology:** 30 confirmed cases of first ever stroke admitted in neurology unit of MGMs medical college and hospital ,Aurangabad were included in study after properly evaluating the exclusion criterias. 30 healthy age matched individuals were selected as controls. Lipid profile (serum total cholesterol, HDL, LDL ,VLDL and TGs) ,serum MDA and SOD levels were compared between cases of ischemic and hemorrhagic stroke and controls. Mean and standard deviation were worked for the quantitative data. Using the student's t-test values, the 'p' value less than 0.01 was considered as statistically significant. **Results:** We found statistical significant difference between all the lipid profile parameters on comparison between cases and controls. On comparing lipid profile parameters between ischemic and hemorrhagic stroke group, mean levels of total cholesterol and LDL were significantly higher and mean HDL levels were lower in ischemic stroke as compared to hemorrhagic stroke. There was no statistically significant difference in levels of TG and VLDL cholesterol. Thus atherogenic lipid profile was seen in patients with ischemic stroke and cholesterol seems to be protective in hemorrhagic stroke. Mean serum MDA levels were increased in both ischemic and hemorrhagic stroke as compared to controls while serum SOD levels were reduced in ischemic and hemorrhagic stroke patients as compared to controls. This explains the role of oxidative stress in pathogenesis of stoke. The present study reveals the importance of determining lipid profile, lipid peroxidation and antioxidant status in CVA patients to enable formulation of specific therapies for early intervention and better management of disease.

Key Words: cerebrovascular stroke, lipid profile, oxidative stress, ischemic stroke, hemorrhagic

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INTRODUCTION

Stroke is the second leading cause of death worldwide and one of the main causes of long term disability.[1]. Globally, in 2013 there were almost 25.7 million stroke survivors, 6.5 million deaths from stroke , 113 million DALYs due to stroke , and 10.3 million new strokes . Improved stroke care, aging, and growth of the population combined with the increased prevalence of many modifiable stroke risk factors are likely to be the main drivers in the increased number of stroke survivors and people affected by stroke.[2] Two third of all strokes occur in people over the age of 65 years, with men more commonly affected as compared to women.[3]. Developing countries like India are facing a double burden of communicable and non-communicable diseases. The estimated adjusted prevalence rate of stroke range is, 84-262/100,000 in rural and 334-424/ 100,000 in urban areas. The incidence rate is 119-145/100,000 based on the population based studies.[4] Stroke is a clinical syndrome divided into two broad categories that define its pathophysiology: Ischemic strokes are caused by sudden occlusion of arteries supplying the brain, either due to a thrombus at the site of occlusion or formed in another part of the circulation accounting for 50%– 85% of all strokes worldwide. Hemorrhagic strokes are caused by

subarachnoid hemorrhage or intra-cerebral hemorrhage accounting for 1%-7% and 7%-27% respectively of all strokes worldwide.[5]The modifiable risk factors for stroke comprise of hypertension, cardiac diseases, diabetes mellitus and dyslipidemia. It is apparent from evidence based data that stroke can be prevented by primary and secondary preventive measures. [6] The potential risk factors which have gained importance in recent years are dyslipidemia and oxidative stress. Several clinical trials showed an association between high concentrations of serum cholesterol, TGs, LDL ,VLDL and ischemic stroke.[7] On the other hand, case-control studies of stroke which examined cholesterol as a risk factor have generally produced negative findings and prospective studies have generally failed to show a direct and strong association.[8] Therefore, the association between cholesterol and stroke may not be as straight forward as for coronary heart disease . [9] Oxidative stress is defined as "an imbalance between oxidants and antioxidants, in favor of the oxidants, potentially leading to damage". is involved in the pathogenesis of acute stroke.[10] The brain cellular membrane lipids are very rich in polyunsaturated fatty acid side chains, which are highly prone to free radical attack resulting in lipid peroxidation and biomarkers such as malondialdehyde (MDA) can be estimated to assess the amount of damage to brain tissue.[11] Endogenous antioxidants involve enzymatic and non-enzymatic antioxidants, all of which consists of the cellular protective antiradical mechanism. Superoxide dismutase (SOD) is the most studied antioxidant enzyme in stroke.[12]Considering the relation of lipid parameters and oxidative stress with cerebrovascular stroke, the present study was undertaken to get a better understanding of their association in stroke patients.

