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OXIDIZED LDL AND PARAOXANASE STATUS IN ISCHEMIC STROKE PATIENTS

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Abstract : Stroke is the leading cause of mortality and long term disability among adults in industrialized countries. Oxidative stress is an independent risk actor by inducing production of oxygen free radicals in ischemic stroke. Because a relationship may be associated between ox-ldl and paraoxanase, the aim of this study was to investigate the association between ox-ldl and paraoxanase in ischemic stroke patients by determining whether ox-ldl is a useful marker for monitoring oxidative stress in ischemic stroke patients. Our study included 100 patients of ischemic stroke and 100 controls. Compared with controls ox-ldl was significantly raised in stroke patients and Paraoxanase activity was low. Our findings suggest that there is a significant association between raised plasma ox-ldl and decrease activity of Paraoxanase with age indicating that plasma ox-ldl may reflect oxidative stress in ischemic stroke patients.

Key words : ischemic stroke ox-ldl paraoxanase

INTRODUCTION

Stroke is the leading cause of mortality and of long term disability among adults in industrialized countries (10, 11). Early detection and control of risk factors such as hypertension, smoking and diabetes mellitus are thought to be crucial in reducing the risk of stroke and providing effective care. However the risk of stroke may differ depending on the etiology of stroke. Oxidative stress is a characteristic of ischemic stroke. The event results in generation of free radicals leading to promotion of lipid peroxidation. This not only affect low density lipoproteins (ldl) and other lipoproteins but also cellular lipids including those in the arterial wall and macrophages (1, 7, 15). An increase in oxidative products can contribute to atherosclerosis and vasomotor tone. The lack of direct evidence on the role of oxidatively modified low density lipoprotein in patients with ischemic stroke partly reflects lack of sensitive tools with which to measure ox-ldl levels. Human serum paraoxanase (Paraoxanase) [(E.C 3.1.8.1) aryldialkyl phosphatase] is a protein of 354 amino acids (molecular mass 43kDa), synthesized in the liver and has antiatherogenic activity due to its protective

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function against low deoxidation. Paraoxanase prevents oxidative modification of low density lipoprotein (9). Because a relationship may be hypothesized between Paraoxanase and ox-ldl the aim of this study was to investigate the association between ox-ldl and Paraoxanase in ischemic stroke patients by determining whether oxidative stress is an useful marker in ischemic stroke patients.

MATERIAL AND METHODS

The study was case controlled in design. We have selected the patients as they have presented. Patients included in the present study were all admitted to the Intensive Care Unit (ICU) or attending the outpatient department of Medicine of Maharaja Yashvantrao Hospital attached to Mahatma Gandhi Memorial College, Indore (M.P). The study group consisted of 100 patients with ischemic stroke between 60–75 years of age and they were undergoing admission to hospital and 100 age and sex matched controls were taken with no family history of stroke. Brief clinical history covering the signs and symptoms, past, personal and family history of concerned risk factors were taken. All participants gave written informed consent and this protocol was approved by ethical and research committee of Mahatma Gandhi Memorial Medical College, lndore. Table-I gives the details of the profiles of the subjects.

Inclusion criteria

Obesity patients were considered with waist circumference >102 cm (40 inch) for men and >88 cm (35 cm) for women. Systemic hypertension was considered to be present if the blood pressure was recorded >140 mm Hg as Systolic blood pressure or >90 mm Hg as diastolic blood pressure. Venous blood samples were collected from all the study subjects after an overnight fast. Plasma ox-ldl was determined by ELISA method. The ldl-c fraction was separated from blood plasma by sequential ultracentrifugation. Diluted ldl-c fractions were added to the microtitre wells that were precoated with anti ox-ldl monoclonal antibody DLHS. After extensive washing the remaining ox-ldl was detected with a sheep antihuman apo B antibody and an alkaline phosphatase conjugated anti sheep IgG antibody.

In each ELISA plate, various concentrations of standard ox-ldl which was prepared by incubating ldl-c with coppersulphate at 37°C for 3 hours were run simultaneously to determine standard curve. Serum Paraoxanase activity was measured by using 5.5 mM/L pnitrophenyl acetate (Sigma chemicals, USA) as a substrate, the increase in the absorbance of p-nitrophenol formed at 412 nm was measured by using ELICO spectrophotometer (3).

The activity of Paraoxanase was measured in 20 mM/L Tris buffer at pH8.0 and which contains 1 mM calcium chloride. The generated product of p-nitrophenol was calculated by using molar extinction coefficient of 17000 per mole per cm at pH 8.0. Results are expressed as U/ml. (1U, 1 nmol p-nitrophenol formed per minute).

