Evaluation of Oxidative Stress and Antioxidant Level of Stroke Patients in Osun State, South-Western Nigeria

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Abstract
Stroke is a leading cause of morbidity and mortality worldwide. Oxidative stress which is as a result of increased activity of free radicals and decreased antioxidant mechanism plays a major role in the pathogenesis of stroke. This study determined the plasma level of malondialdehyde (MDA), a marker of lipid peroxidation and oxidative stress and enzymatic Antioxidants namely, superoxide dismutase (SOD), Glutathione peroxidase (GPx), and Catalase (CAT) in sixty stroke patients of both sexes attending Wesley Guide Hospital, Obafemi Awolowo University Teaching Hospital, Ilesa, Osun State. An equal number of healthy subjects were used as control. The result of this study revealed a significantly (p < 0.05) high plasma level of MDA in stroke patients when compared with the control subjects. Plasma level of SOD in stroke patients was significantly higher (p < 0.05) than the control subjects while plasma GPx and CAT level were found to be significantly lower (p < 0.05) in stroke patients than the control subjects. The result of the study indicated high level of lipid peroxidation and oxidative stress in stroke patients and high degree of free radicals presence as indicated by the high level of SOD.

Keywords
Stroke, Malondialdehyde, Catalase, Superoxide Dismutase, Glutathione Peroxidase, Lipid Peroxidation

1. Introduction
Stroke is a form of cardiovascular disease that decreases blood supply to the brain which results in rapid loss of brain function. This can be due to ischemia (lack of blood flow), or by blockage (thrombosis, arterial embolism) [1] or a hemorrhage. As a result, the affected area of the brain cannot function, which
might result in an inability to move one or more limbs on one side of the body, inability to understand or formulate speech, or an inability to see one side of the visual field [2].

The symptoms experienced by stroke patient will depend on which part of the brain is affected. It is a medical emergency and can cause permanent neurological damage and death. Risk factors for stroke include old age, high blood pressure, previous stroke or transient ischemic attack (TIA), diabetes, high cholesterol, tobacco smoking and atrial fibrillation [3]. Stroke is the second leading cause of death worldwide and also the leading cause of acquired adult disability with high socio-economic impact [4].

Oxidative stress arises from an imbalance between the generation of reactive oxygen (ROS) and nitrogen species (RNS) and the antioxidant defense system in the body so that the latter become overwhelmed [5]. Antioxidant such as reduced glutathione (GSH), vitamin C, vitamin E, SOD, catalase (CAT), GSH peroxidases (GSH-Pxs), are present in cells to remove ROS and RNS. Oxidative stress is a subject of intensive investigation and is considered to be one of the mechanisms involved in neuronal damage due to ischemia/reperfusion in stroke patients [6].

Lipid peroxidation is a free radical related process, which is potentially harmful because its uncontrolled, self-enhancing process causes disruption of membranes, lipids and other cell components. A lot of oxygenated compounds, particularly aldehydes such as Malondialdehyde (MDA) are produced during the attack of free radicals to membranes, lipoprotein and polyunsaturated fatty acids [7]. Thus lipid peroxidation in the blood provides useful information for the prognosis of CVD patients. This imbalance will cause damage to cellular components and tissues in the body leading to oxidative stress and as well as the decrease in total antioxidant capacity.

This work evaluates the plasma levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) in stroke patients and healthy subjects with a view to providing information on diagnosis and treatment.

2. Methodology

This study included 60 freshly diagnosed stroke patients at the outpatient department of Wesley Guide Hospital, Obafemi Awolowo University Teaching Hospital, Ilesa, Osun State, and the same number of normal healthy subjects, without any known disease. Blood pressure was taken on the left arm after 5 minutes’ relaxation, in a sitting position, using a standard mercury sphygmomanometer with appropriate cuff size; systolic (SBP) and diastolic (DBP) blood pressures corresponded to Korotkoff sounds I and V, respectively. The average of three readings, taken at first visit, was used for further analysis. Height and body weight were measured with participants standing without shoes and heavy outer garments. 5 ml of venous blood was collected into a heparin bottle and centrifuged. The plasma was collected and placed in another bottle containing...
no anticoagulant for analysis. The total amount of lipid peroxidation products present in the samples was estimated by the thiobarbituric acid (TBA) method which measured the malondialdehyde (MDA) reactive products according to the method of Ohkawa et al. (1979) [8]. Catalase activity was colorimetrically assayed as described by Sinha (1972) [9]. SOD determination was done by the method of Fukui et al. (2003). Glutathione peroxidase was measured by the method described by Rotruck et al. (1973) [10]. Statistical analysis of result was done using one-way analysis of variance; level of significant difference was set at p < 0.05. All patients gave their informed consent, and this study was approved by the Institutional Review Board of the Hospital.

