Erythrocyte antioxidant enzymes in Parkinson’s disease

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Background & objectives: Oxidative stress is incriminated to play a central role in the pathogenesis of Parkinson’s disease (PD). Oxidative stress, to which neurons are highly susceptible, is also known to induce oxidative changes in human red blood cells (RBCs), in vivo and in vitro. Earlier studies on oxidative stress in RBCs in patients with PD have yielded controversial results claiming unaltered activity to reduced activity. Using RBC as a model, we have undertaken this study to ascertain the possibility of oxidative damage to the RBCs in PD by measuring the cytosolic antioxidant enzymes viz., superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase (G-Px) and glucose-6-phosphate dehydrogenase (G6PD).

Methods: Activities of antioxidant enzymes were measured in erythrocytes of 115 PD patients and 37 normal age-matched healthy persons as controls. Enzymes activities were correlated with age of patients, age of onset of disease, duration of disease, United Parkinson’s Disease Rating Scale (UPDRS) and Hoehn and Yahr stage.

Results: The SOD, CAT, G-Px and G6PD activities were significantly lower in patients with PD compared to the control. A significant (P<0.05) negative correlation of enzyme activities with Hoehn and Yahr stage of the disease and also with UPDRS score was found.

Interpretation & conclusion: Results of the present study showed involvement of oxidative stress as one of the risk factors, which can initiate and/or promote neurodegeneration in PD and was correlated to the severity of the disease.

Key words Antioxidant enzymes - glucose 6-phosphate dehydrogenase - oxidative stress - Parkinson’s disease - superoxide dismutase

Parkinson’s disease (PD) is the second most common neurodegenerative disorder after Alzheimer’s disease, affecting approximately 1 percent of the population older than 50 yr. It is characterized clinically by resting tremor, bradykinesia, rigidity and postural imbalance, and pathologically by the death of dopaminergic neurons in the substantia nigra (SN) with Lewy bodies in surviving neurons. Free radical theory was thought to be as one of the mechanisms involved in the pathogenesis in Parkinson’s disease. Oxidative stress, produced when there is an increased formation or defective inactivation of cytotoxic reactive oxygen species (ROS) can initiate and/or promote degeneration of dopaminergic (DA) neurons in PD. Cytotoxicity of ROS is related to the ability of these molecules to oxidize cell constituents, particularly lipids and nucleic acids which leads to deterioration
of cellular structural architecture and signaling and ultimately death. ROS is also able to trigger both necrotic and apoptotic cell death.

Under normal conditions, the continuous production of free radicals is compensated by the powerful action of protective enzymes. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (G-Px) are the major antioxidant enzymes present in the human body that protect against the oxygen toxicity. Oxidative stress may be a consequence of reduced efficiency of these endogenous antioxidants that may render PD patients more vulnerable to oxidative stress. Glucose 6-phosphate dehydrogenase (G6PD) is essential for keeping glutathione in the reduced state.

There are reports suggesting a decrease in SOD and other antioxidant enzyme activities and increase in various markers of lipid peroxidation in SN of PD patients. Based on the hypothesis that these deficits may not be organ specific, various groups have investigated oxidative stress in red blood cells (RBCs) and yielded controversial results claiming unaltered to reduced activities. The studies by Urakami et al. and Gatto et al. showed that the erythrocyte SOD values were decreased only in treated patients and not in untreated PD group. To ascertain the possibility of oxidative damage to the RBCs in PD, the present study was undertaken in treated PD patients by evaluating the changes in erythrocyte activities of SOD, CAT, G-Px and G6PD and their possible correlation with age, age of onset, duration and stage of the disease was also investigated.

**Material & Methods**

**Samples:** Patients with PD (n=115, 73 males and 42 females) attending movement disorder clinic of Department of Neurology, All India Institute of Medical Sciences, New Delhi between 1999 to 2002 were included in the study. The patients fulfilling our inclusion and exclusion criteria were selected consecutively during the study period. The diagnosis of idiopathic PD was based on the presence of bradykinesia, 4-6Hz resting tremor, rigidity and postural instability not caused by cerebellar or proprioceptive dysfunction. The exclusion criteria for the PD were history of repeated strokes and head trauma, encephalitis, oculogyric crises, neuroleptic treatment within one year of onset of symptoms, more than one affected relatives, cerebellar signs, early severe autonomic disturbance, sustained remission, Balbinski sign, presence of brain tumour and hydrocephalous.

The age (58.2 ± 10.66 yr), disease onset age (54.85 ± 11.55 yr), duration of the disease (4.5 ± 5.19), United Parkinson’s Disease Rating Scale (UPDRS) score (36.46 ± 18.6), modified Hoehn and Yahr staging (1.99 ± 0.89) and England and Schwab activities of daily living (ADL) (80.79 ± 15.53) were collected. All the patients were receiving treatment, spanning from 6 months to 12 yr. Most of the (90%) patients were receiving levodopa treatment either alone or in combination with other drugs. None of the patients were on vitamin E supplement and DA agonist monotherapy. Only two patients were receiving selegeline monotherapy since most of the patients coming here were not in the early stages of the disease. Untreated PD patients could not be included since most of the patients coming to our movement disorder clinic were referred cases. For comparison 37 age-matched normal healthy persons (24 males, 13 females), with mean age 57.17 ± 11.21 yr, were also included. The exclusion criteria were smoking, consumption of alcohol and any medications and vitamins in the previous six months. Since PD is an age-associated disease, we included persons above 45 yr of age and this combined with the exclusion criteria resulted in less number of controls. Blood was collected between 1000 to 1200 in EDTA vacutainers and plasma was removed after spinning the sample at 1200 g. RBCs were washed with normal saline thrice to remove white blood cells and kept at -20°C till analysis. All analysis were carried out within 2 days of blood collection.

