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SHORT COMMUNICATION

Role of Iron and Copper in the Pathogenesis of Parkinson's Disease

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Abstract Parkinson's disease (PD) is an old age disorder of basal ganglia which involves oligomerization of α-synuclein protein and formation of intercellular inclusions known as "Lewy bodies" in substantia nigra and caudate nuclei in brain which is progressive in nature. It is second most prevalent neurodegenerative disorder characterized by tremor at rest, muscle rigidity, slowness of movement (bradykinesia, akinesia), and changes in posture (instability). Both excess and deficiency in levels of transition metals (especially iron, copper) can be detrimental to the central nervous system. Abnormalities in iron (Fe) and copper (Cu) metabolism have been reported to produce oxidative stress which is one of the major cause in pathogenesis of PD. In the present study 35 PD patients and 33 controls of Northern Indian population were included and serum levels of Fe, Cu and ceruloplasmin (Cp) were measured. Serum Fe ($p < 0.01$) and Cu ($p < 0.01$) levels were found to be significantly decreased in PD, whereas there was no significant change in Cp levels in PD patients as compared to controls. These results suggest the existence of a defect in iron which over the time, may hasten the entry of iron into the brain and decrease iron in the extracellular compartment in PD patients.

Keywords Parkinson's disease · Iron · Copper · Ceruloplasmin · Oxidative stress

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Introduction

Parkinson's disease (PD), a disorder of basal ganglia is second most prevalent progressive neurodegenerative disorder characterized by tremor at rest, muscle rigidity and bradykinesia, akinesia. It is closely associated with loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and formation of intercellular inclusions known as "Lewy bodies" in substantia nigra and caudate nuclei.

Transition metals, iron and copper, are obligatory for a number of biochemical and signalling pathways in the central nervous system (CNS) [1]. Copper plays a dual role in PD. On one hand free copper 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 cofactor of important antioxidant enzymes such as Cu/Zn-SOD (Superoxide Dismutase) [3] which reduces oxidative stress. Similarly iron accumulation has been identified in the substantia nigra of PD patients [4]. 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.

Ceruloplasmin is a single chain glycoprotein that transports copper as well as oxidise toxic ferrous (fe^{+2}) ions to nontoxic ferric (fe^{+3}) ion form and incorporate it

into transferrin. Ferroxidase activity of the ceruloplasmin has been reported to be diminished in the plasma of patients with Parkinson's disease leading to the iron overload [5].

In last few years, levels of iron, copper and ceruloplasmin have been investigated in plasma/serum and substantia nigra of PD patients. However, plasma iron levels do not show consistent results. Based on this the present study was undertaken to assess the levels of iron, copper and ceruloplasmin in PD and correlation among them.

Materials and Methods

Study Design

Thirty five PD patients (mean age 57 years) and 33 normal subjects (mean age 50 years) were included in the study. Cases consisted of subjects diagnosed with PD using Unified Parkinson Disease Rating Scale (UPDRS) and Mini Mental State Examination (MMSE). The control samples were collected from community, not having recent history of stroke, cerebrovascular surgery, head injury, depression or any other mental disorder. The study was approved by the ethical committee of IHBAS. Written consent was obtained from all the patients and controls. All participants underwent assays of copper, iron and ceruloplasmin along with routine laboratory tests.

Biochemical Investigations

Blood was collected from subjects as routine sample taking all universal precautions. Serum was separated within 30 min and stored at −20 °C until analysis. Serum copper concentration was measured by colorimetric method using kit from Fortress diagnostics Ltd. UK. Serum iron was measured using Ferene supplied by Fortress diagnostics Ltd. UK. Serumceruloplasmin was assayed by turbidimetric specific reaction using kit from Randox (RX Series).

Statistical Analysis

All values were expressed as mean ± SD. Independent two tailed *t* test was used to analyse continuous variables and correlation between different variables was investigated by Spearman's correlation coefficient (SCC).

Results

The mean age of PD patients was 57 years (SD 12), out of which 68.5 % were males. Smoking and alcohol habits were not found associated with the PD.

Serum Iron, Copper and Ceruloplasmin Levels in PD Patients

Table 1 shows iron, copper and ceruloplasmin levels in PD patients and control. Serum copper and iron levels were found to be significantly decreased in PD patients as compared to controls ($p = 0.001$), whereas ceruloplasmin levels were slightly raised in PD patients ($p = 0.61$). In the male and female subgroups similar trends were observed. The Spearman's correlation coefficient (SCC) was used to investigate the correlation between iron–copper, copper–ceruloplasmin, iron–ceruloplasmin in PD patients and controls (Table 2). No obvious correlation could be found among parametric pairs. This implies that concentrations of iron, copper and ceruloplasmin may be relatively independent from each other (Table 3).

