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Original Article

Biochemistry Section

Relation of Paraoxonase1, Arylesterase and Lipid Profile in Ischemic Stroke Patients

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ABSTRACT

Background: Paraoxonase 1 (PON1) is an enzyme associated with High density lipoprotein (HDL) in blood and it is considered to have antioxidant and antiatherogenic properties. PON1 plays an important role in protecting HDL and especially low density lipoprotein (LDL) from oxidative modification by hydrolyzing lipid peroxides which are known to be associated with many vascular diseases including atherosclerosis and ischemic stroke.

Aim: The aim of the study was to evaluate and correlate serum paraoxonase (PON1) and arylesterase (ARE) activities as well as lipid profile levels in patients with ischemic stroke.

Materials and Methods: The study population was comprised of 50 ischemic stroke patients and 50 healthy controls. The serum PON1 and ARE activities were measured spectrophotometrically by using paraoxon and phenylacetate as substrate respectively by Eckerson method. Serum lipid was measured using routine biochemical method.

Results: The normality of the distribution of the parameters are assessed by Shapiro-Wilk test. Two sample t-test is applied for hypothesis testing. The serum PONI and arylesterase ARE decreased significantly in ischemic stroke patients ($p<0.001$). The PON1 was positively correlated with HDL.

Conclusion: This study strongly suggests that the estimation of HDL-C associated PON1 enzyme gives valuable information for prediction of risk of ischemic stroke due to cerebrovascular thromboembolism. The result shows that PON1 and ARE could be considered as a risk factors for ischemic stroke.

Keywords: Atherosclerosis, HDL, LDL oxidation, Paraoxon

INTRODUCTION

Stroke is one of the leading causes of mortality and morbidity worldwide. Approximately 20 million people suffer from stroke each year [1]. Stroke is the third commonest cause of death after Coronary Heart Disease (CHD) and cancer of all types in worldwide and also in India. Among Asians, the number of people who died from stroke was more than three times than that of people with CHD [2]. Stroke or a cerebral vascular accident or brain attack is defined as the abrupt onset of a neurologic deficit that is attributable to a focal vascular cause or in other words it is the sudden death of brain cells due to inadequate blood flow [3]. Cerebral ischemia or ischemic stroke is caused by a reduction in blood flow that lasts for longer than several seconds or ischemia occurs when one of the arteries that bring blood to a part of the brain becomes blocked by a blood clot or a cholesterol plaque. The resulting lack of oxygen and nutrients in these areas causes neurons to stop functioning. Atherosclerosis of carotid artery is one of the most common causes of ischemic stroke. It is the slowly progressive disease of medium to large sized muscular arteries and large elastic arteries characterized by elevated focal intimal fibro fatty plaque called atheroma (also called atheromatous or atherosclerotic plaque) [4,5]. Inflammatory response to tissue injury is initiated by different inflammatory mediators, includes leucocytes and monocytes induced macrophages which are cellular mediators [4]. The monocytes transforms into macrophages which engulf lipoproteins including oxidized LDL. The lipid oxidation plays a key role in the pathogenesis of atherosclerosis [6].

PON1, aryldialkylphosphatase, (EC 3.1.8.1) is a calcium dependent glycosylated protein consisting of 354 amino acid residues with molecular weight of 43-47 kDa. PON1 is mainly bound to High Density Lipoproteins (HDL) containing apolipoprotein A-1 and clusterin (apolipoprotein J) which is synthesized in the liver, and then secreted into plasma [7-9]. PON1 possess paraoxonase, arylesterase and lactonase activities and hydrolyzes different kinds of substrates. PON1 hydrolyzes oxons like paraoxon, chlorpyrifosoxon and diazoxon which are toxic metabolites of organophosphate insecticides parathion, diazinon and chlorpyriphos [10]. The human PON1 shown to be highly effective in preventing oxidation of LDL and also inactivates LDL-derived oxidized phospholipids as LDL oxidation is the major cause of atherosclerosis [11-13]. It has also been shown that PON1 by inhibiting LDL oxidation, prevents the upregulation of Monocytes Chemotactic Protein-1 (MCP-1) secretion and this may inhibit atherosclerosis at an early stage [14]. PON1 also found to hydrolyze hydrogen peroxide (H_2O_2) which is a major reactive oxygen species produced under oxidative stress during atherogenesis. Thus PON1 preserves antiatherogenic functions of HDL and also protects oxidation of LDL [15].

