Original Article

Oxidant and Antioxidant Status in Parkinson's Disease

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Abstract

Parkinson's disease (PD) is a progressive, neurological disease that mainly affects movements and occurs at older ages and is clinically characterized by resting tremor, rigidity, bradykinesia and postural imbalance. These clinical manifestations of PD are caused by a selective degeneration of dopamine-producing neurons in substantia nigra in the brain stem and the consequent dopamine shortage in the striatum. Oxidants and antioxidants related substances may contribute to the pathogenesis and the progression of Parkinson's disease. Research can make great progress in understanding and further treating the PD. This study demonstrates significant variation of oxidants-antioxidants status in Parkinson's disease. Oxidative stress plays a crucial role in progression of PD; however, oxidative stress is a cause or the consequence of PD is debatable. In our study we observed there is significant increase in the levels of serum Malondialdehyde ($p< 0.001$), Nitric oxide end products ($p< 0.001$), and significant decrease in the activity of Glutathione peroxidase ($p< 0.001$), Superoxide dismutase ($p< 0.001$), and Catalase ($p< 0.001$) in PD patients as compared with controls. Further Vitamin C ($p< 0.05$), Vitamin E ($p< 0.05$) significantly decreased, but Uric acid levels ($p> 0.05$) remain unchanged and this may be due to compensatory mechanism of body against oxidative stress, which not allowed much alteration in other parameter in the PD patients as compared with controls.

Keywords

malondialdehyde, nitric oxide, glutathione peroxidase, superoxide dismutase, catalase, uric acid, parkinson's disease

Introduction

In 1817, James Parkinson published his famous monograph: "An essay on the shaking palsy". In this report, he described a neurological illness — now known as Parkinson's disease — consisting of resting tremor and a peculiar form of progressive motor disability. Parkinson's disease results from the destruction of nerve cells in a part of the brain called the basal ganglia. Different parts of the brain work together by sending signals to each other to coordinate all of our thoughts, movements, emotions, and senses. When we want to move, a signal is sent from the basal ganglia to the thalamus, and further to the cerebral cortex, and all different parts of the brain. Nerve cells in the brain communicate by using chemicals. A chemical (neurotransmitter) called dopamine is produced in a group of cells called the substantia nigra and is essential for normal movement. When the cells die they can no longer produce and send dopamine, so the signal to move doesn't get communicated. Another chemical in the brain, acetylcholine, is controlled by dopamine. When there is not enough dopamine, there is too much acetylcholine, causing the tremors and muscle stiffness that many people with PD experience.

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Oxidative stress in PD

The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules, commonly called "free radicals." Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases [1, 2]. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating.

Parkinson's disease (PD) is one of the major progressive neurological disorders, characterized by the loss of dopaminergic neurons in pars compacta of the substantia nigra. The causes for this is the interactions between external toxins (which arise from environmental, dietary and life style factors) and internal toxins arising from normal metabolism, genetic and epigenetic (mitochondria, membranes and proteins) components. Oxidative stress is one of the intermediary risk factors that could initiate and promote degeneration of neurons. Even though oxidative stress in brain is an important factor in the neuropathology of PD, yet the role of systemic oxidative stress is inconclusive[3].