OBJECTIVES

1. To study the serum levels of total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and triglyceride (TG) in patients of cerebrovascular stroke and controls.
2. To study the serum levels of malondialdehyde (MDA) and superoxide dismutase (SOD) as markers of oxidative stress in patients of cerebrovascular stroke and controls.

MATERIALS AND METHODS

The present study was carried out in the Department of Biochemistry and Central Investigation Laboratory in collaboration with the Department of Medicine at MGM medical college and hospital, Aurangabad. The study was approved by Institutional Ethical and Research Committee to use human subjects in the research study. Informed consent was taken from patient / relative and control subjects. The study was conducted from January 2016 to December 2016. Study Groups: 30 Patients (age 30-90 years) of cerebrovascular stroke referred by various hospitals to MGMs medical college and hospital, Aurangabad were selected as cases based on inclusion and exclusion criteria. 30 normal individuals (age and sex matched) belonging to same socioeconomic status participated as controls.

Inclusion criteria: Patients presenting with focal neurological deficit of acute onset in the form of hemiparesis, hemi anesthesia or aphasia, or having evidence of presence of ischemic or hemorrhagic stroke on CT/MRI scan of brain were included in study complying with the WHO definition of stroke.[13]Patients with new onset of stroke presenting within 72 hours of onset of stroke were included. Clinical examination including vitals and detailed neurological examination were carried by physicians and neurologist.

Exclusion criteria: Patients with history of recent myocardial infarction or acute coronary syndrome(within last three months) , neoplastic disease, hepatic failure, chronic alcoholics and smokers, with infectious diseases, on NSAIDs, statins, hormone replacement therapy, antioxidants , post partum stroke, TIA were excluded

Collection of Blood Sample: About 3-5 ml of venous blood after overnight fasting was collected in vacutainer by means of sterile needle, from anterior anticubital vein. It was allowed to clot for few minutes and was subjected to centrifugation for 10 minutes at 3000 rpm to separate the serum and kept at -20º C until analysis was carried out. Concentration of serum lipids was measured By Dimension Rxl Fully automated analyzer using The kits supplied By Siemens . LDL and VLDL were estimated by Friedwalds formula[14]. Serum Malondialdehyde (MDA) was estimated using the thiobarbituric acid [TBA] method (cited by Nourooz ZadehJ, TajaddiniSarmati J ,Mccarthy) .[15]Super oxide dismutase(SOD) levels in serum were estimated spectrophotometrically using the method by S Marklund and G Marklund .[16]The following parameters were estimated

1. Lipid profile (total cholesterol, HDL,TG,LDL,VLDL).
2. Serum MDA (malondialdehyde)
3. Serum SOD (superoxide dismutase)

The chemicals and reagents used for the procedure were of analytical grade

Statistical analysis: Mean and standard deviation were worked out for estimating the levels of lipid profile, serum MDA and SOD in patients of ischemic and hemorrhagic stroke and age matched controls. Using the student's t-test values, the 'p' values (probability values) were obtained. 'p' value less than 0.01 was considered as statistically significant.

OBSERVATION AND RESULTS

1. The mean age of cases and controls were 58.56 ± 13.51 and 47.36 ± 7.71 years respectively
2. Maximum number of cases were in the age group of 51-60 years (36.6%). (Table No. 1)

Table 1: Age wise distribution of cases

Age group (Years)	No.of cases
31-40	4(13.3%)
41-50	4(13.3%)
51-60	11(36.6%)
61-70	5(16.6%)
71-80	5(16.6%)
81-90	1(3.3%)

3. Present study comprised of 30 cases and 30 controls. Among cases, 20 were male (66.66%), 10 were female (33.33%). Out of 30 controls 16 were male (53.33%) and 14 were female (46.66%).
4. Out of 30 stroke cases 17 were of ischemic type and 13 were of hemorrhagic type.
5. On comparing mean cholesterol, TG, HDL , LDL and VLDL between cases and controls, the p value was < 0.01 in all above test parameters which was found to be statistically significant

Table 2: Comparison of mean cholesterol, TG, HDL , LDL and VLDL between cases and control

