

Role of free radical and antioxidant imbalance in pathogenesis of Parkinson's disease.

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Abstract

Oxidative stress contributes to the cascade, leading to dopamine cell degeneration in Parkinson's disease (PD). However, oxidative stress is intimately linked to other components of the degenerative process, such as mitochondrial dysfunction, excitotoxicity, nitric oxide toxicity and inflammation. It is therefore difficult to determine whether oxidative stress leads to, or is a consequence of, these events. Oxidative stress was assessed by estimating lipid peroxidation [LPO] product in the form of thiobarbituric acid reactive substances [TBARS], and nitric oxide. Enzymatic antioxidants like superoxide dismutase [SOD], glutathione peroxidase [GSHpx], catalase, ceruloplasmin and non enzymatic antioxidant vitamins e.g. vitamin E,C in either serum or plasma or erythrocyte in 22 patients of Parkinson's disease [PD] in the age group 45-75 years. Trace elements e.g. copper, zinc and selenium were also estimated. Plasma TBARS and nitric oxide levels were Significantly high but activity of SOD, GSHpx, catalase, and levels of ceruloplasmin, vitamin-E, vitamin-C, copper, zinc and selenium were significantly low in Parkinson's disease when compared with control groups. Present study showed that imbalance between free radicals and antioxidants may be playing a role in dopaminergic neuronal loss in substantia nigra pars compacta and involved in pathogenesis of the Parkinson's disease.

Key words: Parkinson's disease, Oxidative stress, Antioxidants

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Introduction

Recent advances in both molecular genetics and neurochemistry have shown involvement of excitotoxicity and oxidative stress in cell death [1]. Parkinson's disease is pathologically characterized by loss of catecholaminergic neurons in the brainstem. Number of biochemical processes involved in pathogenesis and progression of neurological disorders. The concept of oxidative stress and antioxidants may be directly or indirectly involved in the pathogenesis of Parkinson's disease [2,3,4].

The pathological hallmarks of Parkinson's disease are presence of Lewy bodies, degeneration of brain stem nuclei and loss of dopaminergic neurons [5,6]. Parkinson's disease can be caused due to exogenous neurotoxins, infectious agents, mitochondrial hypothesis, genetic hypothesis, and endogenous neurotoxins [7].

Number of studies showed that oxidative stress damages neurons by free radicals and play an important role in substantia nigra [8,9]. Antioxidant substances have role to

protect cell from pathogenic oxidation. Enzymatic antioxidants like superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase, and non enzymatic antioxidants ceruloplasmin, vitamin A, vitamin E and vitamin C. Trace elements are required in a small concentration for activation of antioxidant enzymes. Considering all these facts we planned to study certain relevant biochemical parameters for understanding the pathogenesis of Parkinson's disease.

Materials and Methods

The present study was carried out with 22 clinically examined Parkinson's disease patients ranging in age group 45-80 years, admitted in Govt. Medical College Hospital Miraj. Patients having obvious malignancy, hepatic, renal, or cardiac disease and addiction history of alcohol or smoking were excluded from the study. Patients with co-existing neurological disorder like Alzheimer's disease, cerebral ischemia injury (strokes) or any kind of neuro deficit were also excluded.

Written consent was obtained from patients as well as controls. Diagnosis of Parkinson's disease was done by physicians and confirmed by senior neurologist of government medical college; hospital Miraj. Age and sex matched 22 normal healthy persons as per IFCC guidelines were included by excluding history of alcohol or smoking as control subjects.

Blood collection

Fasting blood samples were collected under sterile condition from healthy controls and Parkinson's disease patients, just before starting any drug treatment. 8 ml blood was taken in EDTA vials and 5 ml in plain bulbs; plasma was separated for the estimation of TBARS were estimated in plasma as described by Buege JA.[10] employing MDA as a reference standard. Concentration of vita-

min E was estimated in plasma according to the method of Beaker and Frank [11].

Concentration of vitamin C was estimated in plasma by method of Natelson [12]. Levels of Ceruloplasmin were estimated by colorimetric method by Karl [13]. Erythrocytes were washed with cold isotonic saline and used for estimation of SOD, GSHpx, and catalase. SOD was assayed in RBC according to Kono method [14]. GSHpx was assayed in RBC by method of Beutler. Duron, Kelly [15] Catalase was estimated in erythrocytes hemolysate, according to method of Aebi [16]. Sera were separated and used for nitric oxide (nitrate + nitrite). Serum nitric oxide was estimated as the stable breakdown products, nitrate and nitrite by Cortas and Walkid [17]. Serum Copper, Zinc and Selenium levels were estimated by Atomic Absorption Spectrophotometer (AAS), Parkin Elmer model - 3030.