AIM

Thus, the aim of our study is to estimate serum paraoxonase-1 (PON1) activity and ARE activity and to correlate paraoxonase1and arylesterase with lipid profile and in ischemic stroke patients.

MATERIALS AND METHODS

The study consists of 50 patients diagnosed as having ischemic stroke as cases. The confirmation of diagnosis was done with the clinical symptoms which includes numbness (paresthesia) or weakness (paresis) of the face, arm or leg, usually on one side of the body (hemianesthesia or hemiparesis), difficulty in speaking (expressive aphasia); slurred speech (dysarthria) [3]. Whenever the patient has admitted in the hospital, the next day morning fasting sample was collected. The patients having diabetes mellitus, coronary heart disease, kidney failure, liver diseases, etc. were excluded. The control population consisted of 50 healthy subjects matched for age, gender and attending the routine health check-up in our outpatient department. Written valid informed consent was obtained from all subjects. The study was approved by the institutional ethical committee.

Serum Lipid

The serum cholesterol and HDL-C were determined by the CHOD-PAP method [16,17]. The serum triglycerides were measured by the enzymatic GPO-PAP method [18]. LDL cholesterol was estimated by the Friedewald formula: LDL cholesterol = Total serum cholesterol - (HDL cholesterol +Triglyceride/5) mg/dl [19].

SERUM PARAOXONASE (PON1) ACTIVITY ASSAY: The assay mixture included 2 mM/L paraoxon, 2 mM/L $CaCl_2$ dissolved in 100 mM/L Tris-HCl buffer, pH 8.0. The rate of p-nitrophenol formation was measured spectrophotometrically at 405 nm over 200 s with a 25 s lag time. One unit of paraoxonase activity produces 1 nmol of p-nitrophenol and the activity is expressed as U/L based on the molar absorption coefficient (18050 M-1cm-1) at 405 nm at pH 8.0 [20].

SERUM ARYLESTERASE ACTIVITY ASSAY: The assay mixture contains 4.0 mM/L phenylacetate, 1 mM/L $CaCl_2$ dissolved in 20 mM /L TrisHCl buffer, pH 8.0 at 25^{0}C. The rate of phenol formation was measured at 270 nm following 20s lag time. One unit of arylesterase activity is equal to 1 mM of phenylacetate hydrolysed per min. The activity is expressed as kU/L based on the extinction coefficient of phenol of 1310 M-1cm-1 at 270 nm, pH 8.0, and 25^{0}C after correction for non-enzymatic hydrolysis [20].

STATISTICAL ANALYSIS

Statistical data were analysed with MYSTAT student version. The results were presented as mean ± standard deviation. The continuous variables tested for normality with Shapiro-Wilk test. Student's unpaired t-test used for statistical analysis between cases and controls for numerical variables in guassian distribution. The strength of association between two parameters is expressed by the Pearson's correlation coefficient. The value p<0.001 were considered to be significant.

RESULT

In [Table/Fig-1], it shows serum levels of total cholesterol, and low density lipoprotein- cholesterol are higher in cases than in controls and are statistically significant, triglyceride and very low density lipoprotein levels are higher in cases and not significant.

Parameters	Cases	Control	p-value
Total Cholesterol (mg/dl)	201.66 ± 38.73	177.94 ± 28.79	< 0.001*
Triglyceride (mg/dl)	174.90 ± 36.94	167.12 ± 36.75	NS
High density lipoprotein-Cholesterol (mg/dl)	30.46 ± 7.42	35.48 ± 6.97	< 0.001*
Low density lipoprotein-Cholesterol (mg/dl)	136.40 ± 35.88	108.62 ± 30.06	< 0.001*
Very Low density lipoprotein-Cholesterol (mg/dl)	34.56 ± 7.68	31.84 ± 7.37	NS
Paraoxonase activity (U/L)	73.38 ± 27.39	116.42 ± 30.88	< 0.001*
Arylesterase (kU//L)	87.42 ± 21.49	118.68 ± 23.61	< 0.001*

[Table/Fig-1]: Serum paraoxonase activity of PON1 in cases and control
*- Significant, NS - Not significant

However, serum HDL-C levels are decreased significantly in ischemic stroke patients when compared with control group. Serum paraoxonase (PON1) activity in cases (73.38± 27.39 U/L) is significantly decreased as compared to control group (116.42±30.88U/L). The serum arylesterase activity also showed significant decrease in cases (87.42±21.49 kU/L) as compared to control group (118.68 ± 23.61kU/L).

