Association of atherogenic cardiac index and markers of oxidative stress in non-diabetic dyslipidemia stroke patients in a tertiary care hospital in Eastern India

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ABSTRACT

Background: Stroke is a clinical syndrome consisting of rapidly developing clinical signs of focal or global disturbance of cerebral function. Antioxidants are exogenous or endogenous compounds acting by scavenging reactive oxygen species or their precursors, inhibiting reactive oxygen species formation and binding metal ions needed for the catalysis of its generation. Atherogenic index, a predictor of atherosclerosis, increases in dyslipidemia also shows a positive association with oxidative stress. Aims and Objectives: Our objective was to compare the lipid profile, the markers of lipid peroxidation (malonaldehyde and paraoxonase (PON1), and atherogenic index in non-diabetic stroke patients with healthy controls and to evaluate the correlation between the lipid parameters with markers of lipid peroxidation and atherogenic index in those patients. Materials and Methods: One hundred diagnosed stroke patients from the Neurology department were compared with 100 age-matched healthy controls. Estimation of lipid profile parameters was done by AutoAnalyzer using standardized kits. Estimation of serum malonaldehyde was done based on the principle of utilization of Thiobarbituric acid. Serum PON1 activity was estimated using paranitrophenyl acetate substrate. Results: Among the lipid profile parameters, high-density lipoprotein (HDL) was significantly lower \( (P<0.001) \) and low-density lipoprotein (LDL) was significantly higher among stroke patients. Serum malonaldehyde level was significantly increased and PON1 activity was significantly decreased in stroke patients in comparison to healthy individuals. A significant positive correlation between HDL and PON1 and a negative correlation between LDL and PON1 were found. Atherogenic index is negatively correlated with PON1. Conclusion: Low level of PON1 activity and an increased level of malonaldehyde were found in stroke patients indicating an imbalance between free radical generation and its scavenging action and also associated with more chance of atherosclerosis.

Key words: Paraoxonase1; Malonaldehyde; Antioxidant

INTRODUCTION

Cerebrovascular accidents have affected the richest of the rich to the poorest of the poor in the history of mankind. It is characterized by focal or global neurological disturbances lasting for more than 24 h. It is grossly classified into two groups: Hemorrhagic and ischemic. Though transient ischemic attack is another entity of neurological disturbance it lasts for only a few seconds.\(^1\)

The pathogenesis of stroke is complex and the etiology is multifactorial but hyperlipidemia, metabolic syndrome, and atherosclerosis are a few important risk factors for both hemorrhagic and ischemic stroke.\(^1,2\)
Oxidative stress occurs in an imbalance of oxidative free radical generation and antioxidant defense by which reactive oxygen species accumulate in the body in excess amounts. These highly reactive substances induce cell damage and apoptosis. Hence, it is responsible for a number of pathological conditions including premature aging and cancer. Atherosclerosis which is an inevitable sequelae of uncontrolled dyslipidemia is also associated with oxidative free radical accumulation. Hence, the quantification of oxidative stress is very important not only for the prediction of developing cerebrovascular accidents but also for the prediction of prognosis and complications.

Free radicals that are formed induce lipid peroxidation of polyunsaturated fatty acids and damage biological membranes producing malonaldehyde (MDA) as one of the end products of lipid peroxidation. The pathophysiological processes involve the increased permeability of the blood-brain barrier, energy failure, loss of cell ion homeostasis, acidosis, increased intracellular calcium, apoptosis, and neuronal death.

Human serum paraoxonase 1 (PON1) is an enzyme that has PON as well as arylesterase activity and is physically bound to high-density lipoproteins (HDL). It plays a key role in the biological action of HDL by protecting the lipoprotein, and by preventing the oxidative damage of the HDL biological membrane. Serum PON1 is almost exclusively found in association with HDL particles. The lipid composition of HDL can influence its size and structure and is determined by a combination of various factors including dietary intake, metabolism, and storage. Hence, dietary fats may affect PON1 activity by changing the phospholipids composition of HDL. On the other hand, it seems that any variation in HDL fatty acid composition could alter the function of HDL and the activity of its related enzyme, PON1.

Studies have shown that PON1 contributes to the atheroprotective function of HDL by decreasing lipid peroxidation along with an anti-inflammatory component. Though the exact mechanism is not known, it is widely believed that PON1 inhibits the oxidative inactivation of Lecithin Cholesterol Acyl Transferase, well known for the reverse cholesterol transport enzyme associated with HDL. Recent studies have demonstrated that HDL is extremely potent in protecting LDL against oxidative modification.

