Oxidative Stress Association with Autonomic Dysfunction in Parkinson Patients

Asma'a Khalaf Hamod (MBChB, MSc)[1], **Abdul Kareem Kadim AL- Khazraji** (MBChB, FIBMS)[2], **Isra'a Fayiq Ja'afer** (PhD)[3] and **Farqad Badar Hamdan** (PhD)[4]

Abstract

Background: Parkinson's disease may be caused by oxidative stress within cells due to defective nucleoli. Dopamine producing neurons are particularly sensitive to oxidative stress. The researcher's present evidence showing defective nucleoli within dopamine producing neurons lead to oxidative stress and damage

Objective: To assess antioxidant activity in Parkinson patients with autonomic dysfunction.

Materials and Methods: samples were collected in Al Kadhimiya Teaching hospital and Baquba teaching hospital in a period lasts from 1st January to 1st November 2013. Antioxidant activity had been tested in 44 Parkinson patients with autonomic dysfunction, 23 Parkinson patients without autonomic dysfunction and 25 healthy matched controls; using (Antioxidant Capacity, Total BioAssay™ Kit ;US Biological company, Catalog No. A2298-43).

Results: antioxidant activity showed a reduction in its level in Parkinson patients without autonomic dysfunction (0.75) with further reduction in Parkinson patient with autonomic dysfunction (0.37) compared with the control groups (1.2).

Conclusion: Oxidative stress and antioxidants have a role in pathogenesis of Parkinson disease. Both increase oxidative stress (direct) and a reduction in activity of the antioxidants (indirectly) cause loss of neuron and reinforcing damage mechanisms that play a role in autonomic dysfunction in Parkinson Disease.

Keywords: Parkinson's disease; autonomic dysfunction, antioxidant activity, brain cell death

Corresponding Author: Sma.kalaf@yahoo.com

Received: 3rd March 2015

Accepted: 15th March 2015

[1] Baquba Teaching Hospital - Diyala Health Directorate - Health Ministry - Diyala - Iraq.
[2] Department of Medicine - Collage of Medicine - Al - Nahreen University - Baghdad - Iraq.
[3] Department of Physiology -Collage of Medicine - Baghdad University - Baghdad - Iraq.
[4] Department of Physiology - Collage of Medicine - Al -Nahreen University- Baghdad -Iraq.

Introduction

Parkinson disease is regarded as a common neurodegenerative disorders in the world. This disease was first subscribed in 1817 by James Parkinson in 1817[1].

Parkinson's disease (PD) caused by dopaminergic neurons death in the Substantia Nigra (SN) of the ventral midbrain [2]. This leads to a progressive and chronic loss of motor coordination, tremors, bradykinesia, in addition to non-motor symptoms like sensory problems, cognitive deficits or sleep difficulties [3]. The loss of brain cells may be implicated by genetic and environmental

factors. However, the major contributors to neurodegeneration in PD were inflammation, oxidative stress, and mitochondria dysfunction [4].

Parkinson patients commonly had autonomic dysfunction; this is due to the underlying pathophysiologic status that affects the catecholaminergic neurons of the autonomic nervous system. It includes degeneration and dysfunction of autonomic mediating nuclei functions; in addition to degeneration of cholinergic, monoaminergic, and serotoninergic nuclei [5]. If neurons that produce dopamine are damaged or die, there would be a weakness in the connection between the brain and muscles. So, the brain would be unable to control muscle movement [6]. In addition, break down of the excess of dopamine in the synapses by Mono Amine Oxidase-B would further diminish the dopamine in the Substania Nigra. Furthermore, the reduction in dopamine levels causes the neurons in the Basal ganglia to fire randomly leading to involuntary movements [7].

Many mechanisms had been suggested about the brain cells loss. One mechanism is consists of an abnormal accumulation of the protein alpha-synuclein bound to ubiquitin in the damaged cells [8, 9]. Accumulation of this insoluble protein inside the neurons leads to the formation of inclusions called Lewy Bodies [10]. Also, Inhibition of oxidative phosphorylation complex has been found to induce pathologic symptoms of PD, oxidative stress and mitochondrial dysfunction [11].

Post-mortem brain studies implicate oxidative damage in the pathogenesis of PD; especially oxidative damage to lipids, proteins, and DNA has been observed in the SN of sporadic PD brains [12, 13]. The origin of increased level of oxidative stress may be unclear but it may involve dysfunction of mitochondria, elevated dopamine metabolism that can yield excess reactive oxygen species (ROS) mainly hydrogen peroxide, impaired antioxidant defense pathways and an increase in reactive iron [14].

