ANNALS
RES ARTICLE

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

Thamizh Thenral Subburaman, A. J. Vanisree

Department of Biochemistry, University of Madras, Guindy Campus, Chennai-600 025.

KEY WORDS

Parkinson's disease
Oxidative stress
Antioxidant
biochemical analysis

Corresponding Author:

A. J. Vanisree, PhD
Tel : +914426471075
E-mail : mahithamizh@gmail.com

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.

doi : 10.5214/ans.0972.7531.1118306

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 resting 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 the enrolment. Patients were staged according to the criteria of Hoehn and Yahr[17] 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 oxidation of reduced glutathione.

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]

Table 1: Clinical history of PD patients

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

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

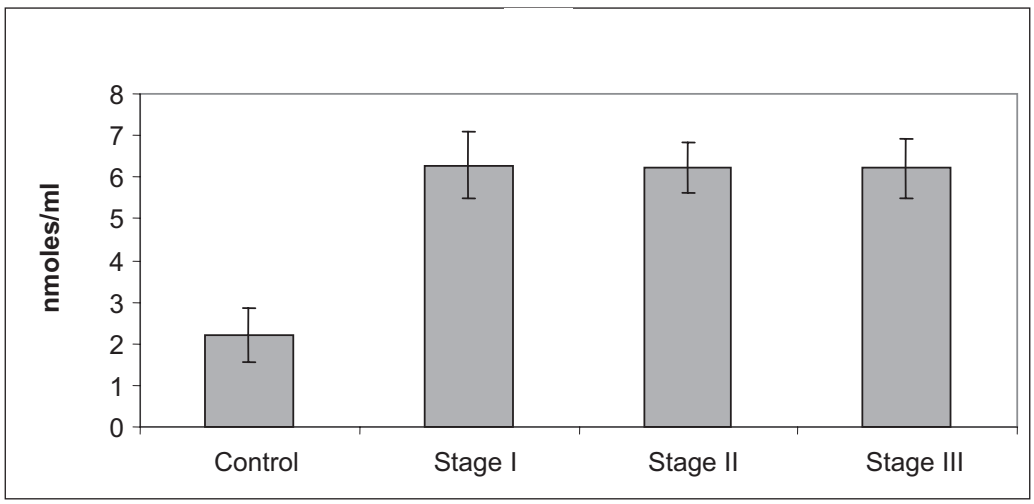

Fig. 1: The level of LPO in the blood of healthy age matched control and PD patients (Hoehn and Yahr stages). The values are given as mean ± S.EM. *p<0.05 control *vs* PD stages.

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'- 'GGAATCCTCTATCTGGTCACA -3' reverse 5'-TGTTTGCTGGGCAGCTTTGCT-3' and β-actin forward 5'- GTGGGGCGCCCCAGGCACCA-3' reverse 5'-CTTCCTTAATGTCACGCACGATTTC-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% agarsoe 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. The levels of SOD and CAT when compared between control and PD groups were found to be decreased in Stage III when compared to Stage I (Figure 2 and 3)