Aabha Sharma *et. al.* concluded from the study carried out on Parkinson's disease patients in Indian population that, increased lipid peroxidation, decreased Glutathione levels and increased Superoxide Dismutase (SOD) activity are due to the deteriorative action of various reactive oxygen species (ROS) which have been generated during the state of oxidative stress present in PD patients. Further they did not find any change in activity of Glutathione reductase (GR), Glutathione Peroxidase (GPX) and Gamma Glutamyl Transpeptidase (GGT) in the hemolysate of PD patients as compared to controls, and justify that the compensatory mechanism of body against oxidative stress not allowed much alteration in other parameter[4]. Monika Vinish *et. al.* also found there was significant increase in malondialdyhyde (MDA) content and SOD activity in peripheral blood parameter of PD patients in comparison with controls, but no changes were observed in GPX and Nitric oxide levels among them[5]. Ahmad Agil *et. al.* found plasma lipid peroxide levels were 33% higher in PD group as compared with controls[6]. Shashikant Nikam *et.al* observed in Parkinson's disease patients that, Plasma thiobarbituric acid reactive substances and Nitric oxide levels were significantly high but SOD, GPX, Catalase (CAT), ceruloplasmin, vitamin-E, vitamin-C, copper, zinc and selenium levels were significantly low in Parkinson's disease when compared with control subjects, and concludes that, the elevated oxidative stress may be playing a role in dopaminergic neuronal loss in substentia nigra pars compacta and involved in pathogenesis of the Parkinson's disease[7]. S.Abraham *et.al* also observed SOD, CAT, GPX activities were significantly lower in red blood cells of patients with Parkinson's disease as compared with controls[8]. K Sudha *et.al* observed Lipid peroxidation, oxidative hemolysis and plasma ceruloplasmin were significantly higher in PD patients as compared to normals. But also found erythrocyte antioxidants in PD patients were not significantly different from the controls. However, they observed plasma vitamin C in PD patients significantly lower than the controls[9]. Usha Adiga and co worker found that significant decrease in total Antioxidant activity in PD patients as compared to controls[3]. Jaya Sanyal *et. al.* concluded that, plasma nitrate levels are higher in PD patients than in controls. But also observed PD patients with short disease duration did not have elevated plasma nitrate levels compared to control subjects[10]. According to Melinda K Kutzing altered serum uric acid concentrations, both above and below normal levels, have been linked to a number of disease states. An abnormally high uric acid level has been correlated with gout, hypertension, cardiovascular disease, and renal disease, whereas a reduced uric acid concentration has been linked to multiple sclerosis, Parkinson's disease, Alzheimer's disease, and optic neuritis[11].

In contrary to above results, Jose Antonio Molino *et. al.* suggests from their study that, serum MDA levels[12] and plasma nitrate[13] levels did not differ significantly between PD patients and controls, and were not influenced by antiparkinsonian therapy in PD patients. Pilar Fernadez Calle[14] and co-workers showed that, serum levels of Vitamin C were similar in PD patients and controls, and there was no correlation with analyzed clinical feature of PD. D king[15] and co-worker showed that, there was no significant difference in serum concentration of Vitamin A and E in patients of Parkinson's disease and controls. However, vitamin C was significantly higher in Parkinson's disease group as compared with control and further concludes that

the deficiency of these antioxidants did not contribute to the onset or progress of PD.

Metabolism of oxidants and antioxidants related substances may contribute to the pathogenesis and the progression of Parkinson's disease. There is inadequate information available regarding the status oxidative stress in PD patients. So, the present work was designed with specific aim to study the following biochemical parameters in the blood of PD patients, and same we have to compare with the blood levels of healthy controls. To study oxidant status in PD patients and controls we have to analyze lipid peroxidation in terms of MDA and levels of serum Nitric oxide end products. To study activity of antioxidant enzymes involved in free radical scavenging in PD and controls we have to analyze, activity of SOD, GPX and CAT. To study status of non enzymatic antioxidant involved in free radical scavenging in PD and controls we have to analyze levels of Plasma Vitamin E, Vitamin C, and serum Uric acid.

Material and Methods

The present study was conducted in Department of Biochemistry, B.J. Govt. Medical College & Sasoon General Hospital Pune. A total 104 subjects, including control, were enrolled in the study and further grouped as, 52 clinically examined Idiopathic Parkinson's disease patients (35 males and 17 females) while remaining 52 were taken as age and sex matched healthy controls. The study was approved by institutional ethical committee and informed consents were obtained from all the patients and controls of the study group. Diagnosis of Parkinson disease done by physicians and confirmed by neurologist by using UK Parkinson's disease society brain bank clinical diagnostic criteria[16], with evidence on neurological examination has at least two of the three cardinal signs rest tremor, rigidity, bradykinesia.

Inclusion criteria:

1) Male and female patients diagnosed as Idiopathic Parkinson's disease aged between 50 to 70 years in the initial stage of disease (1-2 years) without any drug therapy.

2) Willing to participate in study and provide informed consents.

3) Control group included healthy volunteers who were consistent with the patients according to age, sex and body mass index.

Exclusion Criteria:

1) Patients having blood disorders, obvious malignancy, hepatic, renal or cardiac disease and additional history of alcohol or smoking will be excluded from the study.

2) Patients with co-existing neurological disorder like Alzheimer's disease, stroke or any kind of neural deficit was also excluded.

3) Patients on any concomitant medication such as Lipid lowering drug, antioxidants, vitamins, minerals, herbal treatment, or the substance which may alter our study parameters excluded from study.