Statistical analysis

All values are presented as mean±s.d. Statistical significance was analyzed by student ‘t’ test and correlation between

variables were studied by using Pearson's correlation coefficient test. The level of significance was set at P<0.05.

RESULTS

The clinical characteristics of ischemic stroke patients and control subjects are presented in Table I. Among 100 ischemic stroke patients, 70% were males and 30% were females, among 100 controls 64 were males and 36 were females. There are 52 patients with obesity and only 19 subjects among controls were obese and they are statistically significant (P<0.001). Among 100 ischemic stroke patients 58% had the history of hypertension and 26% in control group which was statically significant (P<0.001).

TABLE I: Baseline characteristics of study subjects.

Particulars	*Patients (n=100)*	*Controls (n=100)*
Age (years)	70.0±7.5	68.2±4.1
Male/Female	70/30	64/36
HTN (%)	58%	26%
Obesity	52	19

HTN=Hypertension

Table II shows the plasma ox-ldl levels and Paraoxanase activity status in patients as well as controls. Plasma levels of ox-ldl increased in stroke patients as compared to controls (P<0.001) and serum levels of Paraoxanase activity decreased in stroke patients as compared to controls (P<0.001).

As indicated in Table III ox-ldl showed a positive correlation and paraoxanase showed a negative correlation with age.

TABLE II: Serum ox-ldl and paraoxanase concentration in study subjects.

Particulars	*Patients (n=100) Mean±SD*	*Controls (n=100) Mean±SD*	*P-value*
ox-ldl (mg/dl)	4.62±0.36	2.56±0.32	P<0.001*
Paraoxanase (U/ml)	443.3±0.4	157.9±4.8	P<0.001*

ox-ldl-oxidized-ldl, Paraoxanase-Paraoxanase
*Highly Significant (P<0.001) v/s controls.

TABLE III: Correlation coefficients of ox-ldl and Paraoxanase levels between 60–75 years in ischemic stroke patients v/s controls.

Variable	*Particulars*	*Patients (n=100)*	*Controls (n=100)*
Age	Ox-ldl (r)	0.21	0.46
(60–75)	Paraoxanase (r)	–0.97	–0.68

r=correlation coefficient; ox-ldl=oxidized-ldl; PON=Paraoxanase.

DISCUSSION

The study was conducted on 100 confirmed cases of ischemic stroke and 100 age and sex matched controls. A murine monoclonal antibody has been established (DLH3), which recognizes oxidatively modified lipoproteins in plasma and foam cells in atherosclerotic lesions (5, 6). This antibody reacts with several oxidized products of phosphatidylcholine and it has been observed increased plasma ox-ldl concentrations in patients with ischemic stroke (2, 4, 18). It is speculated that plasma ox-ldl level in patients with ischemic stroke would be increased and could serve as a marker of ischemic stroke. Another explanation for the elevation of plasma ox-ldl in ischemic stroke patients as reported in our study is because that lipolysis is

increased in brain regions subjected to ischemia and that lipid peroxidation products are increased in plasma, the source of ox-ldl detected by DLH3, recognizing oxidized phophatidylcholine, may in part therefore be oxidized phospholipids released from brain tissues into the circulation. Similar results have been reported by Polidori MC and Nishi K et. al. (12, 13).

As indicated in Table-III a positive correlation between ox-ldl and age indicates that old people often have dysfunction in their endothelial cells and are more vulnerable to oxidative stress. Ldl oxidation, which is maintained by an internal defence system against oxidative stress, may overcome the defences after ischemic stroke. As a result, uncontrolled oxidative damage in brain cells may occur after an insult. Similar results have been reported by Sun D et. al. (16). Rajagopalan S et. al. (14) and Taddei S et. al. (17). Significantly lower Paraoxanase activities have been reported after ischemic stroke when compared with age and gender controls. Decreased serum activity has been reported with other states associated with increased atherosclerosis including ischemic stroke and renal diseases. Therefore, paraoxanase activity may reflect the antioxidant and antiatherogenic capacity (8).

In conclusion we noted elevated ox-ldl levels and lower mean serum Paraoxanase activity in ischemic stroke patients. We hypothesised that elevated ox-ldl levels and lower Paraoxanase activity may contribute for the development of oxidative stress. The findings suggest that a raised ox-ldl level reflects oxidative stress in ischemic stroke patients and is an useful independent marker of ischemic stroke but further studies are required to resolve the association between reduced Paraoxanase activity and raised ox-ldl levels in ischemic stroke patients with age. The present study confirms that there is an elevated ox-ldl levels and reduced Paraoxanase activity in ischemic stroke patients with age and emphasizes the importance of these markers for early diagnosis and therapeutic interventions.

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