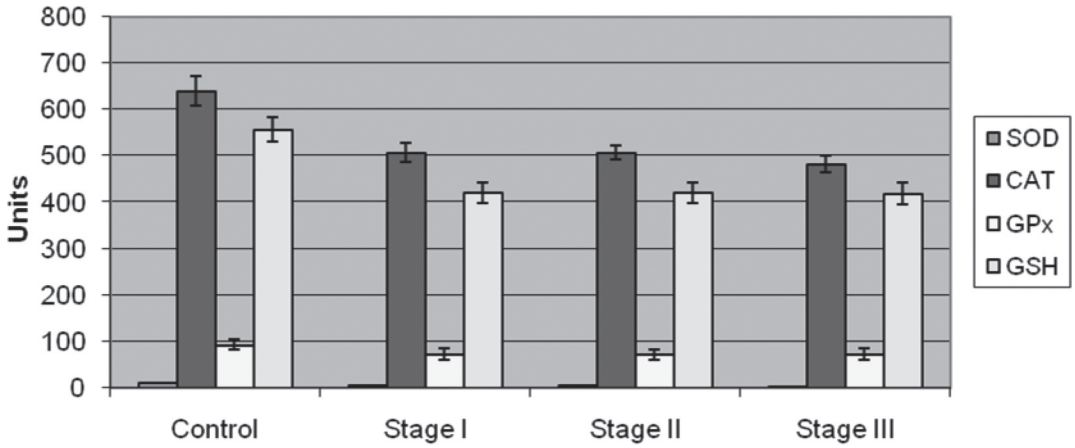

Fig. 2: The activities of SOD, CAT, Gpx and GSH in the blood of healthy age matched control and PD patients (Hoehn and Yahr stages). The units were SOD- U SOD/mg Ptn, CAT- n mole/H_2O_2 decomposed/ min/mg protein, Gpx- nmoles of glutathione oxidized min/mg protein, GSH- μ mol/l. The values are given 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.

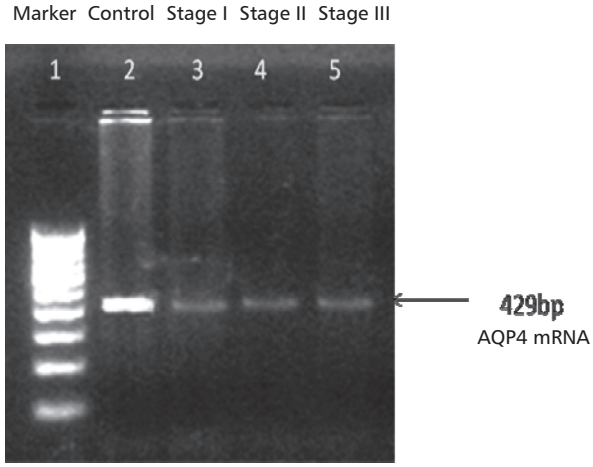

Fig. 3: mRNA expression of AQP4 in control and PD patients.

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_2O_2) to water and molecular oxygen.[23] 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.[24] 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_2O_2 resulting in the generation of highly neurotoxic hydroxyl radicals.[25] 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 substantua nigra region was found in preclinical stages of PD.[26] 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 radicles and also increase the production of highly deleterious H_2O_2 in the blood.[27, 28] 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.[29]

Our earlier observation showed an elevated LPO with attenuated anti oxidants in MPTP treated mice brain[30] along with down regulated AQP4 mRNA (unpublished data). This data and the current observation support the recent theory of involvement of AQP4 in PD pathology.

Acknowledgements

The authors thank University Grants Commission (UGC), New Delhi for financial assistance in the form of Junior Research Fellowship.

The article complies with International Committee of Medical Journal Editor's uniform requirements for the manuscripts.

Competing interests – None, Source of Funding – UGC

Received Date : 12 May 2011; Revised Date: 16 June 2011

Accepted Date : 11 July 2011

References

1. Ebadi M, Hiramatsu M. Glutathione and metallothionein in oxidative stress of Parkinson's disease. Free radicals in brain pathophysiology 2000; 427–465.

