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Serum trace element levels and the complexity of inter-element relations in patients with Parkinson's disease

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Abstract

Trace elements have been postulated to play a role in Parkinson's disease (PD). In order to elucidate whether changes in the serum levels of trace elements reflect the progression of PD, we assessed serum levels of 12 elements (Na, K, Fe, Al, Cu, Zn, Ca, Mg, Mn, Si, P and S) in early PD, severe PD and normal subjects, using inductively coupled plasma atomic emission spectrometry. The concentrations in μmol/ml, the relative mole percentage distribution and inter-element relations were computed. Statistical analysis of these data showed a definite pattern of variation among certain elements in early and severe PD compared to controls. In both early and severe PD serum, Al and S concentrations were significantly decreased ($p<0.05$) compared to the controls. Fe ($p<0.01$) and Zn ($p<0.05$) concentrations were significantly lower in severe PD, while K, Mg, Cu ($p<0.01$) and P ($p<0.05$) concentrations were higher in early and severe PD compared to the controls. The data revealed an imbalance in the inter-element relations in both early and severe PD serum compared to controls, as shown by the direct and inverse correlations. These results suggest a disturbance in the element homeostasis during the progression of PD.

Keywords: Parkinson's disease; Element homeostasis; Inter-element relation; Serum; Early PD; Severe PD

Introduction

Trace elements at optimum biological levels are required for numerous metabolic and physiological processes in the human body. Elements like Na, K, Mg, Ca and P serve as structural components of tissues and as constituents of the body fluids and are therefore essential for the functioning of the cells [1]. Imbalances in the optimum levels of these elements as well as trace metals such as Fe, Cu, Zn and Al may adversely affect biological processes and are associated with many neurological diseases [2–7].

The pathogenesis of neuronal degeneration in the pars compacta of substantia nigra in patients with Parkinson's disease (PD) is still not clearly known. Several studies have suggested the presence of oxidative stress in substantia nigra of PD patients [8–14]. An increase in Fe and other paramagnetic trace metals in substantia nigra could hypothetically elicit oxidative processes.

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Limited data is available concerning the levels of metals in serum during pathological conditions of PD. Studies have suggested that levels of transition metals like Zn, Cu and Fe in serum do not play a role as risk factor indicators for PD [15]. Moreover, most of the available information is limited to a few selected elements [16,17] and there is no study examining inter-element relations with regard to the severity of PD. The aim of the present study was to assess the serum levels of 12 elements (Na, K, Fe, Al, Cu, Zn, Ca, Mg, Mn, Si, P and S) in patients with early and severe PD compared with a control population, and also to understand the possible relevance of inter-element relations for the progression of PD.

Patients and methods

Patients

We assessed the serum levels of 12 elements in early and severe PD patients in comparison with controls. Blood samples were collected from 52 patients with PD (27 early and 25 severe PD) recruited among the outpatients attended in the Neurology Departments of two urban hospitals (Sri Venkateswara Institute of Medical Science and J.S.S. Medical Hospital, India). The PD patient group was graded into early PD and severe PD according to clinical severity of the disease. All patients met the commonly accepted diagnostic criteria for PD [18] and were evaluated by the Unified Parkinson's Disease Rating Scale (UPDRS) [19] and the Hoehn and Yahr staging [20]. The first stage of the Hoehn and Yahr staging of PD was considered as early PD, while the latter stages of Hoehn and Yahr staging were assigned to severe PD. 14 of the 27 early PD patients were untreated, the other 13 patients were treated with one or a combination of antiparkinsonian drugs such as levodopa (11 cases) and anticholinergics (2 cases). Of the 25 severe PD patients, 6 were untreated, and 19 were treated with antiparkinsonian drugs—a single drug or a combination—including levodopa (18 cases) and anticholinergics (3 cases). Cases with occupational exposures to Fe and Mn were excluded from the study. The main clinical features of the PD patients and controls are shown in Table 1.

The control group comprised 25 volunteers with no significant illnesses or medications for at least 3 months before the time of blood collection. Both the control and the PD groups were assessed by a neurologist and a psychiatrist.