Oxidative stress has been suggested as a causative factor in the progression of PD. It is regarded as a result of an imbalance in pro-oxidant/antioxidant homeostasis that ends in the generation of toxic reactive oxygen species [15, 16].

Patients and Methods

Sixty seven patients were admitted to Al Emmamain Al Kademian Teaching hospital and Baquba Teaching Hospital, and were included in the the research after performing exclusion criteria for any disease that may affect dysautonomic features like cardiovascular accident and diabetes mellitus. Then those patients were divided into two groups according to neurological assessment of autonomic nervous system:

Parkinson patients with autonomic dysfunction, who comprised of 44 patients (32 males and 12 females). The other group; Parkinson patients without autonomic dysfunction; include 23 patients (17 males and 6 females). Control group comprised 25 subjects (15 males and 10 females).

Oxidative stress test

In the present study, the level of total blood antioxidants was measured in blood serum. All blood serum samples were collected & stored at –80°C.

Trolox standard curve: we add 0, 4, 8, 12, 16, 20ul of A2298-43D: Trolox Standard to individual wells. Adjust the total volume to 100ul with ddH2O to give 0, 4, 8, 12, 16, 20nmol of Trolox standard.

Regarding sample preparation, the kit (Antioxidant Capacity, Total BioAssay™ Kit ;US Biological company, Catalog No. A2298-43) has been tested with serum. The small molecule TAC is desired, so samples diluted 1:1 with protein mask. Sample volumes between 0-100ul had been assayed

per well and duplicated. All well volumes were adjusted to 100ul with ddH2O.

The amples absorbance was in the linear range of the standard curve (0-20nmol/well). The samples that fall outside of this range, rediluted and rerun. The detection limit of the assay is ~0.1nmole/well (or 1uM) of Trolox.

Then we dilute one part: Cu2+ Reagent with 49 parts of Assay Diluent. Each well requires 100ul of Cu2+ working solution. We add 100ul Cu2+ working solution to all standard and sample wells, then cover the plate and incubate at RT for 1.5 hours. After which, we read the absorbance at 570nm using the plate reader.

For Calculations we plot standard curve: plot absorbance at 570nm as a function of Trolox concentration. Then, we calculate sample antioxidant Trolox equivalent concentrations:

$$\textit{Sample Antioxidant Capacity} = \frac{(\textit{Sample absorbance} - \textit{Blank absorbance}) \times (\mu l\ \textit{of Sample})}{(\textit{Slope of Standard Curve})}$$

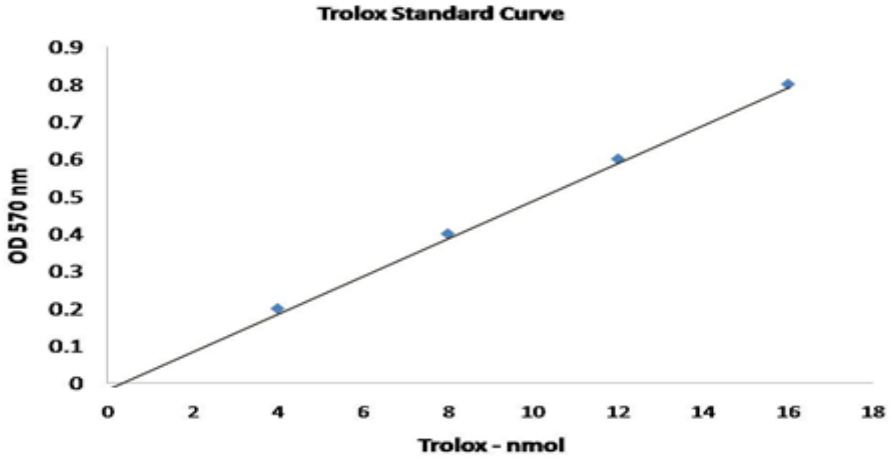

Figure (1): Sample Trolox standard curve.

Statistical analysis

The statistical analysis was done by using Statistical package for Social Sciences (SPSS) version 19**.**

Results

According to age distribution, the present study demonstrated the mean age of Parkinson patient with dysautonomia was 60.4±13.5 years, for Parkinson patients without dysautonomia 58.7±13.1 years, while for control group 56.3±12.4 years, with no significant difference statistically. Out of 67 parkinsonian patients with and without autonomic dysfunction and control group was found to be more in males than in females, but statistical analysis not revealed a significant difference between them (Table1).