2. Dauer W and Przedborski S. Parkinson's disease: mechanisms and models. Neuron 2003; 39: 889–909.
3. Shults CW, Oakes D and Kieburtz K. Effects of coenzyme Q10 in early Parkinson's disease: evidence of slowing of the functional decline. Arch Neurol 2002; 59**:** 1541–1550.
4. Nielsen S, Nagelhus EA, Amiry-Moghaddam M *et al*. Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. J Neurosci 1997; **17**(1): 171–180.
5. Yi Fan, Hui Kong, Xueru Shi, *et al.* Hypersensitivity of AQP4-dificient mice to 1-methyl-4-phenyl1-2-3-6-tera hydropyrindine and astrocytic modulation. Neurobiology of aging 2008; 29: 1226–1236.
6. Dexter DT, Carter CJ, Wells FR, *et al.* Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. J Neurochem 1989; 52: 381–389.
7. Sanchez-Ramos J, Overvik E, Ames BN. A marker of oxyradical-mediated DNA damage (8-hydroxy-2'deoxyguanosine) is increased in nigrostriatum of Parkinson's disease brain. Neurodegeneration 1994; 3**:** 197–204.
8. Yoritaka A, Hattori N, Uchida K, *et al.* Immunoistochemical detection of 4-hydroxynonenal protein adducts in Parkinson's disease. Proc Natl Acad Sci USA 1996; 93: 2696–2713.
9. Perry TL, Yong VW. Idiopathic Parkinson's disease, progressive supranuclear palsy and glutathione metabolism in the substantia nigra of patients. Neurosci Lett 1986; 67**:** 269–274.
10. Sian J, Fexter DT, Lees AJ, *et al*. Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Ann Neurol 1994; 36**:** 348–355.
11. Sofic E, Lange KW, Jellinger K, *et al*. Reduced and oxidized glutathione in the substantia nigra of patients with Parkinson's disease. Neurosci Lett 1992; 142: 128–130.
12. Checkoway H, Costa LG, Woods JS, *et al*. Peripheral blood cell activities of monoamine oxidase B and superoxide dismutase in Parkinson's disease. Neural Transm 1992; 4**:** 283–290.
13. Kushleika J, Checkoway H, Woods JS, *et al*. Selegiline and lymphocyte superoxide dismutase activities in Parkinson's disease. Ann Neurol 1996; 39**:** 378–381.
14. Bostantjopoulou S, Kyriazis G, Katsarou Z, *et al*. Superoxide dismutase activity in early and advanced Parkinson's disease. Funct Neurol 1997; 12**:** 63–68.
15. Kilinc A, Yalcin AS, Yalcin D, *et al*. Increased erythrocyte susceptibility to lipid peroxidation in human Parkinson's disease. Neurosc Lett 1988; 87**:** 307–310.
16. Kalra J, Rajput AH, Mantha SV, *et al*. Oxygen free radical producing activity of polymorphonuclear leukocytes in patients with Parkinson's disease. Mol Cell Biochem 1992; 112: 181–186.
17. Martı´nez-Martı´n P, Carrasco de la Pena JL, Ramo C, *et al*. Inter-observer reproducibility of qualitative scales in Parkinson's disease (I). Arch Neurobiol (Madr) 1987; 50: 309–314.
18. Lowry OH, Rosenbrough NJ, *et al*. Protein measurement with the Folin phenol reagent. J Bio Chem 1951; 193: 265–276.
19. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972 ;247(10):3170–3175.
20. Sinha AK. Colorimetric assay of catalase. Anal Biochemistry 1972; 47: 389–394.
21. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxidase in animal tissues by thiobarbituric acid reaction. Anal Biochemistry 1979; 95: 351–358.
22. Flohe L, Gunzler WA, Schook HH. Glutathione peroxidase:a selenoenzyme. Febs Letters 1973; 32: 132–134. .
23. Sagara JI, Miura K, Bannai S. Maintenance of neuronal glutathione by glial cells. J Neurochem 1993; 61: 1672–1676.
24. Mytilineou C, Kramer BC, Yabut JA. Glutathione depletion and oxidative stress. Parkinsonism Relat Disord 2002; 8: 385–387.
25. Ebadi M, Srinivasan SK, Baxi MD. Oxidative stress and antioxidant therapy in Parkinson's disease. Prog Neurobiol 1996; *48*: 1–19.
26. Zhang J, Graham DG, Montine TJ, *et al*. Enhanced N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity in mice deficient in CuZn-superoxide dismutase or glutathione peroxidase. J Neuropathol Exp Neurol 2000; *59*: 53–61.
27. Urakami K, Sano K, Matsushima E, *et al*. Decreased superoxide dismutase activity in erythrocyte in Parkinson's disease. Jpn J Psychiatry Neurol 1992; *46*: 933–936.
28. Gatto EM, Carreras MC, Pargament GA, et *al*. Neutrophil function, nitric oxide, and blood oxidative stress in Parkinson's disease. Mov Disord 1996; *11*: 261–267.
29. Nakajima K, Hida H, Shimano Y, *et al*. GDNF is a major component of trophic activity in DA-depleted striatum for survival and neurite extension of DAergic neurons. Brain Res 2001; 916: 76–84.
30. Thamizh Thenral S, Uvarajan S, Vanisree AJ. Neuro protective action of *Piper Longum* against MPTP-induced changes in mouse brain. Annals of Neurosciences 2010; 17(1): 18–21.