The following exclusion criteria applied to both PD patients and controls [21,22]: (a) ethanol intake higher than 80 g/day during the last 6 months; (b) previous history of chronic hepatopathy or diseases causing malabsorption; (c) previous history of severe systemic disease; (d) atypical dietary habits (diets constituted exclusively by one type of foodstuff such as vegetables, fruits, and meat, or other special diets because of religious reasons, etc.); (e) previous blood transfusions, anemia and polycytemia; (f) intake of supplements of Fe, Cu, Al, Zn or chelating agents; (g) therapy with chlorotiazides, adrenocorticotropic hormone (ACTH) or steroids; (h) acute infectious disorders, traumatisms or surgery in the last 6 months; and (i) haemolytic anemia.

Ethical approval for the collection of blood samples from PD and control patients was obtained from the research ethical committee of the J.S.S. Medical College and Hospital and Sri Venkateswara Institute of Medical Science, India. A written consent was obtained from the patients/carers prior to the collection of blood samples.

Precautions to avoid cross contaminations during sample collection and storage

10 ml of venous blood was collected from each PD or control patient using intravenous canula to avoid iron contamination, and serum was separated by centrifugation. The serum was frozen at −20 °C and protected

Table 1. Clinical features of the PD patients and the control group

	Early PD ($n = 27$)	Severe PD ($n = 25$)	Control ($n = 25$)
Age (years)	57.15 ± 5.2	59.30 ± 3.9	55.4 ± 6.4
Sex	13 F/14 M	11 F/14 M	12 F/13 M
Duration of PD (years)	2.7 ± 0.9	7.0 ± 1.4	
Hoehn and Yahr stage	1st stage	2nd stage onwards	
Scores of Unified PD Rating Scale:			
Total (items 1–31)	23.1 ± 11.4	57.6 ± 15.6	
ADL[a] subscale (items 5–17)	9 ± 5.2	22.3 ± 7.3	
Motor subscale (items 18–31)	17.8 ± 5.8	29.7 ± 13.6	

Data of quantitative variables are expressed as mean ± standard deviation.
[a]ADL: activities of daily living.

from exposure to light until analysis. Blood collection and serum separation were carried out in dust free environments. All tubes used were polypropylene, no glass material was used to prevent Al and Si contaminations. All precautions to eliminate metal contamination during blood collection and storage were taken in accordance with National Committee for Clinical Laboratory Standards (NCCLS) criteria [23].

Instrumentation and element analysis

The element analysis was carried out using inductively coupled plasma atomic emission spectrometry (ICP-AES) (JY 70, Jobin Yvon, France), either by sequential or simultaneous mode depending on the elements to be analyzed. All dilutions were made with ultra pure Milli Q water (18 MΩ resistance) in dust free environment. For optimization of the ICP-AES method, lines were selected and detection limits evaluated for each element. The lines were chosen for each element in a way to obtain minimum interferences from other elements. The wavelengths used and the detection limits of the elements are summarized in Table 2. Quality control of the analyses was performed by analyzing a serum matrix matched multi-element synthetic standard and certified standard reference material (Bovine liver 1577a) obtained from the National Bureau of Standards, USA [24].

Data analysis

The element concentrations are expressed in μmol/ml as mean ± standard deviation and range of values. The mole percentage (element concentration in mol% = element concentration (μmol/ml) × 100/total element concentration (μmol/ml) of all analyzed elements in each sample) was calculated for the analyzed elements and the relative distribution based on the mole percentage was computed. Mole percentage calculations are essential to understand the relative distribution of each element in relation to other elements in a biological matrix. In addition, a normalization of the data of different samples is achieved in order to obtain clear inter-element relations. Element to element ratios and correlations were calculated based on the mole percentages to find possible element inter-relations (direct and inverse) in control and PD serum samples.

All statistical calculations such as inter-relations, correlation coefficients, and *t*-tests were carried out using Microsoft Excel 2000 and 'graph pad prism' software.

Table 2. Inductively coupled plasma atomic emission spectrometry: wavelenghts and detection limits

Element	Wavelength (nm)	Detection limit[a]	
		μg/ml	μmol/ml
Na	588.995	0.03	0.00130
K	766.49	0.06	0.00153
S	182.98	0.05	0.00156
P	213.618	0.05	0.00162
Ca	393.366	0.002	0.00005
Mg	279.806	0.001	0.00004
Cu	224.7	0.002	0.00003
Zn	213.856	0.002	0.00003
Fe	259.94	0.005	0.00009
Al	396.152	0.002	0.00007
Mn	257.61	0.001	0.00002
Si	251.611	0.08	0.00285

[a]The detection limits were calculated by running a multi-element standard solution containing 500 ng/ml of each element.