3. Discussion

Stroke is a form of cardiovascular disease affecting blood supply to the brain, which is caused either by a blockage or a rupture in the vessel supplying blood to the brain. From the result of this work, as shown in Table 1, there is a significant increase (p < 0.05) in the age and blood pressure of stroke patients respectively when compared with control subjects respectively which confirm the fact that high blood pressure (hypertension) and old age are factors in the development of stroke. This finding supports the report that age is the most important risk factor in developing cardiovascular or heart diseases [11]. It also correlates with data obtained from a study done on stroke patients in Northern Manhattan where the mean age was 68.8% [12]. Multiple explanations have been proposed to explain why age increases the risk of cardiovascular/heart diseases. One of them is related to serum cholesterol level. In most populations, the serum total cholesterol level increases as age increases [13]. The plasma level of MDA in stroke patient as shown in Table 2 is significantly higher (p < 0.05) when compared with the control. MDA which is a breakdown product of lipid peroxidation has been found to increase in stroke patient [14] [15]. The significantly high level of MDA in stroke patients as revealed in this study agrees with the outcome of a study by Abdulkaldar et al. (2007) [16]. who reported a high MDA level in the serum of stroke patients when compared with the control group. This he attributed to the fact that cellular membranes in the brain are very rich in polyunsaturated fatty acids, which are especially sensitive to free radical attack. The result of the study in Table 2 reveals low Glutathione (GPx) activity in stroke patients when compared with the control. This was consistent with findings of Demirkaya et al. (2001) [17], who reported that GPx in the erythrocyte of patients with hemispheric ischemic stroke decreased significantly within 24 hrs after the onset of stroke symptoms. The results also agree with the report of Seema et al. (2010) [18], who carried out a study on the role of antioxidants in cardiovascular diseases reported a decreased level of GPx in stroke patients and ischaemic heart disease patients The activity of SOD was significant (p < 0.05) higher in stroke patient than the control subjects as shown in Table 2. The increase in the activity of SOD as revealed in this study consisted with the report of Gruener et al. (1994) [19].
Table 1. Parameters showing the anthropometric measurement of stroke patients and control subjects.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Stroke Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (YRS)</td>
<td>24.50 ± 1.63b</td>
<td>61.0 ± 13.28a</td>
</tr>
<tr>
<td>Weight (KG)</td>
<td>53.66 ± 22.6b</td>
<td>83.5 ± 17.52a</td>
</tr>
<tr>
<td>Height (M)</td>
<td>1.48 ± 0.2a</td>
<td>1.62 ± 0.20a</td>
</tr>
<tr>
<td>B/P (mm/HG)</td>
<td>116.6/68.6b</td>
<td>193.4/105.3a</td>
</tr>
<tr>
<td>BMI (KG/M²)</td>
<td>24.5b</td>
<td>31.6a</td>
</tr>
</tbody>
</table>

Results are presented as means ± standard deviation. Values with different superscript are significantly different. B/P (Blood pressure), BMI (Body mass index).

Table 2. Parameters of blood plasma showing the mean malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) value of stroke patients and control subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Stroke Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/L)</td>
<td>206.95 ± 30.23b</td>
<td>217.3 ± 16.11a</td>
</tr>
<tr>
<td>CAT (U/L)</td>
<td>50.46 ± 12.55a</td>
<td>36.9 ± 16.33a</td>
</tr>
<tr>
<td>GPx (pg/ml)</td>
<td>533.3 ± 180.9a</td>
<td>186.91 ± 48.82b</td>
</tr>
<tr>
<td>MDA (nmol/L)</td>
<td>1.98 ± 0.42a</td>
<td>6.32 ± 1.36a</td>
</tr>
</tbody>
</table>

Results are presented as means ± standard deviation. Values with different superscript are significantly different.

There is a significant decrease in the activity of catalase in the stroke patients when compared with the control subjects. The increase or decrease in the activity of antioxidant enzymes in stroke patients may result from the degree of damage by reactive oxygen species (ROS).

4. Conclusion

This study confirms the fact that high blood pressure is a major factor in the prognosis of stroke; it also reveals a possibly high level of free radicals and lipid peroxidation in stroke patients which are factors that could be monitored to control and manage this life threatening disease.

Acknowledgements

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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