Sample collection: 10 ml fasting blood samples from patients and controls were collected from anticubital vein, with all aseptic precaution. 4 to 5ml blood was collected in the heparinised vacutainers and remaining 5 to 6 ml blood was collected in plain vacutainers. Heparinised whole blood was used for the estimation of SOD and GPX activity and hemoglobin concentration. Plasma was used for estimation of Vitamin C and Vitamin E levels. Serum separated from plain blood used for the estimation of MDA, Nitric oxide end products, Uric acid and activity of CAT. All the spectral analyses were carried out on fully automated UV-Visible spectrophotometer and for reagent preparation glass distilled water used throughout the study. Lipid peroxidation measured in terms of Serum MDA by K.Satoh method[17]. Serum Nitric oxide measured in terms of nitrite by using Cortas and Wakid method[18]. Activity of GPX and SOD measured by using RANSEL and RANSOD kits respectively[19, 20]. Serum CAT activity estimated by using L Goth method[21]. Concentration of Plasma vitamin C measured by Chaterjee and Banerjee method[22] while Plasma vitamin E measured by Baker and Frank method[23]. Serum Uric acid estimated by using kits from Erba diagnostics [24].

Results

Table 1
Showing clinical background of Controls and PD patients

	Control (n = 52)	PD patients (n = 52)
Men	35	35
Women	17	17
Mean age ± SD	60.05 ± 5.4	59.97 ± 5.4 #

p > 0.05 No significant difference in age of Parkinson's disease patients as compared with controls.

Table 2
Showing comparison of various parameters between Controls and PD patients

No.	Parameter	Control	PD Patient
1	Serum MDA nmol/ml	2.15 ± 0.3	4.28 ±0.8 **
2	Serum Nitric oxide end products µmol/l	39.72 ± 4.3	56.36 ± 4.8 **
3	Glutathione peroxidase U/g Hb	63.73 ± 4.1	43.73 ± 2.3**
4	Superoxide dismutase U/g Hb	1463 ± 131	1004 ± 120**
5	Serum Catalase kU/l	45.61 ± 14.2	23.25 ± 5.8**
6	Plasma Vitamin C mg/dl	0.90 ± 0.1	0.82 ± 0.1*
7	Plasma Vitamin E mg/l	11.25 ± 1.7	10.51 ± 2.0*
8	Serum Uric Acid mg/dl	4.47 ± 1.0	4.45 ± 1.0 #

Values are expressed as Mean ± S.D, * $p < 0.05$ as compared with control, ** $p < 0.001$ as compared with control, and # $p > 0.05$ as compared with control.

Discussion

Table 1 Depicts the clinical background of subjects participated in present study. We include total 104 subjects; among these 52 Parkinson's disease patients (35 males and 17 females) and 52 healthy controls (35 males and 17 females). Mean age ± SD for PD patients 59.97 ± 5.4 and for control 60 ± 5.4. As from **Table 1** there is no significant difference in age of Parkinson's disease patients as compared with control ($p > 0.05$).

As from **Table 2**, levels of oxidants such as serum MDA ($p < 0.001$) and Nitric oxide end products levels ($p < 0.001$) were significantly increased among PD patients as compared with controls, clearly indicates involvement of oxidative stress in PD.

Lipid peroxidation:

Exposure of cell membrane to oxygen radicals stimulates the process of lipid peroxidation. In this process the fatty side chains of membrane lipids, especially those containing two or more carbon- carbon double bonds are oxidized. Insertion of an oxygen molecule is catalysed by free radicals called non-enzymatic lipid peroxidation and by enzymes called as enzymatic lipid peroxidation[2].

The free radicals continuously form in all aerobic cell, and consist of the superoxide radical, and hydroxyl radical. These metabolites are responsible for lipid peroxidation, which is described as conglomeration reaction of the polyunsaturated fatty acids found in cell membrane (phospholipid, glycolipid, glyceride and sterol) to various products such as peroxides, alcohols, aldehydes, hydroxy fatty acids, ethane and pentane[25].

All of major classes macromolecules may be attacked by free radicals but lipids are probably the most susceptible. Cell membranes are rich sources of PUFA, which are readily attacked by oxidizing radicals. The oxidative destruction of PUFA, known as lipid peroxidation, is particularly damaging because it proceeds as self-perpetuating chain reaction. It was also considered that the lipid peroxide formed in primary sites would be transferred via blood to other organs or tissue where the damage would be provoked by the propagation of lipid peroxidation[25, 26].