Table-I: Levels of lipid peroxidation and antioxidants in Parkinson's disease and control.

S. No.	Parameters	Healthy Control (n=22 Mean \pm SD)	Parkinson's disease (n=22) Mean \pm SD
1	LPO n moles/ml	2.35 \pm 0.640	5.80** \pm 0.875
2	Nitric Oxide (NO) μ mole/Lit	280.35 \pm 1.09	333.03* \pm 103.83
3	SOD U/gm of Hb	3.0 \pm 0.82	2.0** \pm 0.605
4	GSHpx U/gm of Hb	56.28 \pm 5.58	34.55** \pm 3.98
5	Catalase nmole/H ₂ O ₂ decomposed/min	630 \pm 110.05	445.77 ** \pm 115.0
6	Ceruloplasmin U/L	110.50 \pm 18.15	93.85* \pm 12.18
7	Vitamin-E mg/dl	7.96 \pm 2.52	5.00* \pm 2.77
8	Vitamin-C mg/dl	1.66 \pm 0.46	0.67* \pm 0.14
9	Copper μ gm/dl	105.0 \pm 14.45	0.86* \pm 9.3
10	Zinc μ gm/dl	96.50 \pm 8.25	68.50* \pm 9.20
11	Selenium μ gm/dl	19.05 \pm 1.42	14.58* \pm 0.98

Values are expressed in mean \pm SD. (n) = Number of observations.

Student's 't' Newman Keul test was applied to judge the significance of difference between two groups. ** indicates $p < 0.001$ * indicates $p < 0.01$

Results and Discussion

In comparison to the control group, plasma levels of TBARS, nitric oxide were significantly raised where as erythrocyte SOD, GSHpx and catalase activities were significantly lowered in the patients of Parkinson's disease. Levels of vitamin E & vitamin C in plasma and serum

Ceruloplasmin, copper, zinc & selenium were significantly lowered in comparison with control groups.

Free radicals have been accepted into the biochemical and medical orthodoxy. Their existence and importance in living systems was ignored. Oxidative stress has been implicated in the pathophysiology of many neurological disor

ders [18,19,20].

Thiobarbituric acid reactive substances, the indicator of lipid peroxidation were significantly elevated in Parkinson's diseases (Table 1). In Parkinson's disease the metabolism of dopamine by action of enzyme monoamine oxidase is accelerated and excessive formation of hydrogen peroxide (H_2O_2) takes place. In subsequent reactions of H_2O_2 hydroxyl radicals are generated. As per reports, the activity of monoamine oxidase is increased in Parkinson's disease.[21]. This increased activity of monoamine oxidase may further metabolize dopamine to produce excessive formation of hydrogen peroxide. The polymerization of auto-oxidative products of dopamine may lead to the formation of characteristic pigmentation of the substantia nigra. These released free radicals might be responsible for the loss of dopaminergic neurons.

Activity of nitric oxide synthase (NOS) is enhanced by N-methyl – D – aspartate, which in turn leads to enhanced generation of nitric oxide. It combines with superoxide radical to form peroxynitrate [22,23]. This peroxynitrate increases oxidative stress, which might be causing loss of dopaminergic neurons.

Enzymatic antioxidant status was studied by estimating erythrocyte SOD, GSHpx, catalase activity and serum ceruloplasmin levels.

Zinc stabilizes the structure of SOD and hence when zinc ions are removed, it results in loss of SOD activity [24]. Thus low zinc levels might be responsible for reduced SOD activity and increase concentration of superoxide radicals. This suggests that an increased formation of superoxide radicals in proximity to mitochondria inducing an increase in superoxide dismutase activity. These superoxide radicals combine with nitric oxide and elevate oxidative stress.

GSHpx contains selenium in the form of single selenocysteine residue [24]. Thus decreased selenium concentration may decrease the activity of GSHpx. The increased oxidative stress may oxidize hemoprotein subunit of catalase. Due to oxidation, there may be dissociation of tetrameric hemoprotein molecule and results in loss of catalase activity. Thus elevated oxidative stress may decrease the activity of catalase. Oxidative stress may inhibit synthesis of ceruloplasmin, which might be responsible for reduced levels of Ceruloplasmin in PD.

Due to increased oxidative stress in PD there may be increased consumption of vitamin E and C. Vitamin E traps free radicals and interrupts the chain reaction that damage the cells [25]. Decreased vitamin E and C might be causing oxiradical mediated injury and thus may contribute to nigral neurodegeneration.

This study indicates that, oxidative stress and antioxidants may play an important role in PD. Elevated free radicals may cause neuronal loss and play a role in pathogenesis of Parkinson's disease. The decreased activity of these antioxidant may be indirectly responsible for neuronal loss and probably plays a role in pathogenesis of Parkinson's disease.

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