Oxidative pathology and AQP4 mRNA expression in patients of Parkinson’s disease in Tamil Nadu

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KEY WORDS

Parkinson’s disease Oxidative stress Antioxidant biochemical analysis

ABSTRACT

Background: Parkinson’s disease (PD) typically appears in late middle aged and elderly persons and progresses over a period of several years. It is characterised by defective motor and cognitive function. Oxidative stress is believed to play a central role in the pathogenesis of PD. Purpose: The objective of the study was to assess the oxidative burden and mRNA expression of AQP4 related to oxidative pathology of PD related symptoms of Hoehn and Yahr stages. Methods: The study included 30 healthy controls and 90 PD patients who were undergoing treatment. The blood samples were collected and analyzed for biochemical assays and whole blood DNA was used for mRNA expression of AQP4 using RT-PCR. Results: The level of SOD, CAT and Gpx were found to be decreased while there was increase in LPO when compared to the healthy controls. The levels of SOD, CAT in stage III were significantly decreased when compared with stage I. The mRNA expression of AQP4 was found to be reduced when compared with that of healthy control samples. There was no variation in observed oxidative burden and the AQP4 mRNA expression among the different stages of disease. Conclusion: Based on the results obtained this study may be helpful in validating novel approach to treatment of PD by advancing antioxidant strategies.

Introduction

PD is the second most common neurodegenerative disorder after Alzheimer’s disease, affecting approximately 1 per cent of the geriatric population. 1 It is characterized clinically by rest tremor, bradykinesia, rigidity and postural imbalance. 2 The etiology of PD still remains unknown. Various factors implicated in the pathogenesis of PD, include the oxidant stress hypothesis which has been implicated to play a major role in neuronal cell death associated with PD. 3 The studies of the CNS are beset with the complexity of direct investigation because of the inaccessibility of the neural tissue, and hence the difficulty in obtaining a brain biopsy, until after the death of the affected individual. It is, therefore, imperative to search suitable biomarkers, which can help in the diagnosis of PD during life. Aquaporin (AQP4) is a water channel protein in mammalian brain, which is expressed in plasma membrane of astrocyte assisting in astrocytic migration and proliferation. AQP4 plays an important role in water homeostasis in the brain and brain edema. 4 Recently, AQP4 expression has been reported to be involved in the pathophysiology of the development of PD using MPTP in mice. 5 Further, various markers of lipid peroxidation and oxidative damage to DNA are increased in the substantia nigra of Parkinsonian patients. 6-8 These phenomena may be the consequence of reduced efficiency of endogenous antioxidants, such as glutathione that may render PD patients more vulnerable to oxidative stress. Indeed, concentrations of reduced or total glutathione are decreased in the substantia nigra of this patients 9-11 while the activity of SOD is increased. Slight increase in the concentrations of cytosolic and mitochondrial isoforms of SOD (Cu/Zn SOD and Mn SOD, respectively) have been reported in lymphocytes of PD patients. These increases, however, were mostly related to the intake of the MAO-B inhibitor selegiline. 12,13 On the other hand, certain other authors have reported decreased total SOD activity and increased concentrations of malondialdehyde (a product of lipid peroxidation) in erythrocytes, serum, and plasma of PD patients. 14-16 To ascertain the possibility of oxidative damage to the blood in PD, the present study was undertaken in treated PD patients by evaluating the changes of SOD, CAT, Gpx, GSH and LPO in blood samples of PD patients. Further, mRNA expression of AQP4 was also assessed to elucidate its possible association with redox status of PD patients.

Methods

Subjects were recruited after the approval by the Institutional Ethical Committee. Written informed consent was obtained from all patients. 90 PD patients under treatment with L-DOPA+Carbidopa and 30 healthy volunteers were enrolled for control (Table 1). All patients had been previously diagnosed with idiopathic PD at the Centre for Parkinson’s disease and Movement Disorders of the Neurological Institute Madras Medical College (MMC), Chennai. They were being followed as outpatients at the moment of enrolment. Patients were staged according to the criteria of Hoehn and Yahr17 and evaluated with the Unified Parkinson’s Disease Rating Scale (UPDRS) 17 for the assessment of PD severity. The control subjects who had any evidence of other neurological, cerebrovascular, metabolic or endocrine diseases were excluded from the study. All subjects underwent venipuncture for treated PD patients, this corresponded to an interval of 3 to 4 hours from the last dose of L-DOPA.

Sample collection

Heparinised blood (5 ml) was collected aseptically from the antecubital vein and exposure to air was minimized to avoid oxidative damage to the blood in PD. This study was undertaken in treated PD patients by evaluating oxidative damage to the blood in PD, the present study was undertaken in a treated PD patients by evaluating the changes of SOD, CAT, Gpx, GSH and LPO in blood samples of PD patients. Further, mRNA expression of AQP4 was also assessed to elucidate its possible association with redox status of PD patients.

