Original Article

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Oxidised LDL and Serum Paraoxanase Activity in Ischemic Stroke Patients: A Case Control Study

Biochemistry Section

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ABSTRACT

Introduction: Oxidative stress by producing lipid peroxidation products like oxidised Low Density Lipoprotein (LDL) acts as independent risk factor in ischemic stroke. When lipid peroxidation overwhelms antioxidative defense mechanism; it results in enhanced formation of oxidized LDL which promotes atherosclerosis. Human serum Paraoxanase (PON 1) which is an ester hydrolase synthesized in the liver prevents the oxidative modification of LDL.

Aim: To investigate the level of oxidized LDL as oxidative stress marker and paraoxonase activity as an antioxidant enzyme in ischemic stroke patients and also the relation between Oxidized-Low Density Lipoprotein (Ox-LDL) level and Computed Tomography (CT) findings in ischemic stroke patients.

Materials and Methods: The subjects in this study comprised of 40 ischemic stroke patients and 40 age and gender matched controls. Fasting plasma glucose, serum paraoxanase activity, plasma oxidised LDL level, fasting lipid profile were determined. Data was analysed using Statistical Package for Social Sciences (SPSS) version 16.0.

Results: Ischemic stroke patients had significantly lower paraoxanase activity (<53 kU/L), elevated levels of oxidized LDL (>2.26 µg/ml), total cholesterol (>200 mg%) and fasting plasma glucose levels (>126 mg%).

Conclusion: Oxidised LDL level >2.26 µg/ml, paraoxanase activity <53 kU/L and FBS >126 mg% were independent risk factors for stroke. Significantly higher levels of oxidized LDL were seen in patients with infarct in anterior circulation. This study implies that raised oxidized LDL level and low serum paraoxanase activity are independent risk factors of ischemic stroke in patients with no other risk factors.

Keywords: Antioxidant, Hyperglycaemia, Oxidative stress

INTRODUCTION

Data from Global Burden of Disease Study 2010 has shown that stroke is the second leading cause of mortality and third leading cause of disability [1]. There are regional variations as well as changes over time. Stroke is the third leading cause of death in industrialized countries [2] with decrease in the incidence of stroke. Stroke is currently ranked the third leading cause of Disability Adjusted Life Years (DALY) in 2010 and was ranked fifth in 1990 [3]. The crude prevalence of stroke in India is high which varies from 126 to 165 per 100000 population [4,5] with the exception of higher prevalence in Kolkata [6]. Case fatality rate in the first 30 days reported in India is also high which varies from 27% to 42% [7-9]. Ischemic stroke accounts for 70-80% of all strokes.

Lipid peroxidation is a natural process essential for cell growth. Normal cell metabolism produces lipid peroxidation products leading to an excess of free radicals that can react with unsaturated fatty acids, in particular, LDL. Oxidation of LDL occurs when LDL particles react with free radicals.

When the oxidative stress overwhelms the antioxidative cell defense, the balance is disturbed resulting in enhanced formation of lipid peroxidation products. Oxidative stress by inducing the production of free radicals and lipid peroxidation has an important role in the pathophysiology of stroke. Studies have provided evidence that oxidative stress resulting in lipid peroxidation and protein modification is involved in the pathogenesis of atherosclerosis. Ox-LDL can produce inflammation in arteries promoting atherosclerosis and increases the risk of having a stroke. Normally, receptor mediated uptake of LDL by macrophages is suppressed through down-regulation of LDL receptor expression in response to increased cholesterol levels. After oxidation of LDL, it can be internalized by macrophages through scavenger receptors whose expression is not controlled by cholesterol loading. This leads to cholesterol accumulation in macrophages transforming them into lipid loaded 'foam cells' [10-13]. Extensive cell proliferation and elaboration of extracellular matrix components leads to genesis and progression of atherosclerosis by promoting endothelial damage and amplifying the inflammatory response within the vessel wall. The Ox-LDL itself becomes more reactive with the surrounding tissues producing tissue damage. Stroke patients have elevated plasma Ox-LDL from increased lipolysis in brain regions subjected to ischemia [14].