Table (1): Distribution of the studied groups according to age and gender

Variables	Patient with autonomic symptoms No. = 44	Patients without autonomic symptoms No. = 23	Control No. = 25	P-value
Age (year), Mean ± SD	60.4 ± 13.5	58.7 ± 13.1	56.3 ± 12.4	0.464
Gender				0.472
Male	32 (72.7)	17 (73.9)	15 (60.0)	
Female	12 (27.3)	6 (26.1)	10 (40.0)	

Regarding the antioxidant activity as shown in figure (2), all the studied groups show highest activity in control groups while the least in Parkinson patients with dysutonomia and in between the Parkinson disease without dysautonomia.

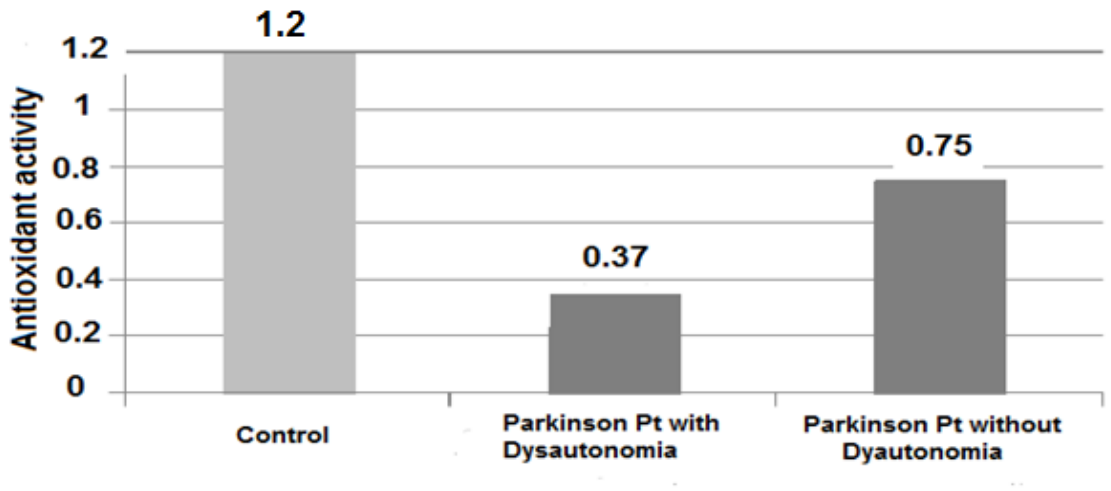

Figure (2): the antioxidant activity in the three groups.

Table 2 shows the levels of antioxidant activity in patients of Parkinson disease with dysautonomia, patients of Parkinson disease without dysautonomia and control group were studied statically using Independent samples t test. The highest level of oxidative stress found in control group, followed by patients of Parkinson disease without dysautonomia, then in patients of Parkinson disease with dysautonomia, the difference was statistically highly significant (P< 0.01).

Table (2): The comparison of oxidative stress levels in the studied groups.

	Antioxidant Activity	**P value**
Parkinson disease with dysautonomia	0.70 + 0.32	0.001
Parkinson disease without dysautonomia	0.82 + 0.34	
Control	1.08 + 0.30	

The difference was not statistically significant in level of oxidative stress between patients of Parkinson disease with autonomic dysfunction and patients of Parkinson disease without autonomic dysfunction, P> 0.05. Whereas, the difference was statistically significant in level of oxidative stress between patients of Parkinson disease with autonomic dysfunction (0.7 + 0.3 pg/ml) and control group (1.1 + 0.3 pg/ml), P< 0.01. The difference was also statistically significant in level of oxidative stress between patients of Parkinson disease without ANS (0.8 + 0.3) and control group (Table 3).

Table (3): Post hoc test for comparison between levels of oxidative stress in each two group in study population.

Study population	Oxidative stress Mean + SD	P value
Parkinson disease with ANS Parkinson disease without ANS	0.7 + 0.3 0.8 + 0.3	0.310
Parkinson disease with ANS Control group	0.7 + 0.3 1.1 + 0.3	0.001
Parkinson disease without ANS Control group	0.8 + 0.3 1.1 + 0.3	0.018

The Correlation between antioxidant activity levels in patients of Parkinson disease with and without dysautonomia as shown in figure (2), there is a weak correlation between these two groups.

