Association of Parkinson's disease with altered serum levels of lead and transition metals among South Indian subjects

Nadella Kumudini¹, Addepally Uma¹, Yalavarthy Prameela Devi², Shaik Mohammad Naushad³, Rukmini Mridula⁴, Rupam Borgohain⁴ and Vijay Kumar Kutala⁵*

¹Centre for Biotechnology, Jawaharlal Nehru Technological University, Hyderabad; ²Department of Zoology, Kakatiya University, Warangal; ³School of Chemical and Biotechnology, SASTRA University, Tirumalaisamudram, Thanjavur; ⁴Department of Neurology, ⁵Department of Clinical Pharmacology and Therapeutics, Nizam’s Institute of Medical Sciences, Hyderabad, 500082, A.P, India

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Several epidemiologic studies have suggested an association between the Parkinson’s disease (PD) and exposure to heavy metals, such as lead, iron, copper, manganese, etc. A growing body of evidence suggests that heavy metals stimulate free radical formation in the brain and can lead to neurodegeneration. In the present study, we investigated whether such association exists in PD cases from rural and urban areas in our study population. The plasma levels of copper, iron, manganese and lead in PD cases (n = 150) and controls (n = 170) were determined by inductively coupled plasma mass spectrometry (ICP-MS) and correlated with the oxidative stress markers like malondialdehyde (MDA), protein carbonyl and total glutathione. Results indicated significant increase in the levels of copper (17.73 ± 4.48 vs. 13.0 ± 3.22 ng/ml) and iron (554.4 ± 123.8 vs. 421.7 ± 126.1 ng/ml) in PD cases compared to controls, whereas no significant differences in the levels of manganese and lead were observed. Further, the data based on urban or rural residence showed that plasma copper, iron, manganese levels were comparatively higher in rural subjects, whereas plasma lead levels were significantly higher in urban subjects. Increased plasma iron showed positive correlation with marker of lipid peroxidation (MDA), suggesting that increased iron levels induced oxidative stress in PD. These results substantiated the earlier observations about the role of environmental exposure and metal-induced oxidative stress in the etiology of PD.

Keywords: Parkinson’s disease, Iron, Copper, Lead, Manganese, Oxidative stress

Parkinson’s disease (PD) is a multi-factorial disease with the involvement of age, genetic and environmental factors¹. Moreover, the contribution of genetic factors in the incidence of the disease is very less (10%) with majority being sporadic². Epidemiological studies have revealed the association of PD with herbicides/pesticides³, rural residence⁴, metal exposure⁵, higher intake of dietary fats, genetic predisposition, free radicals and accelerated ageing⁶. One of the major hallmarks of PD is the presence of intracellular inclusions called "Lewy bodies", consisting of aggregates of the presynaptic soluble protein α-synuclein⁷. However, it is reported that the major underlying factor in the pathophysiology of PD is increased oxidative stress, wherein reactive oxygen species (ROS) induce modifications of protein side-chains as well as enhance lipid peroxidation, thereby mediating the consequences of neuronal cell death⁸. It has also been suggested that exposure to pesticides and metals would accelerate fibrillation of α-synuclein, thereby mediating the formation of inclusion bodies⁹.

Metals act as co-factors for a number of biochemical and signaling pathways. In the brain, iron acts as a co-factor for a large number of enzymes, especially tyrosine hydroxylase, the rate-limiting enzyme of dopamine synthesis¹⁰. However, excess of free iron is toxic to the cell by mediating conversion of H₂O₂ to hydroxyl radical via Fenton reaction, thereby increasing oxidative stress¹¹. Accumulation of iron in the substantia nigra of PD patients has also been identified in the few studies¹²,¹³. Exposure to manganese has long been known to produce clinical effects similar to PD¹⁴,¹⁵. Manganese is known to exert its neurotoxic effect by mediating the conversion of dopamine to 6-hydroxydopamine, thereby increasing oxidative stress¹⁶. Copper plays a key role in cell metabolism by acting as co-factor for

*Corresponding author:
E-mail: vijaykutala@gmail.com
Ph: +91-9395532288

Abbreviations: ICP-MS, inductively coupled plasma mass spectrometry; MDA, malondialdehyde; PD, Parkinson’s disease; ROS, reactive oxygen species.
several detoxifying enzymes and proteins (superoxide dismutase, ceruloplasmine, metallothioneine, etc.), wherein altered homeostasis would result in increased free radical production, thereby mediating cell death\(^{17}\). The reports on association between PD and manganese are conflicting. While an association between the PD and managenese exposure in some studies\(^{18,19}\), some other studies have shown null association\(^{20,21}\). Lead is neurotoxic and known to induce multiple clinical phenotypes, including Parkinsonism in lead-exposed individuals\(^{22}\).