Human serum paraoxanase [15] or aryl esterase (PON 1) is an ester hydrolase which is synthesized in the liver and prevents the oxidative modification of LDL. Serum paraoxanase, which is located in a sub fraction of HDL that contains Apo A1 and clusterin (Apo J) causes breakdown of lipid peroxides and protects against lipoprotein oxidation. Studies showed that PON 1 works in conjunction with HDL, affecting two critical steps in atherogenesis. PON1 reverses oxidation of LDL cholesterol and thereby inhibits LDL mediated formation of foam cells. PON 1 has also been shown to protect HDL associated phospholipids from oxidation. Decreased paraoxanase activity seen in cardiovascular and stroke patients may reflect the atherogenic potential in them. Human studies on Ox-LDL level indicate oxidative stress and paraoxanase activity as an antioxidant enzyme in ischemic stroke patients are limited in India. The present was done to investigate the level of oxidized LDL as oxidative stress marker and paraoxanase activity as antioxidant enzyme in ischemic stroke patients.

MATERIALS AND METHODS

The study was conducted on ischemic stroke patients diagnosed

by CT scan, clinical signs and symptoms in the age group 40-65 years who attended the Neurology Department of Trivandrum Medical College, Kerala, India. The Institutional Ethics Committee approved the study. Informed consent was obtained from all subjects. The study was conducted from January 2012 to January 2013. The sample size was estimated to be 40 newly diagnosed ischemic stroke patients based on a previous study [14], with equal number of age and gender matched healthy volunteers from health department with no family history of stroke as controls. Patients with renal insufficiency, hepatic disease, on antioxidants or vitamin supplements, cardiac failure, respiratory diseases, cardiogenic source for embolism, haemorrhagic stroke, other neurodegenerative diseases etc were excluded from the study.

Under strict aseptic precautions about 7 mL fasting blood sample was collected from all subjects. Blood for FBS estimation was collected in bottles containing sodium fluoride and for Ox-LDL in EDTA bottle. The tubes with blood samples were centrifuged at 2000 gm for 20 minutes for clear separation of serum and plasma. Plasma for Ox-LDL and serum for paraoxanase activity were kept at -20°C until analysis was carried out. Blood glucose was estimated by glucose oxidase method. Serum cholesterol was determined by end point estimation using cholesterol oxidase-phenol 4-aminoantipyrine peroxidise (CHOD-PAP) method. TG was determined by Gycerol Phosphate Oxidase (GPO-Trinder method), High Density Lipoprotein (HDL) by enzymatic method and LDL value was derived using Friedewald's equation. Analysis was done on fully automated analyser (EM360) from Transasia Biomed. Oxidized LDL estimation was done by ELISA method on ELx800MS, ERBA MICROSCAN ELISA machine. Serum paraoxanase activity was estimated by a chemical method using phenyl acetate, calcium chloride and tris acetate and its absorbance measured using Jasco UV spectrophotometer at 270 nm at 25°C.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS for windows version 16.0 and values were expressed in mean and standard deviation. The difference in the group means of quantitative variables was compared by student's t-test. Multivariate analysis was done for final selection of independent variables for ischemic stroke. A p-value <0.05 was considered significant.

RESULTS

We enrolled 40 cases and an equal number of age and gender matched controls. Anterior circulation stroke was seen in 34 patients (85%) compared to only six patients (15%) with posterior circulation. Maximum number of patients (47.5%) was in the age group between 51-55 years followed by 56-60 years (27.5%). Males (57.5%) were more as compared to females (42.5%).