Results

Element concentrations

Element concentrations for control, early PD and severe PD serum samples are given in Table 3. The data are presented in μmol concentrations in order to calculate mole ratios of the elements and to determine inter-element correlations. The difference in percent in serum element concentrations between early or severe PD patients and controls, as well as between early and severe PD patients are presented in Table 4. The results clearly show that serum levels of K, Mg, Cu and P were statistically significantly higher ($p < 0.01$) in both early and severe PD compared to the controls. Serum S and Al concentrations were significantly lower ($p < 0.05$) in both early and severe PD, while Fe and Zn concentrations were decreased significantly ($p < 0.01$) only in severe PD compared to controls, which may reflect the severity of PD. Interestingly, in early PD serum samples, the concentrations of P, Cu, K and Ca were higher than in control and severe PD samples. However, there was only a marginal increase in the total concentration (μmol/ml) of the measured elements in early and severe PD serum compared to the controls. There was no correlation of these values with age at onset, sex or antiparkinsonian therapy. These findings are in agreement with previous studies [15,17].

The element concentrations determined in the present study in serum of control human subjects were compared with reference values from the 'Handbook on metals in clinical and analytical chemistry' [25] and from the study of Muniz et al. [26] (Table 3). The values matched for most of the elements, except for K, Al and Zn, where larger variations were observed. Normal

Table 3. Element concentrations (in μmol/ml ± standard deviation) in control, early and severe PD serum

Element	Reference values from Ref. [25] or [26]*	Control	Early PD	Severe PD
Na	139.2* (134.8–147.8)	135.4 ± 4.1 (126.5–141.3)	142.6 ± 11.4 (125.0–164.5)	142.9 ± 9.8 (127.2–168.5)
K	(4.09–4.48)*	3.54 ± 0.6 (2.4–4.9)	4.46 ± 0.4 (3.3–5.4)	3.79 ± 0.4 (2.8–4.7)
S	Not available	36.6 ± 3.7 (31.1–44.5)	32.0 ± 5.0 (25.3–45.6)	31.1 ± 3.3 (26.5–38.0)
P	3.56	3.2 ± 0.4 (2.3–4.0)	4.12 ± 0.9 (2.7–6.8)	3.65 ± 0.5 (2.8–4.5)
Ca	2.35 (2.2–2.6)	2.2 ± 0.2 (1.8–2.5)	2.41 ± 0.3 (1.8–3.0)	2.23 ± 0.2 (2.18–2.69)
Mg	(0.6–1.07)	0.9 ± 0.09 (0.78–1.1)	1.05 ± 0.1 (0.82–1.3)	1.05 ± 0.08 (0.86–1.19)
Cu	0.017	0.014 ± 0.003 (0.009–0.019)	0.022 ± 0.008 (0.007–0.035)	0.02 ± 0.006 (0.011–0.035)
Zn	0.013	0.009 ± 0.001 (0.006–0.01)	0.008 ± 0.002 (0.006–0.012)	0.007 ± 0.001 (0.005–0.009)
Fe	(0.012–0.030)	0.023 ± 0.009 (0.016–0.047)	0.02 ± 0.004 (0.01–0.028)	0.017 ± 0.007 (0.004–0.035)
Al[a]	< 0.2	0.59 ± 0.04 (0.4–0.68)	0.45 ± 0.05 (0.34–0.5)	0.37 ± 0.1 (0.29–0.5)
Mn	Not available	< DL (DL:0.00002)	0.001 ± 0.0002 (0.0005–0.034)	0.001 ± 0.0002 (0.0004–0.045)
Si	Not available	< DL (DL: 0.00285)	0.032 ± 0.01 (0.002–0.06)	0.009 ± 0.002 (0.004–0.014)
Total		181.8 ± 6.3 (170.3–195.5)	186.7 ± 15.9 (162.7–218.4)	184.8 ± 10.5 (168.1–205.9)

DL: detection limit.
Values in parentheses indicate the range. Reference values of elements (in μmol/ml) in control human serum from Ref. [25] or [26] (the latter marked with an asterisk) are given in the second column for comparison.
[a]In nmol/ml.