Parameter	Cases	Controls
Total cholesterol(mg/dl)	217.93±40.80	191.83±15.70
TG(mg/dl)	179.46±20.97	126.96±21.33
HDL(mg/dl)	32.8±5.19	46.8±7.36
LDL(mg/dl)	149.24±43.38	99.44±17.48
VLDL(mg/dl)	35.90±4.17	25.39±4.26

On comparing, levels of total cholesterol, HDL cholesterol and LDL cholesterol between ischemic and hemorrhagic stroke, p value was statistically significant while difference between triglyceride level and VLDL was not significant

Table 3: Comparison of mean cholesterol, TG, HDL,LDL and VLDL between cases of ischemic stroke and cases of hemorrhagic stroke

Parameter	Ischemic stroke	Hemorrhagic stroke	P value
Total cholesterol (mg/dl)	246±25.70	181.23±23.80	0.0001
TG(mg/dl)	183.52±21.57	174.15±19.71	0.2315
HDL(mg/dl)	30.70±3.72	35.53±5.69	0.009
LDL(mg/dl)	178.58±26.41	110.86±28.32	0.001
VLDL(mg/dl)	36.70±4.31	34.86±3.89	0.2373

On comparing, the difference between mean MDA values of ischemic stroke and controls was found to be statistically significant ($p<0.01$). Also on comparison between hemorrhagic stroke and control the difference in mean MDA values was found to be statistically significant ($p<0.01$).

Table 4: Comparison of mean serum MDA between ischemic stroke, Hemorrhagic stroke and control group

Parameter	Cases		Controls
	Ischemic stroke Mean±SD	Hemorrhagic stroke Mean±SD	Mean±SD
MDA(nmol/ml)	3.07±0.43	2.84±0.70	1.56±0.34

On comparison by student t test, the difference between mean SOD values of ischemic stroke and controls was found to be statistically significant ($p<0.01$). Also on comparison between hemorrhagic stroke and controls the difference in mean SOD values was found to be statistically significant($p<0.01$).

Table 5: Comparison of mean serum SOD between ischemic stroke, Hemorrhagic stroke and control group

Parameter	Cases		Controls
	Ischemic stroke Mean±SD	Hemorrhagic stroke Mean±SD	Mean±SD
SOD(U/ml)	2.68±0.28	2.82±0.18	3.17±0.20