DISCUSSION

Ischemic stroke is a heterogeneous pathophysiological entity. The hyperlipidemia is one of risk factor related to acute cerebral infarction or ischemic stroke and having relative risk of 1.8-2.6 of developing stroke [3]. Dyslipidemia is the condition in which lipoprotein pattern is altered or deranged due to existing pathological conditions. It is well-known that the concentration of cholesterol in High-Density Lipoproteins (HDL) has an inverse correlation while serum total cholesterol, triglyceride and LDL-C levels are positively correlated with that of atherosclerosis. In the present study, there was significant change in lipoprotein pattern in cases as compared to controls. Serum Total Cholesterol (TC) levels and serum LDL-C levels were found significantly higher in cases than in the controls and significant lower levels of HDL-C in cases than in controls. These deranged serum lipoprotein values in ischemic stroke shows that it may promote the development of atheroma in the carotid artery wall. There are number of studies which support our findings of hypercholesterolemia. In a large case-control study of Tirschwell DL et al., showed higher total cholesterol were associated with increased risk of ischemic stroke [21].

The present study evaluated the role of PON1 in ischemic stroke. The paraoxonase and arylesterase activities of PON1 in ischemic stroke cases and controls were assessed. It has been suggested that PON1 is associated with apolipoprotein (Apo) A1 in high-density lipoprotein (HDL) and is capable of preventing HDL and LDL oxidation by hydrolyzing lipid peroxides in the lipoprotein [22]. This is because the antioxidant property of HDL is due to paraoxonase enzyme. Mackness MI and colleagues have shown that HDL has capacity to inhibit LDL oxidation caused by transition metals and also prevents formation of lipid hydroperoxides and oxidation of HDL revert its protective anti-atherogenic effects [23]. G Zuliani et al., in study of the anti-atherogenic properties of HDL particles showed that the HDL exert anti-inflammatory activity by inhibiting the expression of adhesion molecules by endothelial cells and the transmigration of monocytes [24]. HDL has antioxidant activity through the anti-oxidative properties of apolipoprotein A1 and the presence of enzymes such as paraoxonase, glutathione-peroxidase, and platelet activating factor (PAF) acetylhydrolase. It has also an antithrombotic effect by inhibiting platelets aggregation and beneficial effect on endothelial function.

Aviram M et al., have demonstrated that the human serum paraoxonase has the capacity to reduce oxidized lipids in human atherosclerotic lesions derived from either carotid or coronary artery specimens [25]. The findings of the present study show that HDL associated paraoxonase and arylesterase activities were significantly decreased in cases as compared to control subjects. This suggests that decrease in antioxidant capacity in ischemic stroke. Moreover HDL-C levels were significantly positively correlated with PON1 activity (r=0.328, p<0.001). Interestingly similar finding were observed in case-control study by Kim NS et al., have evaluated the serum activity and concentration of PON1 in Korean ischemic stroke and in age and gender matched control healthy subjects [26]. They found significantly lower activity and concentration of serum PON1 in cases than control group. Additionally, they observed PON1 activity was negatively correlated with age whereas it was positively correlated with HDL-C in stroke group. The measures to decrease oxidation of HDL can reduce the disease conditions in stroke patients.

LIMITATION

The limitation of our study is small sample size.

CONCLUSION

The present study clearly shows changes in the lipoproteins with increase in total cholesterol, triglyceride, LDL-C, and decrease in HDL-C levels in ischemic stroke. The decreased antioxidant defense due to decreased activity of PON1 further deteriorates the condition leading to increased risk for ischemic damage. So PON1 assays seem to add more important information than the simple HDL quantity assessment. Thus we conclude that the estimation of HDL-C associated PON1 enzyme and arylesterase activity gives

valuable information for prediction of risk of ischemic stroke due to cerebrovascular thromboembolism while further studies are needed. This can also be used as promising novel biomarker applied for monitoring of future antioxidant treatment.

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