Hypertension (72.5% Vs 42.5%), and diabetes mellitus (37.5% Vs 12.5%) were more in the study group compared to controls. The difference in mean diastolic BP (90.5 Vs 83.1 mmHg) and systolic BP (144.4 Vs 131.7 mmHg) between the cases and controls were statistically significant [Table/Fig-1]. Similarly, the difference in mean oxidized LDL, paraoxanase activity, total cholesterol, and FBS were more in the study group compared to controls [Table/Fig-1]. The proportion of subjects with Oxidised LDL >2.26 µg/mL, paraoxanase activity <53 kU/L, systolic BP >140 mm of Hg, FBS >126 mg% and alcoholism were found associated to stroke [Table/Fig-2]. These study variables found significant in univariate analysis were subjected to multivariate analysis and was found to be independently associated with the exception of alcoholism. Risk estimates (adjusted odds ratio) are given in [Table/Fig-3].

In [Table/Fig-4], oxidized LDL level was found to be significantly higher in patients having an infarct in anterior circulation region when compared to those with an infarct in posterior circulation region.

Parameters	Stroke (Mean±SD)	Controls (Mean±SD)	p-value
Age (years)	53.6±3.4	52.3±4.6	0.147
BMI (kg/m^2)	24.1±2.3	24.2±2.0	0.799
Ox- LDL (µg/mL)	6.8±4.8	1.8±1.4	<0.001*
Paraoxanase activity (kU/L)	43.4±26.1	78±33.5	<0.001*
Total cholesterol(mg/dL)	206±43.1	188.3±34.4	0.046*
LDL-cholesterol(mg/dL)	135.5±35.3	121.2±32.4	0.062
Triglyceride(mg/dL)	136.2±50.3	124.6±49.8	0.299
HDL-cholesterol(mg/dL)	43.8±12.6	42.8±11.9	0.520
Fasting blood sugar (mg/dL)	124.9±35.5	109.1±23.1	0.021*
Systolic BP (mmHg)	144.4±23.2	131.7±10.9	0.002*
Diastolic BP (mmHg)	90.5±13.9	83.1±10.7	0.009*

[Table/Fig-1]: Study parameters among cases and controls.
Value represented as mean±SD, *p-value <0.05 was considered significant

Parameters	Number (%) among stroke patients	Number (%) among controls	χ^2	p-value
Ox LDL >2.26 µg/mL	30 (75.0)	9 (22.0)	22.06	<0.001
Paroxanase <53 kU/L	29 (72.5)	7 (17.5)	24.44	<0.001
SBP >140 mmHg	29 (72.5)	17(42.5)	7.366	0.007
FBS >126 mg%	15 (37.5)	5 (12.5)	6.67	0.010
Alcoholism	19 (47.5)	6 (15)	9.833	0.002

[Table/Fig-2]: Percentage distribution of risk factors for stroke.
*p-value <0.05 was considered significant

Parameters	Beta Co-efficient	Adjusted Odds ratio	95% C.I. for Exp(B)		p-value
			lower	upper	
Oxidised LDL >2.26 µg/mL	1.31	3.71	1.02	13.52	0.047*
Paraoxanase activity <53 kU/L	2.28	9.79	2.43	39.18	0.001*
SBP >140 mm Hg	1.46	4.30	1.21	15.29	0.024*
FBS >126 mg%	1.63	5.09	1.09	23.89	0.039*

[Table/Fig-3]: Multivariate analysis.
* p-value <0.05 was considered significant

Ox-LDL level	Anterior circulation infarct		Posterior circulation infarct		Total
	n	%	n	%	n
>2.26 µg/mL	28	82.4	2	33.3	30
<2.26 µg/mL	6	17.6	4	66.7	10
Total	34	100	6	100	40

[Table/Fig-4]: Relation between Ox-LDL level and CT findings
α^2 =6.536; df =1; p-value=0.011*.

DISCUSSION

In the study, an age and gender matched hospital based cross sectional study with adequate sample size has shown that high levels of Ox- LDL and low levels of paraoxanase activity is associated with stroke.