The mechanisms involved in metal-induced toxicity have one thing in common i.e. increased oxidative stress, either directly or indirectly\(^{23}\). Transition metals like copper, iron, manganese and zinc catalyze redox reactions within biological systems and hence mediate the oxidative stress\(^{11}\). Whereas redox inactive metals like lead, chromium etc. are indirectly involved in the production of ROS by depleting glutathione and protein-bound sulfhydryl groups\(^{24}\). Studies related to elemental alterations of metals in PD cases are very sparse and conflicting. This is the first comprehensive analysis of lead and transition metals in the sera of PD cases from Indian subcontinent.

We hypothesized that occupational exposure, rapid industrialization and environmental pollutants might contribute to higher serum levels of lead and transition metals, which in turn might lead to inflating the risk for PD. Thus, we have conducted a case-control study to explore association of PD with altered serum levels of iron, copper, manganese and lead with specific emphasis on the occupational exposure and urban/rural residence.

**Materials and Methods**

**Enrollment of subjects**

A case-control study was conducted enrolling 150 PD cases and 170 age- and ethnicity matched controls from the Department of Neurology, Nizam’s Institute of Medical Sciences (NIMS), Hyderabad, India. Cases were diagnosed and confirmed by consultant neurologist based on UK Parkinson’s disease rating scale (UKPDRS). Clinical diagnosis was confirmed based on the presence of two of the four cardinal signs i.e. resting tremor, cogwheel rigidity, bradykinesia and postural instability. In addition, good response to levodopa, pill roll tremor of the hand and asymmetric onset were considered as key criteria for differentiation from Parkinsonian like disorders. All cases of secondary Parkinsonism were excluded from the study. Healthy individuals comprising of patient’s attendants coming from urban and rural areas without any neurological disease were recruited as controls.

Demographic data relating to age, gender, farming occupation, chemical exposure and duration of the disease were collected from all the cases. NIMS, a premier tertiary care hospital of South India receives referrals from all regions of South India. Depending on the place of residence of the subject, data were segregated into rural and urban. The study protocol was approved by the Ethical Committee of NIMS (EC/NIMS/1289/2011). Written informed consent was obtained from all the subjects participated in the study.

Six ml of whole blood samples were collected in EDTA vacutainers from all the subjects (cases and controls) participated in the study. Plasma was separated immediately, following centrifugation at 3000 RPM and stored at -80°C until analysis.

**Biochemical analysis**

Plasma malondialdehyde (MDA), a marker of lipid peroxidation was estimated by thiobarbituric acid (TBA) method\(^{25}\), while protein carbonyl, a protein oxidation product was measured 2,4-DNPH method\(^{26}\). Total glutathione content in plasma was determined by Ellman’s method\(^{27}\).

**Estimation of plasma iron, copper, lead, manganese by ICP-MS method**

Plasma samples were diluted with 0.05 M nitric acid and 1.0 ml of diluted sample was used for the analysis. After processing of plasma samples, the levels of copper, iron, lead and manganese were quantified by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies 7700 Series). Argon gas flow rate was maintained at 15 L/min (plasma) and 1 KW power was used. The standards of copper, iron, manganese or lead of 10, 25, 50, 100, 200 µg/L were prepared and calibrated. The concentration of the test was calculated by using the standard curve plot.

**Statistical analysis**

All values were expressed as mean ± SD. Student t-test was used to analyze continuous variables. Analysis of variance (ANOVA) was used to analyze more than one continuous variable. Correlation between different variables was tested using non-parametric Pearson correlation coefficient (r) and statistical significance was considered at \(P<0.05\).
Results

As shown in Table 1, the demographic characteristics, such as age, gender, smoking habits, farming occupation showed no statistically significant differences between cases and controls. PD cases showed lower body mass index (BMI), compared to controls. Subjects originating from urban areas showed higher risk for PD, compared to subjects from rural upbringing.