Laboratory technique

The protein content was determined by Lowry et al. 18 SOD activity was measured by the method of Misra and Fridovich. 19 Catalase was assayed according to the method of Sinha et al. 20 Lipid peroxidation was assayed by the method of Ohkawa et al. 21 Gpx activity was measured by the method of Flohe et al. 22

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Table 1: Clinical history of PD patients

|                           | Control | PD patients | Clinical signs in PD patients |
|---------------------------|---------|-------------|-------------------------------|
|                           | No of Patients | 30         | 90                           |
| Age onset (years)         | –       | 62.0± 1.4   | 10-Speech disturbance         |
| Gender                    | M-25    | M-56        | 5-Confusion                   |
|                           | F - 5   | F -w 4      | 40-Lethargy                   |
| Disease Duration (year)   | –       | 4.0±5.4     | 12-Weight loss                |
| Daily dose of L-DOPA (mg) | –       | 330-440     | 20-Appetite loss              |
|                           |         |             | 25-Constipation                |
|                           |         |             | 32-Sleep disturbance           |
|                           |         |             | 60-Resting tremor              |

Age and disease duration, mean ± SD. M- Male; F-Female.

RNA extraction and RT-PCR for AQP4

Total RNA was extracted using RNA isolation kit and used for RT-PCR analysis. The primer used for AQP4 was as follows: Forward 5’- ‘GGAATCTCTATCTGTCACA -3’ reverse 5’-TGTTGCTGCCCAGCTTTGCTTGG-3’ and β-actin forward 5’- GTGGGGCGCCCCAAGGACCACTGCAACGCAGATTC-3’. The thermal cycling conditions of the PCR were for 94°C 5 min, followed by 23-35 cycles for 20s at 94°C, 20 min at 60°C, 1 min at 72°C and a final extension at 72°C for 7 min. The amplified products were separated by electrophoresis in 2% agarose gel containing 0.1 µg/ml eithidium bromide.

Statistics

The data were evaluated with SPSS/10 software. Hypothesis testing methods included one way analysis of variance followed by least significant difference test. p values of less than 0.05 were considered to indicate statistical significance. All these results were expressed as mean ± SEM.

Results

The PCR results showed following trend: Beta actin served as internal control. Lane 1: Marker; Lane 2: Control; Lane 3: Stage I PD patient’s blood mRNA expression; Lane 4: Stage II PD patient’s blood mRNA expression; Lane 5: Stage III PD patient’s blood mRNA expression; AQP4 was decreased in PD patients. No significant correlation of LPO was observed between Hoehn and Yahr stages of PD (Figure 1). The values are given as mean ± S.EM. *p<0.05 control vs PD stages.
Fig. 2: The activities of SOD, CAT, Gpx and GSH in the blood of healthy age matched control and PD patients (Hoehn and Yahr stages). The units were SOD- U SOD/mg Ptn, CAT- n mole/H$_2$O$_2$ decomposed/ min/mg protein, Gpx- nmoles of glutathione oxidized min/mg protein, GSH- µ mol/l. The values are given as mean ± S.EM. *p<0.05 control vs PD stages. SOD, CAT was significantly (p<0.05) decreased in stage III when compared with stage I.

Discussion
Reduced glutathione is an important intracellular free radical scavenger which is synthesized in the brain by both neurons and glial cells, although it is more abundant within astrocytes. Its role is to detoxify hydrogen peroxide (H$_2$O$_2$) to water and molecular oxygen. This important role for glutathione has been proposed in the pathogenesis of PD because a decrease in total glutathione concentration in the substantia nigra has been observed in preclinical stages in PD patients. Such depletion of glutathione triggers cascades of events, which may ultimately result in cell death, which could be attributed to concurrent elevation in glutathione. An increased level of LPO was also observed in PD patient’s blood sample when compared with healthy controls. It is said that the loss of dopaminergic neurons in PD would lead to enhanced metabolism of dopamine, augmenting the formation of H$_2$O$_2$, resulting in the generation of highly neurotoxic hydroxyl radicals. This argument, however, is well suited for the brain tissue and could not justify the current observation of LPO levels in a blood of the patients. It is interesting to note similar GSH-LPO status of substantia nigra region was found in preclinical stages of PD. Further, decreased activities of SOD, CAT and Gpx were found in the patient’s blood which could be interpreted as a compensatory response to enhanced formation of superoxide radicals and also increase the production of highly deleterious H$_2$O$_2$ in the blood. The study shows the redox status in blood of PD patients which is quite similar to that brain of PD patients occurring due to astrocytic/dopaminergic variations in them. AQP4 which is redox regulated channel, also showed a marked variation in the current study in the blood of PD patients. The role of AQP4 in PD is not fully understood. In this study the down regulation of AQP4 mRNA was seen in PD patients when compared with age matched healthy control and this change in the expression of AQP4 is not influenced by the stages between Hoehn and Yahr. This deficiency of AQP4 has been speculated to be the factor involved in mediating enhanced sensitivity of dopaminergic neurons to neurotoxicity/oxidant burden, by the modulation of astrocytes which provide precursors for redox-modulating components and also neurotrophic factors.

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