Impaired Systemic Iron Homeostasis and Parkinson's Disease

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Abstract
Introduction: Parkinson's disease is second most prevalent neurodegenerative old age disorder of basal ganglia characterized by tremor at rest, muscle rigidity, slowness of movement (bradykinesia, akinesia), and changes in posture (instability) which involves α-synuclein protein oligomers and intercellular inclusions known as "Lewy bodies" in substantia nigra and caudate nuclei that is progressive in nature. Both excess and deficiency in levels of transition metals (especially iron) can be detrimental to the central nervous system (CNS). Abnormalities in iron metabolism have been reported to produce oxidative stress which is one of the major cause in pathogenesis of Parkinson's disease.

Material & Method: In the present study 79 PD patients and 80 controls of Northern Indian population were included and serum levels of Transferrin, TIBC, iron, Transferrin saturation, Ferritin were measured.

Results: In this study, we observed elevated levels of Transferrin (p < 0.001), TIBC (p < 0.001) while reduction in iron (p < 0.001), Transferrin saturation (p < 0.001), Ferritin (p < 0.023) levels in serum of PD patients compared to the controls.

Conclusion: These results suggest the decreased iron concentrations in the peripheral blood, indicating that the iron accumulation in the brain is not dependent of an iron overload, but probably due to impaired iron homeostasis.

Keywords: Parkinson’s Disease, Transferrin, TIBC, iron, Transferrin saturation, Ferritin, Oxidative Stress.

INTRODUCTION
Parkinson’s disease is most common motor disorder and second most common neurodegenerative disorder, after Alzheimer’s disease. PD is disorder of elders with mean age of onset about 60 years, but also has been reported in the patients in 20s, or even younger. It is a progressive disorder clinically characterized by cardinal symptoms like tremors at rest, rigidity, bradykinesia/akinesia, and gait impairment/postural instability. PD is heterogeneous diseases with regard to clinical, neuropathological, biochemical and molecular features. There are two forms of the disease which can be distinguished: the familial form, most frequently with early onset of symptoms, and the sporadic form, where the symptoms usually appear in older age.

Iron, transition metal, is obligatory for a number of biochemical and signalling pathways in the central nervous system (CNS) [3]. It plays a dual role in PD. On one hand free iron is associated with increased oxidative stress[2], oligomerization
of alpha-synuclein protein, and formation of Lewy bodies via Fenton and Haber–Weiss reaction while on other hand it acts as a co-factor for tyrosine hydroxylase, enzyme that limits the dopamine synthesis, as well as free iron is toxic to the cell. Iron accumulation has been identified in the substantia nigra of PD patients [3]. Transferrin is a apotransferrin Fe³⁺ complex. Atpotransferrin is a beta globulin synthesized in liver which binds to two atoms of ferric iron and carries it to different tissues via the circulatory network. Apart from being a soluble ferric iron transporter, it prevents iron from reacting with other molecules by attenuating its redox activity. TIBC (Total Iron Binding Capacity) is the capacity of transferrin to bind to iron while Transferrin saturation, measured as a percentage, is the value of serum iron divided by the total iron-binding capacity, a measure of iron in transit in the serum. Elevated levels of Transferrin and TIBC while reduced value of Transferrin saturation indicate towards the iron deficiency which may increase the risk of PD [4]. Ferritin is an iron storage complex composed of 24 protein subunits consisting of heavy (H) chains and light (L) chains. The L-chains are dominant in ferritin in organs that store iron, where it is responsible for iron nucleation into the safe ferric state. It not only stores iron in nontoxic ferric ion form but also acts as antioxidant. It can store up to 4500 ferric ions. Decreased level of L-chain-ferritin has been demonstrated in the substantia nigra of Parkinson’s disease patients compared to that of controls [5].