Among all the metals quantified, copper and iron showed marked elevation in the PD cases compared to controls, whereas plasma lead levels showed no significant difference between cases and controls (Table 2). Decreased plasma manganese levels were observed in cases, when compared with controls; however, this decrease was not statistically significant. PD cases exhibited elevated plasma MDA (7.1 ± 2.1 vs. 4.5 ± 1.5 µmol/L) and protein carbonyls (2.0 ± 1.3 vs. 1.0 ± 0.4 nmol/mg protein) levels and depleted antioxidant GSH levels (407 ± 209 vs. 649 ± 319.0 µmol/L), compared to controls.

Table 1—Demographic characteristics of cases and controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n = 150)</th>
<th>Controls (n = 175)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD) in yrs</td>
<td>55.7 ± 10.6</td>
<td>53.73 ± 10.9</td>
<td>0.10</td>
</tr>
<tr>
<td>Female: Male ratio</td>
<td>43:107</td>
<td>55:120</td>
<td>0.68</td>
</tr>
<tr>
<td>BMI (mean ± SD) in kg/m²</td>
<td>23.7 ± 4.08</td>
<td>24.61 ± 3.7</td>
<td>0.04*</td>
</tr>
<tr>
<td>Smoking (Yes/No)</td>
<td>63/87</td>
<td>61/114</td>
<td>0.23</td>
</tr>
<tr>
<td>Age at onset</td>
<td>47.9 ± 11.0</td>
<td>NA</td>
<td>----</td>
</tr>
<tr>
<td>Mean disease duration</td>
<td>7.9 ± 4.7</td>
<td>NA</td>
<td>----</td>
</tr>
<tr>
<td>Early onset PD</td>
<td>63 (42%)</td>
<td>NA</td>
<td>----</td>
</tr>
<tr>
<td>Late onset cases</td>
<td>87 (58%)</td>
<td>NA</td>
<td>----</td>
</tr>
<tr>
<td>Familial cases</td>
<td>23 (16%)</td>
<td>NA</td>
<td>----</td>
</tr>
<tr>
<td>Head injury</td>
<td>24 (16%)</td>
<td>NA</td>
<td>----</td>
</tr>
<tr>
<td>Chemical exposure</td>
<td>46 (30.7%)</td>
<td>NA</td>
<td>----</td>
</tr>
<tr>
<td>Farming occupation</td>
<td>20/130</td>
<td>35/140</td>
<td>0.15</td>
</tr>
<tr>
<td>Rural background</td>
<td>61/89</td>
<td>108/67</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Urban background</td>
<td>89/61</td>
<td>67/108</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

* statistically significant

Table 2—Plasma levels of copper, iron, manganese and lead levels in PD cases and controls

<table>
<thead>
<tr>
<th>Metal</th>
<th>Mean ± SD (ng/ml)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>17.73 ± 4.48</td>
<td>13.0 ± 3.22</td>
</tr>
<tr>
<td>Iron</td>
<td>554.4 ± 123.8</td>
<td>421.7 ±126.1</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.098 ± 0.05</td>
<td>0.11 ± 0.065</td>
</tr>
<tr>
<td>Lead</td>
<td>0.43 ± 0.36</td>
<td>0.22 ± 0.23</td>
</tr>
</tbody>
</table>

r: Pearson correlation co-efficient, *statistically significant.

When the markers of oxidative stress were correlated with plasma iron, copper, lead and manganese, the plasma iron levels showed a positive association with MDA (r = 0.35, P = 0.05) (Table 3). The data were further analyzed by ANOVA, wherein plasma MDA levels were segregated with iron levels into 3 tertiles (<420, 420-530 and >530 µg/ml) and a significant increase in MDA was observed with increase in iron levels as shown in Fig. 1 (P<0.05). This association had 80% power with type I α error < 0.05.

The influence of metal exposure in PD subjects living in rural/urban areas was also investigated. As shown in Fig. 2, the plasma copper, iron, manganese levels were higher in rural subjects, whereas plasma lead levels were significantly higher in cases from urban areas. Increased risk of PD was
observed between the manganese and rural living ($P = 0.02$), whereas lead showed increased risk with urban inhabitancy ($P = 0.04$). Copper and iron also showed association with rural living, but not statistically significant (Fig 2); however, no significant difference was observed in control group from urban and rural areas.