Atherogenic index of plasma (AIP) is defined as the logarithm of the ratio of plasma triglyceride concentration to HDL concentration (\( AIP = \log \frac{\text{TG}}{\text{HD}} \)). It is based on TG and HDL concentrations, both of which are independent risk factors for coronary artery disease. Recently, AIP has been used as a predictor of atherosclerosis. Individuals with AIP values of \(-0.3 \sim 0.1\) are categorized as low risk, those with \(0.1 \sim 0.24\) are categorized as medium and individuals with an AIP of more than 0.24 are categorized as patients with high cardiovascular risk.

**Aims and objectives**

Our aim was to find the relation of oxidative stress and atherosclerosis to of in stroke. The objectives of the study are to find the association between the atherogenic index and the markers of oxidative stress in non-diabetic dyslipidemia stroke patients attending tertiary care hospitals in Eastern India.

**MATERIALS AND METHODS**

This observational analytical study was done in the Department of Biochemistry, IPGME&R, Kolkata, with collaboration of the Department of Neuromedicine of Bangur Institute of Neurosciences Kolkata from January 2019 to March 2021 after obtaining the approval of the Institutional Ethics Committee (Memo No. IPGME&R/IEC/2017/069 dated February 06, 2017). One hundred diagnosed stroke patients from the outpatient department and inpatient department of the department of neuromedicine were compared with 100 age-matched (18–65 years) healthy controls. Informed consents were taken from the patients before inclusion in the study. Open EPI version 3 software was used to calculate the sample size. Patients with any history of diabetes mellitus, or malignancy, on any hypolipidemic and antipsychotic medications, and pregnant women were excluded from the study.

Estimation of lipid profile parameters and fasting and post-prandial blood glucose was done in AutoAnalyzer ERBA XL 640 using standardized kits.

NCEP ATP III guideline and ADA guideline were used, respectively, for the diagnosis of dyslipidemia and diabetes.

The estimation of malonaldehyde was done by utilization of thiobarbituric acid (TBA) and is based on the acid-catalyzed decomposition of lipid hydroperoxides to malonaldehyde (MDA) that reacts with TBA to form a chromogen (pink colored) evaluated spectrophotometrically at 532 nm.

For estimation of PON activity, paranitrophenol acetate of strength 5.5 mmol/l and activator solution (calcium chloride 20 mmol/L and sodium chloride 155 mmol/L) mixed in Tris HCL buffer (0.1 mmol/L, pH 8) were taken. After adding a diluted serum sample, absorbances were taken at 0, 1, and 3 min at 412 nm in a UV-Vis spectrophotometer T60 manufactured by LABINDIA, Thane, India. The
calculated delta absorbances were finally multiplied with a molar extinction coefficient of 17000/mol/cm to quantify the enzymatic activity.\textsuperscript{11}

The calculation of atherogenic index was performed by the formula $\log_{10}(\text{Triglyceride/HDL})$ for each dyslipidemia individuals (atherogenic index).\textsuperscript{6}

**RESULTS**

Continuous data were expressed as (Mean±SD) and the normality was tested by the Shapiro–Wilk test. Student’s t-test was carried out to compare the parameters of lipid profile, fasting blood sugar (FBS), postprandial blood sugar PPBS, malonaldehyde, PON1, and atherogenic index (AIP) between cases (stroke patients) and healthy controls.

**DISCUSSION**

Cerebrovascular accidents are a rising health problem all over the world. Damaging effect of cerebrovascular accidents on neural tissue arises out of decreased perfusion, hematoma-related mass effect, release of excitatory neurotransmitters (glutamate) release, or developing of free radicals such as ROS, RNS due to oxidative stress. This in turn can activate apoptotic pathways and lead to neuronal cell death. These relate to the poor post-stroke outcome both immediate as well as distant.\textsuperscript{1}

The present study was designed to know whether there is a role for atherosclerosis, as reflected by the lipid profile of the patients, in the occurrence of stroke. Not only that the objective of the present study was also to know if there is any association between developing oxidative stress and dyslipidemia in the pathogenesis of stroke.

We collected the samples of 143 stroke patients (79 were ischemic and the rest were hemorrhagic) out of which 42 patients were found to be diabetic after the estimation of FBS and PPBS and hence excluded from the study (as increased chance of oxidative stress has already been seen in many previous studies). An equal number of apparently healthy controls were chosen to compare.

Significantly increased level of LDL and malonaldehyde has been seen in stroke patients in comparison to the control group where there is a significant fall in HDL and PON1 levels in them (Table 1) in the present study.

Similar findings have also been seen in other previous literature.

The study done Kontos by shows HDL and HDL/ApoA1 ratio is significantly lower and LDL/HDL ratio is higher in ischemic stroke patients in comparison to healthy controls.\textsuperscript{12}

Table 2 in our study shows a significant positive correlation between HDL and PON1 and a negative correlation between LDL and PON1. Malonaldehyde is negatively correlated with HDL and positively correlated with LDL.