Several studies had shown raised Ox-LDL in stroke patients especially ischaemic stroke. A case control study from China showed higher levels of Ox-LDL levels in patients with atherosclerotic thrombotic cerebral infarction involving cortical branches and not perforating artery infarcts [16]. Jinhua C et al., confirmed that Ox-LDL was elevated significantly in acute phase of cerebral infarction compared to the convalescence [17]. Elevated levels of Ox-LDL during acute phase in cerebral infarct patients, especially those with large cortical infarcts were reconfirmed by Uno from Japan [18]. A higher level of Ox-LDL has been shown in patients with cerebral infarct from India [14]. A recent study by Abd-Al-Farag E et al., and Kasab SA et al., has demonstrated high fasting oxidised LDL in participants with stroke due to atherosclerosis [19,20].

Low levels of serum paraoxanase activity have been documented in patients with ischemic stroke. Case control study in Korea has shown lower levels of paraoxanase activity in ischemic stroke patients [21]. Along with the low levels of paraoxanase, higher levels of malondialdehyde are associated with ischemic stroke [22]. Low levels of paraoxanase, as well as HDL cholesterol levels, are associated with ischemic stroke [23,24]. Paraoxanase gene polymorphism is associated with stroke which suggests a biological plausibility [25]. Walsh found low levels of paraoxonase as well as apo A1 in patients with ischemic stroke [26].

In the present study, only systolic hypertension was associated with ischaemic stroke which was consistent with the Framingham [27] and the Copenhagen City Heart Study [28] results. On the contrary, the Oslo study showed that stroke was associated with diastolic blood pressure [29]. The more recent INTERSTROKE study confirmed that history of hypertension is a risk factor for stroke [30].

Hyperglycaemia is a known risk factor for stroke [31,32] and the mean FBS levels were elevated in stroke patients. Hypercholesterolemia is a conventional risk factor for stroke. Asia Pacific Cohort study has shown a significant association between cholesterol and risk of fatal and nonfatal ischemic stroke [33]. But the Framingham study reported that no direct relationship either long term or short term exists between ischemic stroke and total /LDL cholesterol [27]. INTERSTROKE study also did not show any association of ischemic stroke with total cholesterol but showed association with apolipoprotein A1 and HDL cholesterol [30].

There was no difference in the distribution of family history of stroke, smoking, sedentary life style, Body Mass Index (BMI), or psychosocial factors among patients with ischaemic infarcts and control. A meta-analysis found that heavy alcohol consumption >60 gm per day increases relative risk of stroke while light (<12 gm per day) or moderate alcohol intake (12 to 24 gm per day) may be protective against ischemic stroke [34].

The study results indicate that existence of an abnormal balance between oxidative stress and protective mechanism can be a causative factor for acute cerebral infarction in these patients. This implies the use of Ox-LDL as a specific marker in the absence of other risk factors of stroke. It is a potential target for prevention of stroke and reducing the clinical severity. Studies on the role of statins and antioxidants on Ox-LDL and paraoxanase activity may be of importance in a therapeutic point of view. Dietary or pharmacological interventions which may increase paraoxonase activity may be studied which may prove to have application in preventing atherosclerosis.

LIMITATION

The study not explored the relation between Ox-LDL and infarct volume.

CONCLUSION

Elevated Ox-LDL and lower serum paraoxanase activity in patients with ischemic stroke. Ox-LDL levels were found to be elevated more in stroke patients with anterior circulation infarct compared to those with posterior circulation infarct. This implies the use of Ox-LDL as a specific marker in absence of other risk factors for stroke. Standardization of assays, sensitivity, specificity and cost benefit issues will need to be further determined before Ox-LDL can be recommended as available biomarker for clinical practice. This also implies the importance of using antioxidants and statins in the prevention of stroke and reducing the clinical severity. Fasting blood sugar and systolic BP was found to be significant risk factors in the pathogenesis of ischemic stroke in the present study.

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