Discussion

The present study shows that serum iron and copper can discriminate between PD patients and controls. It has been an accepted fact iron and copper 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. Logroscino et al. [8] has also reported decreased serum iron levels in PD whereas Jimenez-Jimenez et al. [9] found no significant difference of serum iron levels between PD and controls. Few studies partly explain such variations. In study by Hedge et al. [10] serum iron levels

Table 1 Serum copper, iron and ceruloplasmin levels in PD patients and control group

Category	Parameter	Control Mean ± SD	PD Mean ± SD	*p* value
Total	Iron (μmol/l)	23 ± 5	13 ± 2	0.001
Copper (μg/dl)		147 ± 33	112 ± 11	0.001
Ceruloplasmin (mg/dl)		41 ± 8	42 ± 6	0.61
Male	Iron	24 ± 5	13 ± 3	0.001
	Copper	151 ± 29	111 ± 10	0.001
	Ceruloplasmin	40 ± 8	40 ± 5	0.82
Female	Iron	22 ± 5	13 ± 3	0.001
	Copper	143 ± 36	113 ± 13	0.01
	Ceruloplasmin	42 ± 8	45 ± 7	0.34

Table 2 Spearman correlation coefficients between concentration of different parameters in serum

Subjects	Category	Iron–copper	Iron–ceruloplasmin	Copper–ceruloplasmin
PD	Total (35)	−0.029	−0.021	−0.205
Controls	Total (33)	0.563**	0.304	0.431*

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

Table 3 Biochemical profile of PD patients and controls

Category	Parameter	Control Mean ± SD	Patient Mean ± SD	*p* value
Total	SGOT (U/L)	32 ± 19	21 ± 5	0.001
	SGPT (U/L)	32 ± 18	13 ± 6	0.001
	Albumin (g/dl)	4 ± 0.5	4 ± 0.4	0.114
	Urea (mg/dl)	24 ± 9	32 ± 12	0.003
	Creatinine (mg/dl)	0.91 ± 0.33	0.97 ± 0.2	0.37
	Uric acid (mg/dl)	5 ± 1	5.3 ± 1.5	0.35
Male	SGOT (U/L)	35 ± 24	20 ± 5	0.001
	SGPT (U/L)	36 ± 19	13 ± 6	0.001
	Albumin (g/dl)	1 ± 0.4	1 ± 0.4	0.62
	Urea (mg/dl)	26 ± 10	33 ± 13	0.05
	Creatinine (mg/dl)	1 ± 0.4	1 ± 0.1	0.74
	Uric acid (mg/dl)	5.2 ± 1	5.5 ± 1.4	0.54
Female	SGOT (U/L)	30 ± 12.5	22 ± 5.6	0.14
	SGPT (U/L)	28.5 ± 15.8	14.3 ± 7	0.004
	Albumin (g/dl)	4.2 ± 0.6	4.3 ± 0.34	0.25
	Urea (mg/dl)	23 ± 8	31 ± 9	0.02
	Creatinine (mg/dl)	0.9 ± 0.17	0.9 ± 0.27	0.08
	Uric acid (mg/dl)	4.8 ± 1.2	4.8 ± 1.6	0.82

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. [11] showed protective role played by increased serum iron levels in PD suggesting that with every 10 μg/dl increase in iron in serum, there is 3 % decrease in risk of PD. In present study relation couldn't be studied due to low sample size. Also severity of PD was not assessed in PD patients.

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. It has been shown that trace metals cross blood brain barrier (BBB) by selective uptake mechanism e.g. iron is transported from blood to brain by carrier protein Transferrin. Iron and transferrin are transported by means of a transferrin receptor mediated transcytosis through the BBB. This also explains correlation of iron levels in brain with the severity of neuropathological change in PD due to increased transport through BBB [12].

Similar pattern was observed with serum copper levels in PD patients. Serum copper levels in PD were found significantly low as compared to controls which was in agreement with the findings of Zhao et al. [13] but in contrast with Hegde et al. [10]. Hedge et al. reported increased serum copper concentration in both early and severe PD cases, but copper concentration was marginally increased in early PD as compared to controls. In contrast to this study, Zhao et al. [13] reported reduction of copper level in PD patient with age ≥65 years. Studies have revealed weak participation of copper in PD development and progression as indicated by decreased copper levels in PD brain which may be casually related to the increase of iron concentration [13]. Hence, it can be concluded that decreased copper level in PD brain is a consequence of disease and not the cause of it.

It has been an established fact that multicopper enzyme ceruloplasmin plays an active role in iron haemostasis due to its oxidative (ferroxidase) activity [14]. Hence, attempt was made to assess the levels of ceruloplasmin in serum of PD patients and controls and its correlation with iron and copper levels. Ceruloplasmin levels were found marginally increased in PD patients as compared to controls which is in agreement with the findings of Arnal et al. [15]. This finding may be explained by the fact that in disease, increased copper concentration may represent a compensatory mechanism for low copper oxidative activity [16]. It is further suggested by studies showing negative correlation between ceruloplasmin concentration and the oxidative activity in serum and iron deposits in SN in brain [17]. However, no correlation could be established between serum ceruloplasmin with copper and iron in the present study which may indicate the need for measurement of ceruloplasmin oxidative activities along with ceruloplasmin levels in serum of PD patients.

Conclusions

At present it is not clear whether decreased iron and copper serum levels of PD patients are a cause or consequence in the pathology of disease, but there is definite change in

haemostasis of these metals but fall short of any correlation of these with each other or ceruloplasmin levels.

Compliance with Ethical Standards

Conflict of interest Mohit Kumar Gangania declares that he has no conflict of interest. Dr. Jyoti Batra declares that she has no conflict of interest. Dr. Suman Kushwaha declares that she has no conflict of interest. Dr. Rachna Agarwal declares that she has no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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