In the study of Kesavulu et al., the serum malondialdehyde in spontaneous intracerebral hemorrhage patients pointed out increased MDA levels in them which is significant when compared to healthy control (P<0.001).\textsuperscript{13}

Lipid peroxidation is a well-established mechanism of cellular injury in humans and is used as an indicator of oxidative stress in cells and tissues. It is suspected that increased levels of lipid peroxides may be due to oxidation of blood or neural lipids by ischemia. Brain nucleic acids may be metabolized to purine and nucleoside bases resulting in excess of adenosine which then becomes substrate for xanthine oxidase pathways that are important in the generation of free radicals. The involvement of lipid peroxidation in CVA was confirmed by the significantly higher concentration of MDA observed in stroke patients compared with controls. This increased level is because

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases (Mean±SD)</th>
<th>Controls (Mean±SD)</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS (mg/dl)</td>
<td>84.66±24.27</td>
<td>75.25±±8.120</td>
<td>3.67</td>
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<tr>
<td>PPBS (mg/dl)</td>
<td>149.3±65.413</td>
<td>119.1±9.210</td>
<td>2.17</td>
<td>0.872</td>
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<td>Cholesterol (mg/dl)</td>
<td>157.1±44.88</td>
<td>139.7±37.501</td>
<td>2.10</td>
<td>0.037</td>
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<tr>
<td>Triglyceride (mg/dl)</td>
<td>127.5±19.075</td>
<td>124.5±19.34</td>
<td>1.98</td>
<td>0.422</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>41.4±10.562</td>
<td>41.0±10.316</td>
<td>1.18</td>
<td>0.843</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>41.8±8.686</td>
<td>52.8±8.510</td>
<td>5.45</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>119.2±19.769</td>
<td>91.55±12.33</td>
<td>6.94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Malonaldehyde (U/L)</td>
<td>34.45±10.060</td>
<td>16.99±6.57</td>
<td>6.82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Paraoxonase1 (U/L)</td>
<td>137.4±26.19</td>
<td>170.5±32.31</td>
<td>6.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atherogenic index</td>
<td>2.11±0.11</td>
<td>0.23±0.09</td>
<td>147.4</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

FBS: Fasting blood sugar, PPBS: Postprandial blood sugar, VLDL: Very low-density lipoprotein, HDL: High-density lipoprotein, LDL: Low-density lipoprotein.
those lipid peroxidation products are a key mediator of apoptosis induced by oxidative stress and antioxidants that suppress lipid peroxidation have been shown to protect against apoptosis induced by oxidative insults.\textsuperscript{1,13}

Serum PON1 is a glycoprotein synthesized mainly by the liver and it circulates along with HDL molecule. However, without binding with HDL, PON 1 is catalytically inactive.\textsuperscript{14} The enzyme also plays an important role in decreasing oxidative stress by free radical scavenging in the human body. It plays a significant role in inhibiting the oxidation of both LDL and HDL particles; thus, PON1 has a protective role against atherosclerosis and cardiovascular disease.\textsuperscript{14}

Table 2 also shows that there is a significant negative correlation between PON1 and malonaldehyde. The finding is quite obvious because PON1 neutralizes the oxidative stress which is in contrast increased by the malonaldehyde as stated in many other previous literature.\textsuperscript{1,13}

In Table 1, we have also seen that the AIP was higher and PON1 was lower in cases. Table 2 also shows a significant negative correlation between AIP and PON1. In dyslipidemias, it is therefore evident that there is a high risk of atherosclerosis as well as cerebrovascular diseases as corroborated by many other similar studies.\textsuperscript{17} Here, it may be pointed out that the levels of PON1 activity were also very low in stroke patients. Therefore, it may be safely concluded that low levels of PON1 activity may be associated with high cerebrovascular risks. In the future, the risk of CVA may be predicted by estimation of PON1 activity in the serum.

**Limitations of the study**

The most important limitation of the study is the lack of established reference ranges of PON1 in the population and the fact that there are no gold standard methods for the estimation of PON1 activity so far.

**CONCLUSION**

Low PON1 activity is associated with high chances of atherosclerosis and increased chances of cerebrovascular diseases.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r-value</th>
<th>P-value</th>
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<tbody>
<tr>
<td>HDL and PON1</td>
<td>0.152</td>
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<td>LDL and PON1</td>
<td>−0.427</td>
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<tr>
<td>Malonaldehyde and HDL</td>
<td>−0.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Malonaldehyde and LDL</td>
<td>0.031</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Malonaldehyde and PON1</td>
<td>−0.031</td>
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<tr>
<td>AIP and PON1</td>
<td>−0.425</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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