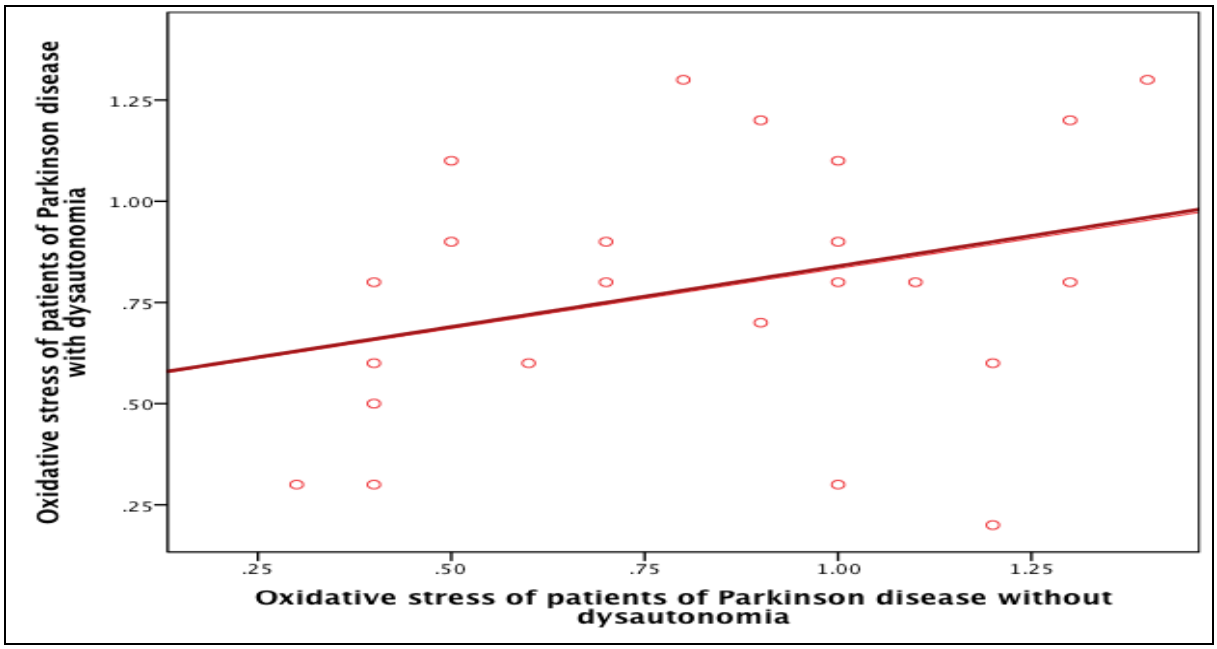

Figure (2): Correlation between antioxidant activity in patients of Parkinson disease with and without dysautonomia

Discussion

The antioxidant activity were studied in all the three groups (Parkinson patients with dysautonomia, Parkinson patients without dysautonomia, and the controls) showing the highest activity was in control groups, followed by Parkinson patients without dysutonomia, then Parkinson patients with dysautonomia.

In the comparison of the antioxidant status the entire three groups. Again the highest level in control group while the lowest level in parkinson patients with dysautonomia & in between was parkinson patients without dysautonomia. The difference was statistically highly significant. The antioxidant status is affected by the autonomic dysfunction. This result mostly demonstrates that oxidative stress level is one of probable causes of Parkinson disease as a whole and dysautonomia in specific, depending on its mechanism.

Polidori and his colleagues suggest that, high amounts of reactive oxygen species

were produced during production of ATP (example; hydrogen peroxide, superoxide anion, O2, H2O2 and hydroxyl radicals). Production of ROS within a physiological range, signaling pathway will occur, this includes; transcription of antioxidant enzymes, (like superoxide dismutase, catalase and glutathione peroxidase, glutathione *S*-transferase, heme oxygenase, thioredoxin, glutathione peroxidase *etc.*). Additionally, ROS are associated with depletion of dopamine and tissue antioxidants. ROS are cytotoxic molecules and key mediators in signaling cascades. In the brain of Parkinson patients, oxidative damage is common, caused by coupling of sustained oxygen consumption and inefficient anti-oxidant defense system [17]. The difference was not statistically significant in level of oxidative stress between patients of Parkinson disease with autonomic dysfunction and patients of Parkinson disease without dysautonomia, P> 0.05. This may be related with limited sample size or type of test used in this study or other cause.