The study protocol was approved by the ethics committee of the institute, and written consent was obtained from all patients and controls.

**Assays of antioxidant enzymes:** SOD was extracted by the method of Winterbourn et al. from RBCs. The enzyme present in the clear top layer was assayed by the method of Nishikimi et al. modified by Kakkar et al. CAT was measured by the method of Aebi. G-Px by the method of Flohe and Gunzler, and G6PD by a commercially available kit (Sentinel, Italy).
**Statistical analysis:** The difference between PD and control groups was analyzed using unpaired Student t-test with log transformation whenever necessary. For PD patients, correlation coefficients were determined for relationship between values of different enzymes with age, age of onset, duration of the disease, UPDRS score and Hoehn & Yahr stage.

**Results & Discussion**

The SOD, CAT, G-Px and G6PD activities were found to be significantly lower \( (P<0.001, P<0.005, P<0.005 \text{ and } P<0.001 \text{ respectively}) \) in patients with PD compared to the controls (Table). No correlation was observed between the age, age of onset and duration of disease and enzyme values \( (r=0.091, 0.146 \text{ and } -0.036 \text{ respectively}) \). Curvilinear relationship was also analysed by plotting the antioxidant enzyme levels against these parameters but found no correlation \( (r^2=0.0083, 0.021 \text{ and } 0.0013 \text{ respectively}) \). A significant negative correlation was observed between Hoehn & Yahr stage and enzyme activities \( (r=-0.216, P<0.05) \). UPDRS score was also negatively correlated \( (r=-0.364, P<0.05) \) to the enzyme activities. No correlation was observed in the curvilinear relationship analysis \( (r^2=0.133 \text{ and } 0.047 \text{ respectively}) \).

PD may serve as an excellent example to discuss the significance of oxidative processes as a central but not an initiating event for the development of clinical disease\(^{21}\). The concept that oxidative stress occurs in PD derives primarily from the realization that the metabolism of dopamine, by chemical or enzymatic means, can generate free radicals and other reactive oxygen species via autoxidation and dopamine oxidation by monoamine oxidase B\(^{22}\). The loss of dopaminergic neuron in PD results in enhanced metabolism of dopamine, augmenting the formation of \( \text{H}_2\text{O}_2 \) thus leading to the generation of highly neurotoxic hydroxyl radicals\(^{23}\). Low activity of mitochondrial complex 1 in PD also results in generation of oxygen species\(^{24}\). Further, any reduction in antioxidant enzymes may result in ineffective removal of ROS.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>PD</th>
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<tbody>
<tr>
<td>SOD (U/g HB)</td>
<td>1295.33 ± 271.6</td>
<td>763.43 ± 285.43**</td>
</tr>
<tr>
<td>CAT (K/g HB)</td>
<td>196.66 ± 20.74</td>
<td>165.19 ± 31.06*</td>
</tr>
<tr>
<td>G-Px (U/g HB)</td>
<td>67.86 ± 16.95</td>
<td>46.11 ± 31.01*</td>
</tr>
<tr>
<td>G6PD (mU/10⁹ erythrocytes)</td>
<td>52.78 ± 22.59</td>
<td>26.02 ± 15.04**</td>
</tr>
</tbody>
</table>

Values are mean ± SD \( (n=37 \text{ controls and } 115 \text{ PD cases}) \)

PD, Parkinson’s disease
SOD, superoxide dismutase
CAT, catalase
G-Px, glutathione peroxidase
G6PD, glucose 6-phosphate dehydrogenase

SOD showed a significant reduction in activity in patients leading to an increase of superoxide radical. Zhang et al\(^{25}\) found that some of the deleterious effects of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on striatal dopaminergic nerve terminals are mediated by both superoxide and hydroperoxides and they occur prior to dopaminergic neurodegeneration in the SN. Superoxide radicals can also react with NO to generate peroxynitrite \( (\text{ONOO}^-) \), a putative neurotoxin\(^{26}\). A significant reduction in both CAT and G-Px activities in effect can increase the production of highly deleterious \( \text{H}_2\text{O}_2 \). G6PD is a key enzyme involved in the synthesis of NADPH, which is essential for keeping the normal level of reduced glutathione\(^{5}\). G6PD deficient erythrocytes are particularly sensitive to oxidative stress\(^{27}\) and reduction in G6PD activity may result in reduction of GSH level. The reduction in GSH level has already been reported in PD patients\(^{23}\). Sian et al\(^{28}\) measured the reduced and oxidised glutathione levels in various brain areas in PD and in few related disorders and found that GSH level in SN was significantly reduced only in PD.
Our results showed no correlation between enzyme activities and age of patients or age of onset of PD. In some studies, a negative correlation was reported between the SOD activity and the duration of illness\textsuperscript{12,29}. We could not establish this in our study. We have found a significant negative correlation between the Hoehn and Yahr stage and enzyme activity. UPDRS score was also found to be negatively correlated to the activity of SOD. Bostantjopoulou \textit{et al}\textsuperscript{29} reported a significant decrease of SOD activity in whole blood and in RBCs in stage III and IV PD patients while there was no relationship between L-Dopa treatment and SOD activity. Sudha \textit{et al}\textsuperscript{11} reported that erythrocyte antioxidants in initial stage PD patients without any drug therapy were not significantly different from the controls.

The findings of our study showed the involvement of oxidative stress in PD. The neuronal degeneration may result from an increased exposure to free radicals coupled with a deficit of antioxidant mechanisms. The antioxidant enzyme levels are negatively correlated to the severity of the disease but independent of age and age of onset.

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**References**


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