DISCUSSION

Stroke is an illness of escalating socioeconomic importance. There is increasing evidence that stroke incidence rates in developing countries have increased by more than 100% during the last four decades.[17] Dyslipidemia is a known risk factor for stroke. Improved detection, modification or control of risk factors, life style modification, diet and physical activity could significantly reduce the incidence of ischemic stroke and reduce the impact of this disease. [18]Lipid peroxidation, with accumulation of thiobarbiturate reactive material, is consistently found in cerebral ischemia. Amount of oxidative stress and acute changes of antioxidant capacity might influence the prognosis of cerebral ischemia. [10]Keeping this in view, we measured lipid profile (total cholesterol, HDL cholesterol, TG, LDL cholesterol, VLDL cholesterol), serum MDA and serum SOD levels in patients of cerebrovascular stroke. The age distribution of the stroke patients in this study was between 30 and 90 years and mean age of cases was 58.56±13.51 with maximum number of patients in age group of 51-60 years of age and men were more commonly affected as compared to women 66.66% vs 33.33% .These findings matched with most of the previous studies.[7,9,18] We compared serum levels of total cholesterol, HDL cholesterol, TG, LDL cholesterol, VLDL cholesterol in cases of stroke (217.93 ± 40.80, 179.46 ± 20.97, 32.8 ± 5.19, 149.24 ± 43.38, 35.90 ± 4.17 mg/dl respectively) and healthy controls (191.83 ± 15.70, 126.96 ± 21.33, 46.8 ± 7.36, 99.44 ± 17.48, 25.39 ± 4.26 mg/dl respectively). We found statistical significant difference in above parameters. Comparing lipid profile parameters between ischemic and hemorrhagic stroke, we found that mean levels of total cholesterol and LDL were significantly higher and mean HDL levels were lower in ischemic stroke as compared to hemorrhagic stroke. There was no statistical significant difference levels of TG and VLDL cholesterol. Ahmed W *et al.*[7] ,Sreedhar et[19] al, Younis *et al.*[20] who showed in their study that, the difference in values of TC, HDL, LDL, TG in study group and controls was found to be highly significant ($p<0.001$). This reflects the anti atherogenic role, of HDL cholesterol in facilitating reverse cholesterol transport. In contrast high LDL and total cholesterol levels favors atherogenesis and plaque formation. Muhammed nazim *et al.*[18] in their study on 370 stroke patients found that Ischemic stroke patients had significantly higher frequency of hypercholesterolemia and reduced HDL-Cholesterol levels than patients of hemorrhagic stroke**.** Asad mehmood *et al.*[9] in their study on comparison of serum lipid profile between two categories of stroke showed a raised serum total cholesterol and low HDL ischemic stroke as compared to hemorrhagic stroke No statistical significance was found on comparing serum values of triglycerides, LDL-cholesterol and VLDL-cholesterol in ischemic and hemorrhagic. Thus difference in lipid profile parameters between ischemic and hemorrhagic stroke should be taken into consideration while initiating lipid lowering therapy for primary prevention in high risk cases and secondary preventive measure in cases of ischemic stroke.[9] Thus our study matched with Muhammed nazim *et al.***,** Asad mehmood *et al.*, Ahmed W *et al.*, Sreedhar *et al.*, Younis E *et al.* ,suggesting that Variation in lipid profile with respect to ischemic and hemorrhagic stroke arises by the fact that cerebral ischemic patients have a more atherogenic lipid profile whereas in intracerebral hemorrhage, lipids apparently play a protective role. In our study we observed that mean serum levels of MDA were increased in both ischemic 3.07±0.43 nmol/mL and hemorrhagic stroke 2.84±0.70 nmol/mL as compared to controls 1.56±0.34 nmol/mL. InimioaraM *et al.*[21] Sarkar *et al.*[22] **,** Beg M*et al.*[23] observed significantly higher concentration of MDA in stroke patients compared with controls, similar to our study and suggested that increased level of lipid peroxides may be due to oxidation of blood or neural lipids by ischemia. They suggested that increased level of lipid peroxide may be due to oxidation of blood or neural lipids by ischemia and rise in lipid peroxide in hemorrhagic stroke was due the compressive effects producing ischemia. These studies match with findings of our study, that serum MDA levels are increased after stroke suggesting involvement of lipid peroxidation in the pathophysiology of ischemic as well as hemorrhagic stroke. We found reduced mean level of serum SOD in cases of ischemic (2.68±0.28 U/ml) and hemorrhagic stroke (2.82±0.18 U/ml) as compared to controls (3.17± 0.20 U/ml). Srikrishna R *et al.*[24] observed reduced SOD in cases 4.04±0.03U/ml as compared to 9.01±1.04 U/ml in controls. Spranger M *et al.*[25] found that mean serum levels of SOD in cases of both ischemic and hemorrhagic stroke were significantly lower as compared to controls suggesting that antioxidants are depleted as a consequence of an excessive production of oxygen free radicals very early after the onset of stroke. Cherubini *et al.* [26] found that antioxidants including SOD are reduced immediately after an acute stroke, possibly as a consequence of increased oxidative stress and A specific antioxidant profile is associated with a poor early outcome .Thus our findings of serum SOD levels matched with previous studies. Hence increasing the anti oxidative capacity in serum within the first day after the onset of symptoms might be a therapeutic option to minimize the oxidative injury caused by oxygen free radicals until the endogenous free radical scavenging systems recovers.

CONCLUSION

The difference in lipid profile between ischemic and hemorrhagic stroke should be taken into consideration while starting statin therapy as primary preventive measure to reduce incidence of first stroke in high risk patients, as well as during secondary prevention in case of ischemic strokes. Antioxidants are depleted as a consequence of an excessive production of oxygen free radicals very early after the onset of stroke. Antioxidant therapy in addition to conventional treatment strategies could be a therapeutic option to reduce injury caused by oxidative stress.
However, we had a small sample size, a larger sample size study is needed to endorse our observations and analyze further.

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