MATERIALS AND METHODS

Subjects
The study was conducted enrolling 79 PD cases and 80 controls in the Department of Neurobiochemistry, Institute of human behaviour and allied sciences (IHBAS), New Delhi, India. Cases were diagnosed and confirmed by specialist neurologist based on the Unified Parkinson Disease Rating Scale (UPDRS) and Mini Mental State Examination (MMSE). Clinical diagnosis was confirmed based on the presence of cardinal signs – Resting Tremors, Cog wheel Rigidity, Bradykinesia and Postural instability. Healthy individuals without any history of stroke/ cerebrovascular surgery/ Intercranial surgery/ Head injury/ Meningitis/ Encephalitis/Hypertension/ Diabetes/ Hypercholesterolemia/Hyperthyroidism/ CAD/ Depression or any mental disorder were recruited as controls in the study. Sociodemographic data related to age, gender, occupation, habitat were collected from all the cases. The study was approved by the ethical committee of IHBAS. Written consent was obtained from all the patients and controls.

Sample collection and Preparation
Considering all universal precautions, blood samples were collected in plain evacuation tubes from BD life sciences (U.S.) and centrifuged for 15 min at 1500 rpm at room temperature. After centrifugation, serum samples were collected and stored at -20°C until analysis. Hemolyzed samples were excluded from the study.

Biochemical Investigations
Serum level of iron (by Spectrophotometric method using reagents from Fortress diagnostics ltd., UK), Transferrin (by turbidimetric method using reagents from..) were measured on fully automated Olympus AU480 by Beckman coulter inc. TIBC was calculated mathematically from the estimated serum value of Transferrin using the formula: TIBC µmol/L = 25.0 × TRF g/L. Transferrin saturation was also calculated mathematically by the estimated serum iron values and calculated TIBC values using formula: Transferrin Saturation = (Serum Iron / Total Iron Binding Capacity) x 100%. Serum Ferritin was estimated by Electrochemiluminescence immunoassay “ECLIA” using reagents from Roche Diagnostic india Pvt. Ltd.

Statistical Analysis
All values were expressed as mean ± SD. Independent two tailed t-test was used to analyse continuous variables. Statistical significance was considered at P<0.05.
RESULT
Serum levels of transferrin were estimated to be increased in Parkinson's disease cases in contrary to that of controls. Results observed were highly significant with p value < .001. TIBC levels were observed to be significantly increased in PD patients in comparison to the controls by (p value < .001). Iron was observed to be significantly reduced in the PD cases in comparison to the controls by (p-value < .001). Values of Transferrin saturation were observed to be 47.5% reduced in cases of PD than in controls which were statistically significant with P-value < .001. Significant lower levels of Ferritin were observed in PD cases in contrary to the levels of controls (p value < .05).

Table 1: Socio-demographic profile of PD patients and control group

|                   | PD (n=79) | Control (n=80) | P-value | COR | 95% Class Interval Lower | Upper |
|-------------------|-----------|----------------|---------|-----|--------------------------|-------|
| Age (mean ± SD)   | 54.57 ± 10.01 | 52.11 ± 8.75 | 0.101   | -5.403 | 0.488                    |
| Gender            |           |                |         |     |                          |       |
| Male              | 48        | 48             | 0.922   | 1.032 | 0.547                    | 1.949 |
| Female            | 31        | 32             |         |      |                          |       |
| Habitat           |           |                |         |     |                          |       |
| Rural             | 16        | 20             | 0.475   | 1.313 | 0.622                    | 2.769 |
| Urban             | 63        | 60             |         |      |                          |       |
| Alcohol           |           |                |         |     |                          |       |
| No                | 74        | 61             | 0.002   | 0.217 | 0.077                    | 0.615 |
| Yes               | 5         | 19             |         |      |                          |       |
| Smoking           |           |                |         |     |                          |       |
| No                | 68        | 61             | 0.113   | 0.519 | 0.229                    | 1.178 |
| Yes               | 11        | 19             |         |      |                          |       |
| Diet              |           |                |         |     |                          |       |
| Non-Veg           | 46        | 27             | 0.002   | 0.365 | 0.192                    | 0.696 |
| Veg               | 33        | 53             |         |      |                          |       |
| Water             |           |                |         |     |                          |       |
| Tap               | 63        | 60             | 0.475   | 0.762 | 0.361                    | 1.607 |
| Underground       | 16        | 20             |         |      |                          |       |