When the influence of occupational exposure on the plasma levels of the metals was evaluated, a positive association was observed with iron ($r = 0.29$, $P < 0.0001$), lead ($r = 0.16$, $P = 0.007$) and copper ($r = 0.11$, $P = 0.047$). However, no association was observed between occupational exposure and manganese ($r = -0.09$, $P = 0.11$).

**Discussion**

In the current study, elevated copper and iron levels were observed in the serum samples of PD cases. Studies on the role of copper in PD are conflicting; twin studies have reported decreased serum copper levels in PD cases$^{28,29}$. In contrast, another study$^{30}$ has reported increased copper levels in PD cases compared to controls, which was in agreement with the present study. On the other hand, another study$^{31}$ has reported no significant difference in copper levels in serum of PD cases, compared to controls.

The present study showed decreased levels of manganese in cases compared to control, which corroborated with previous studies$^{32,33}$, although another study has found no significant variation in cases and controls$^{34}$. Lead also showed no variation in cases and controls, which was in accordance with the previous study$^{35}$. Long-term exposure to lead sulfate or other sulfur compounds is also found to develop Parkinsonism in postal workers$^{32}$.

A significant increase in iron was observed in cases, when compared with controls. Studies on the association of iron toxicity with PD are controversial. Few studies have reported decreased iron levels in cases$^{36}$, whereas in one of the study higher iron is reported in serum of the patients$^{37}$. Our results corroborated with the study on Chinese population reporting increased levels of iron and also manganese in PD patients, compared to controls$^{38}$. Increased iron levels have also been identified in the substantia nigra of the post-mortem brain tissues of PD patients$^{12,39}$.

The role of iron in neurodegeneration has been substantiated by the finding that iron chelation prevents degeneration of dopaminergic midbrain neurons$^{40}$. Consistent with our observation, iron overload is reported to mediate lipid peroxidation via free radical generation$^{41}$. Our results corroborated with the study of previous study in demonstrating increased oxidant species and lowered anti-oxidant capacity in PD$^{29}$.

Several epidemiologic studies have found a positive association between rural living, well water drinking and PD$^{42,43}$. On the contrary, a few studies have reported no association of rural living and well water drinking with PD risk$^{44,45}$, while decreased risk is reported in two studies$^{46}$. However, the relation between rural inhabitancy and drinking well water could be explained by the increased exposure to pesticides and other environmental contaminants$^{47}$. We found a significant association between the manganese exposure and rural living, which was in agreement with previous studies on rural residence. The current study showed decreased risk in rural living and increased risk in urban living, which might be attributed to increased industrial and vehicular pollution, as evidenced by increased lead levels in urban living. We also observed a positive contribution of occupational exposure towards increased serum levels of iron, lead and copper.

Studies on the association of occupational exposure to various metals suggest that welders exposed to various metals are at increased risk of developing PD at an earlier age$^{48}$. It is also reported that occupational exposure to various metals like copper, iron and lead would alter PD risk in cases with family history$^{49}$. Whereas a recent meta-analysis conducted on different populations has failed to confirm the association between the welding workers and
manganese exposure in altering PD risk. Combinatorial exposure to manganese and iron is observed to inflate PD risk, however, in another study, lack of association has been found between PD and manganese exposure. A case-control study has reported an association of PD in those who experienced >20 yrs of exposure to transition metals, such as copper, manganese and also combinatorial exposure to lead, iron and manganese would alter the risk. Several contradictory results relating to occupational exposure and PD can be ascertained to criteria used for diagnosis, as well as methods for exposure assessment. Consistent with a recent meta-analysis, PD cases showed lower BMI than controls in the current study. The role of other environmental factors also needs to be established because of the involvement of multiple factors in the etiology of PD.

To summarize, the present study emphasized the role of metal exposure on the risk of PD in different epidemiological perspectives (urban and rural living). There was a positive association between metals and oxidative stress, indicating the role of metal-mediated oxidative stress in the etiology of PD.

Acknowledgement
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References
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