Table 4. Differences in percent of element concentrations between control and early or severe PD, and between early and severe PD. + and − signs indicate increasing or decreasing trends

Metals	Control/early PD	Control/severe PD	Early/severe PD
Na	+5.3[a]	+5.6[a]	+0.2
K	+26.0[a]	+7.2[b]	−15.0[a]
S	−12.6[a]	−15.1[a]	−2.8[b]
P	+30.0[b]	+15.4[a]	−11.4[b]
Ca	+8.7[b]	+0.6	−7.5[b]
Mg	+16.2[a]	+16.2[a]	0.0
Cu	+63.2[a]	+62.1[a]	−9.1[b]
Zn	−8.4[a]	−19.4[b]	−12.5[b]
Fe	−13.9[b]	−29.5[a]	−15.0[a]
Al	−23.7[a]	−37.3[a]	−17.8[a]

Statistical significance, a: $p < 0.01$; b: $p < 0.05$.

values for Al in serum samples are a contentious issue. Even though it has been agreed that Al levels should be lower than 0.005 μg/ml in control human serum, in many studies values of around 0.010–0.020 μg/ml have been reported. The Al level in control human serum in the present investigation was 0.016 μg/ml.

Relative mole percentages

To elucidate the inter-element relations within the data sets of the control, early PD and severe PD samples, the concentrations (in μmol/ml) were normalized by calculating the mole percentage for each element in a sample. The relative distribution is presented in Fig. 1. The mole percentage data show that levels of Al, Fe and S levels were higher in control serum compared to early and severe PD samples. Na and K were higher in both PD groups than in controls. Regarding the divalent elements, mole percentages of Mg and Cu were higher, while Zn values were lower in early and severe PD serum compared to the controls. No significant difference was observed for Ca.

Inter-element correlations and mole percent ratios

The inter-element correlations for the analyzed elements in control, early PD and severe PD samples showed a distinct pattern of direct and inverse correlations for selected elements. The correlation co-efficients and the statistical confidence levels at which the correlations were determined are given in Table 5. Na was inversely correlated ($r \geqslant -0.95$) with S in all groups.

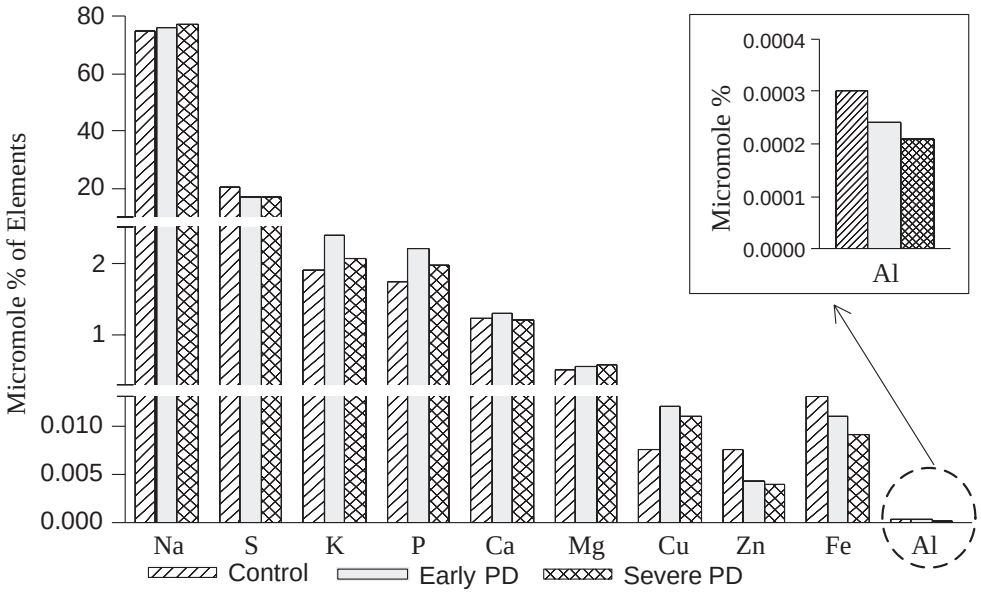

Fig. 1. Comparison of relative mole percentages of elements in control, early and severe PD human serum samples.