Table 2: Serum Copper, Iron, Ceruloplasmin, Transferrin, TIBC, Transferrin Saturation and Ferritin levels in PD patients and control group

| PARAMETERS         | GROUP | N   | Mean   | S.D  | P-value |
|--------------------|-------|-----|--------|------|---------|
| TRANSFERIN (g/l)   | PD    | 79  | 3.64   | .62  | .001    |
|                    | CONTROL | 80  | 3.29   | .48  |         |
| TIBC (µmol/L)      | PD    | 79  | 91.48  | 15.67| .001    |
|                    | CONTROL | 80  | 82.74  | 12.02|         |
| IRON (µmol/L)      | PD    | 79  | 13.50  | 2.49 | .001    |
|                    | CONTROL | 80  | 23.25  | 4.44 |         |
| TRANSFERRIN SATURATION (%) | PD | 79  | 15.09  | 3.34 | .001    |
|                    | CONTROL | 80  | 28.72  | 7.06 |         |
| FERRRTIN (µg/l)    | PD    | 79  | 65.62  | 45.09| .023    |
|                    | CONTROL | 80  | 95.71  | 107.21|         |

DISCUSSION
The present study shows that serum iron, Transferrin, TIBC, Transferrin Saturation and serum ferritin can discriminate between PD patients and controls. It has been an accepted fact iron interacts with α-synuclein, a major component of Lewy bodies that leads to protein aggregation and cross linking in PD\(^6\). Among trace metals, iron is most widely studied as iron deposits in SN of PD patients have been found consistently in many studies\(^7\) leading to oxidative stress, mitochondrial dysfunction, neuro inflammation and protein accumulation. However, information regarding its level in serum/plasma are inconclusive.
One of the major findings of this study was decreased iron levels in PD patients as compared to controls. Jimenez-Jimenez et al. [8] found no significant difference of serum iron levels between PD and controls whereas Logroscino et al. [9] has also reported decreased serum iron levels in PD. Few studies partly explain such variations. In study by Hedge et al. [10] serum iron levels showed decreasing trend with the severity of PD and found 14% fall in iron concentration in early PD as compared to 30% in sever PD as compared to controls. Furthermore, Pichler et al. [4] demonstrated protective role played by increased serum iron levels in PD suggesting that with every 10µg/dl increase in iron in serum, there is 3% decrease in risk of PD. There is not much information available explaining the source of excessive iron in brain and whether source of the increased iron content in brain is serum or not.

Studies related to the indicators of iron level in blood like Transferrin, TIBC, Transferrin saturation, ferritin are very few. Further the observations in these studies are not very conclusive. Logroscino et al. [11] reported reduced levels of systemic ferritin and transferrin and the total iron binding capacity which is parallel to the observations in a Parkinson’s disease brain but decreased value of Transferrin Saturation was unexpected. G Tórsdóttir et al. [12] observed elevation in levels of ferritin and transferrin saturation while reduced TIBC and transferrin levels in PD cases in comparison to controls. In this study, we observed elevated levels of transferrin and TIBC while reduction in levels of ferritin and transferrin saturation which indicates towards the iron deficiency in the peripheral blood.

A possible explanation of systemic iron deficiency could be the impaired control of iron homeostasis [13] due to a possible restriction of iron intake during life [14], frequent blood donation leading to decreased ferritin levels. Although iron accumulation in the SN is considered as a risk factor for the development of PD, a low level in peripheral blood may also be associated with an increased risk because it possibly reduces the functioning of neuronal enzymes, since it acts as a cofactor of tyrosine hydroxylase and plays a role in the synthesis of neurotransmitters.

Furthermore, reduced peripheral iron may decrease ferritin storage in neurons, thus decreasing the pool of iron available for neuronal enzymes and consequently leading to accumulation of free iron in the SN [4]. The role of systemic iron in the accumulation of iron in brain is far from resolved. More knowledge about the factors and mechanisms involved in the disturbed iron homeostasis will probably add significantly to the understanding of how iron can cause damage to neurons, and probably also to the identification of putative pharmaceutical targets to improve neuronal iron-handling.

**CONCLUSION**

Iron is considered a risk factor for PD because it accumulates in the SN of patients. However, we found decreased iron concentrations in the peripheral blood, indicating that this accumulation is not dependent of an iron overload, but probably due to iron homeostasis dysregulation. More studied are needed to be conducted to have the better understanding of relationship between the serum iron levels and brain iron accumulation with the aim to diagnose PD as early stage.

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**CONFLICTS OF INTERESTS**

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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