Nitric Oxide:

Nitric oxide is a regulatory molecule that has come under increasing interest and scrutiny due to its role as an important mediator of homeostatic processes and immunity [27]. Nitric oxide is believed to participate in the regulation of the oxidation/reduction potential of various cells and may be involved in "either the protection against or the induction of oxidative stress within various tissues, depending upon its concentration" [27]. Emerging evidence suggests that some diseases are related to either an inadequate or excessive production of Nitric oxide [28].

Nitric oxide synthesize from amino acid L-arginine by Nitric oxide-synthase (NOS). On the other hand, Nitric

oxide is neurotoxic if produced in excess. In degenerative neurological diseases, NOS-containing neurons preferentially survive. The remaining NOS neurons can be the source of Nitric oxide that mediates neurotoxicity. According to the hypothesis of Youdim, Nitric oxide could be implicated in the pathogenesis of PD in at least three ways: Nitric oxide mediated involvement of corticostriatal glutamatergic neurons; Nitric oxide interaction with the iron storage protein ferritin resulting in the release of iron, formation of iron-nitrosyl complexes and promotion of free radicals, triggering lipid peroxidation; and Nitric oxide induced impairment of mitochondrial function. Thus, Nitric oxide could damage the iron-dependant mitochondrial NADH- coenzyme Q reductase in a similar way to that described in parkinsonian substantia nigra[10]. Increase in the lipid peroxidation in PD patients as compared with controls was reported in previous data[4, 5,6,7,9], our results also consistent with them. Further few researchers observed elevated nitric oxide end products concentration in PD patients as compared with controls [7, 10], our findings also similar with them.

Further as indicated in **Table 1** that, enzymatic antioxidant such as GPX ($p< 0.001$), SOD ($p< 0.001$), and CAT ($p< 0.001$) activities were significantly decreased in PD patients as compared with healthy controls.

Antioxidant Enzymes:

Under normal conditions, the continuous production of free radicals is compensated by the powerful action of protective enzymes. SOD, CAT, and GPX 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.

SOD (EC 1.15.1.1) is the antioxidant enzyme that catalyses the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species hydrogen peroxide (H_2O_2). Peroxide can be destroyed by CAT or GPX reactions [29, 30]. In humans, there are three forms of SOD: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD) [31, 32]. CAT (EC 1.11.1.6) is a tetrameric enzyme consisting of four identical tetrahedrally arranged subunits of 60 kDa that contains a single ferriprotoporphyrin group per subunit, and has a molecular mass of about 240 kDa [33]. CAT reacts very efficiently with H_2O_2 to form water and molecular oxygen; and with H donors (methanol, ethanol, formic acid, or phenols) with peroxidase activity. In animals, H_2O_2 is detoxified by CAT and by GPX. CAT protects cells from hydrogen peroxide generated within them. The selenium-containing peroxidase, GPX (EC 1.11.1.19) contains a single selenocysteine residue in each of the four identical subunits, which is essential for enzyme activity [34]. GPX (80 kDa), catalyse reduction of hydroperoxides by using reduced glutathione (GSH), thereby protecting mammalian cells against oxidative damage. In fact, glutathione metabolism is one of the most essential antioxidative defense mechanisms [35, 36, 37, 38]. As previous data reported, enzymatic antioxidant such as Superoxide dismutase SOD, CAT,GPX were significantly decreased in PD patients as compared with healthy controls [7, 8], our results regarding enzymatic antioxidants was consistent with them.

Further **Table 1** shows that, Vitamin C ($p< 0.05$) and Vitamin E ($p< 0.05$) significantly decreased, but serum Uric acid levels ($p> 0.05$) remain unchanged in PD patients as compared with healthy controls.

Non enzymatic antioxidants:

Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids, as it is capable of neutralising oxidants in the aqueous phase before lipid peroxidation is initiated. Vitamin C acts as a prooxidant at low concentration, including lipid peroxidation in presence of metal ion such as Fe^{3+} or Cu^{2+}. However, high concentration, it is potent antioxidant, and is capable of reacting with superoxide and hydroxyl radicals to prevent their toxic action [14]. As an antioxidant, vitamin E acts as a peroxyl radical scavenger, preventing the propagation of free radicals in tissues, by reacting with them to form a tocopheryl radical, which will then be reduced by a hydrogen donor (such as vitamin C) and thus return to its reduced state. As it is fat soluble, it is incorporated into cell membranes, which protects them from oxidative damage. The fat soluble nature of the vitamin E indicates that its absorption and transport in the body is very complex and regulated by the level of fat intake. In humans, its absorption occurs in the proximal part of the intestine and requires bile acid secretion and micellarization. Vitamin E is mainly distributed in adipose tissue, and in the subcellular membrane fractions, the major concentration of Vitamin E is found in the Golgi apparatus and lysosomes [39]. Uric acid

is a natural antioxidant, accounting for up to 60% of the free radical scavenging activity in human blood. Uric acid can scavenge superoxide, the hydroxyl radical, and singlet oxygen. Uric acid may assist in the removal of superoxide by preventing against the degradation of SOD, the enzyme that is responsible for clearing superoxide from the cell. Removal of superoxide helps to prevent its reaction with nitric oxide, blocking the formation of peroxynitrite. Uric acid is also very effective at preventing peroxynitrite from nitrating the tyrosine residues of proteins, thereby preventing the inactivation of cellular enzymes and modification of the cytoskeleton. Uric acid also has the ability to bind iron and inhibit iron-dependent ascorbate oxidation, preventing an increased production of free radicals that can further contribute to oxidative damage. Thus, a reduced Uric acid concentration may decrease the ability of the body to prevent peroxynitrite and other free radicals from acting on cellular components and damaging the cell [11]. As reported by researchers that, Vitamin C and Vitamin E levels were significantly fall in PD patients as compared with controls [7,9], our results regarding this non-enzymatic antioxidant consistent with them. Further we did not find any change in levels of serum Uric acid of PD patients as compared to control, and this may because, the compensatory mechanism of body against oxidative stress not allowed much alteration in other parameter.

Oxidative Stress and Parkinson's Disease

Reactive oxygen species and oxidative stress contribute in the pathogenesis of Parkinson's disease. The discovery[40] of 1-methy1-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) found in some kinds of synthetic heroin, reveals that it can cause parkinsonian symptoms in humans. MPTP is selectively toxic to nigrostriatal dopaminergic neurons provided an important model of parkinsonism and clues to the possible pathogenic mechanism in PD. MPTP toxicity could also be due to inhibition of adenosine triphosphate (ATP) formation through an impairment of mitochondrial function coupled with the generation of ROS capable of exerting neurotoxicity [41]. The discovery of MPTP toxicity led to the discovery of many analogues of MPTP, some of which also exert toxicity to the substantia nigra [42]. Furthermore, the MPTP story leads to the recognition that several naturally occurring molecules, such as isoquinolines and p-carbolines, can exert MPTP-like toxicity [43, 44]. Some of these are found in food; others may be generated within the brain itself. Extensive investigation [45, 46] of the properties of isoquinolines and p-carbolines have shown that they possess a range of MPTP-like actions including selective uptake by dopamine neurons, impairment of mitochondrial function, and the potential to cause degeneration of nigral cells in rodent and primate species. These are relatively weak neurotoxins, and their relevance to PD is unknown, although it is possible that long-term exposure may lead to progressive neurodegeneration.

Oxidative stress in PD may also arise from the metabolism of dopamine itself by both chemical and enzymatic mechanisms. The autoxidation of dopamine leads to the production of semiquinones, which themselves toxic and which may also lead to the generation of ROS [47, 48]. Increased dopamine turnover in the early stages of PD might generate excessive H_2O_2 which would normally be inactivated by GSH in a reaction catalyzed by GPX. However, if the GSH system were impaired or deficient, H_20_2 might be converted by the iron-mediated Fenton reaction to form highly reactive hydroxyl radical, so initiating lipid peroxidation and cell death[49].

Oxidative stress plays a crucial role in the pathogenesis of PD concludes from our results because, serum MDA, serum Nitric oxide end product levels were significantly increased, in PD patients as compared with controls. We observed activity of enzymatic antioxidant such as GPX, SOD, and CAT was significantly decreased in PD patients compared with controls. Further we found, non-enzymatic antioxidant such as Vitamin C, Vitamin E levels were significantly decreased in PD patients as compared with controls.

In light of our findings and additional data from literature we emphasis that along with clinical examination, the said biochemical investigations in our research project, will be supportive marker for prognosis and diagnosis of PD. This could help physicians, neurologist to find out risk of initiation and progression of PD. It also might allow them to find treatments that will halt the disease process in the early stages.

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