The Correlation between oxidative stress levels in patients of Parkinson disease with and without dysautonomia in figure (2) shows there is a weak correlation between these two groups. This can be explained in that both of the diseased groups (Parkinson patients with and and without dysautonomia) are affected by the same mechanism (oxidative stress) in addition to other causes of dopamine depletion (mitochondrial dysfunction & inflammation), although the effect of these problems are more pronounces in Parkinson patients with autonomic dysfunction than Parkinson patients without autonomic dysfunction. This suggestion had been agreed also by Lin and Beal. [18, 13]

In conclusion. Oxidative stress is important risk factor that may initiate or promote degeneration of dopaenergic neurons in Parkinson disease as a general & autonomic dysfunction related to Parkinson disease. Antioxidants participates in preventing the formation of free radicals and scavenging of these radicals and other potentially toxic oxidizing species. Oxidative stress has been hypothesized to be linked to both the initiation and the progression of PD with and without autonomic dysfunction.

In our study we recommend. Encourage usage of antioxidants especially for persons who were at risk of developing parkinson disease like person with family history of Parkinson disease and others.

References

[1] Lees AJ. Unresolved issues relating to the shaking palsy on the celebration of James Parkinson's 250th birthday. Mov Disord.2007; 22(Suppl 17):S327-34.

[2] Schapira AH. Mitochondrial diseases. Lancet. 2012b; 379:1825-1834.

[3] Barnett-Cowan M, Dyde RT, Foxe SH, Moro E, Hutchison WD, Harris LR. Multisensory determinants of orientation perception in Parkinson's disease. Neuroscience. 2010; 167(4):1138-50.

[4] Mao P, Meshul CK, Thuillier P, Reddy PH. Neurotransmitter CART as a new therapeutic candidate for Parkinson's disease. Pharmaceuticals.2013; 6:108-123.

[5] Poewe W. Clinical measures of progression in Parkinson's disease. Mov Disor.2009; 24(Suppl 2):S671-676.

[6] Anglade P, Vyas S, Javoy-Agid FP, Herrero MT, Michel PP, Marquez J, Mouatt-Prigent A, Ruberg M, Hirsch EC and Agid Y. Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. Histol Histopathol. 1997; 12:25-31.

[7] Goldstein DS, Holmes C, Bentho O, Sato T, Moak J, Sharabi Y, *et al.* Biomarkers to detect central dopamine deficiency and distinguish Parkinson disease from multiple system atrophy. Parkinsonism Relat Disord. 2008; 14:600–7.

[8] Schulz-Schaeffer WJ. The synaptic pathology of alpha-synuclein aggregation in dementia with Lewy bodies, Parkinson's disease and Parkinson's disease dementia. Acta Neuropathol. 2010; 120(2):131-43.
[9] Kasuga K, Nishizawa M, Ikeuchi T. Alpha-synuclein as CSF and blood biomarker of dementia with Lewy bodies. *Int J Alzheimer's Dis.* Article 2012; ID 437025.
[10] Aarsland D, Londos E, Ballard C. Parkinson's disease dementia and dementia with Lewy bodies: different aspects of one entity. Int Psychogeriatr.2009; 21(2):216-9.
[11] Zhou C, Huang Y, Przedborski S. Oxidative stress in Parkinson's disease: a mechanism of pathogenic and therapeutic significance. Ann NY Acad Sci. 2008; 1147:93-104.
[12] Jenner P. Oxidative stress and Parkinson's disease. Handb Clin Neurol. 2007; 83:507-520.
[13] Seet RC, Lee CY, Lim EC, Tan JJ, Quek AM, Chong WL, Looi WF, Huang SH, Wang H, Chan YH, Halliwell B. Oxidative damage in Parkinson disease: Measurement using accurate biomarkers. Free Radic Biol Med.2010; 48:560-566.
[14] Moore DJ, West AB, Dawson VL, Dawson TM. Molecular pathophysiology of Parkinson's disease. Ann Rev Neurosci. 2005; 28:57-87.
[15] Jenner P, Dexter DT, Sian J, Schapira AH, Marsden CD. Oxidative stress as a cause of nigral cell death in Parkinson's disease and incidental Lewy body disease. Ann Neurol. 1992; 32:S82-S87.
[16] Lucking CB, Durr A, Bonifati V, Vaughan J, De Michele G, Gasser T, Harhangi BS, Meco G, Denèfle P, Wood NW, Agid Y, Brice A. Association between early-onset Parkinson's disease and mutations in the parkin gene. French Parkinson's Disease Genetics Study Group. N Engl J Med. 2000; 342:1560-1567.
[17] Polidori MC, Griffiths HR, Mariani E, Mecocci P. Hallmarks of protein oxidative damage in neurodegenerative diseases: focus on Alzheimer's disease. Amino Acids.2007 ; 32(4):553-9.
[18] Lin MT, Beal MF. Mitochondrial Dysfunction and Oxidative Stress in Neurodegenerative Diseases. Nature.2006; 443:787-795.