Table 5. Comparison of inter-element relations between control, early PD and severe PD serum samples

Correlation between elements		Correlation co-efficient		
1	2	Control ($n=25$)	Early PD ($n=27$)	Severe PD ($n=25$)
Na	S	−0.99*	−0.95*	−0.95*
Na	Ca	−0.46**	0.0	−0.59**
Na	K	0.47**	−0.32	−0.22
Na	Al	0.49**	−0.1	−0.05
K	S	−0.51**	0.11	0.36
K	Zn	−0.42**	0.42**	0.48**
K	Ca	−0.29	0.49**	0.36
S	Al	−0.49**	0.09	0.05
S	Fe	0.40**	0.03	0.12
Cu	Zn	−0.09	0.29	0.49**
P	Fe	−0.58**	−0.12	0.39

Confidence levels: *>99.9%, **>95%. At 99.9% and 95% confidence levels, the expected correlation co-efficients for a sample size of 25 are 0.613 and 0.396 respectively. −signs indicate an inverse correlation.

This correlation was also found in our previous studies on cerebrospinal fluid of Alzheimer's patients [27]. Na was directly correlated with K and Al in the control group, but showed a tendency towards inverse correlation in both early and severe PD groups. K was inversely correlated with S and Zn, and S with Al in the control group, but showed a tendency towards direct correlation in both PD groups. A direct correlation was found between S and Fe in the control group, and between Cu and Zn in the severe PD group.

The data were further analyzed in terms of element-to-element mole percentage ratios in control, early and severe PD serum. The ratios were calculated in order to understand the inter-relations of elements in biological systems [24,27]. The ratios Na/K, Na/Cu, Fe/Cu, S/Mg, and S/Cu were significantly decreased in early and severe PD serum compared to the controls, while the ratios K/Al, S/Al, Mg/Al, P/Al, K/Fe, K/Zn and Cu/Zn showed an increasing trend in PD serum compared to the controls. Only moderate differences were observed for other element ratios.

Discussion and conclusions

In the last decade, there has been an increasing interest for the possible role of metals in the pathogenesis of PD [15,28–30]. Substantial information is available on the trace metal distribution in brains of controls [27,31] and patients with neurodegenerative diseases like Parkinson's, Alzheimer's and Huntington disease [27,29,32,33]. Previous investigations [34] have shown an increase in Fe and Zn concentrations in the substantia nigra, lateral putamen and caudate nucleus in PD brain. However, limited data is available on some selected elements in serum of PD affected patients. Moreover, there has been a controversy regarding the Zn and Cu levels in PD serum. Abbot et al. and Pan et al. [35,36] reported decreased serum Zn concentrations in PD serum, while Jimenez et al. [16] found no significant difference in Zn concentrations in PD serum compared to controls. The present study shows decreased Zn levels in both early and severe PD serum, which is in agreement with Abbot et al. and Pan et al. [35,36]. Serum Cu concentrations were found to be normal in previous studies [16,37,38], or decreased in another study [36]. In contrast, in the present study, increased Cu concentrations were observed in both early and severe PD serum ($>$45–65%) compared to the controls.

Furthermore, Jimenez-Jimenez et al. [15] reported that the serum levels of Fe and Mn did not differ significantly between PD patients and controls. However, other studies [35,39] reported decreased serum Fe levels. In the present study, a significant decrease in Fe and a moderate increase in Mn concentrations were observed in PD serum compared to control serum. Interestingly, Fe showed a gradual decreasing trend with the severity of PD. The Fe concentration was lower by ~14% and ~30% in early and severe PD, respectively, compared to the controls.

There are no previous studies on serum levels of elements like Na, K, S, P, Ca, Mg, Si and Al. In the present study, mole percentage ratios and the correlation patterns of the elements indicated that there is an imbalance in the element-to-element inter-relations in serum of PD affected individuals (Table 5).

There has been a controversy regarding metal levels in PD serum and the possible role of metals as risk factors for PD. It is not clear whether the alterations in metal homeostasis is a cause or consequence in the pathology of the disease. So far, there is no detailed or comprehensive database on metal homeostasis and inter-relations. The available reports only indicate changes in the levels of a few elements, but fall short to correlate the element-to-element inter-relation pattern with the progression of the disease. In this perspective, the present study provides a comprehensive database on concentrations of 12 elements (the majority being essential elements) in PD serum in comparison with a control group.

There is limited information concerning a correlation of the element homeostasis in brain, cerebrospinal fluid (CSF), serum and other vital organs. Pall et al. [38] and Pan et al. [36] reported increased Cu levels in the CSF of patients with PD, the former group further suggesting that the concentration of this metal might be elevated in the brain as well. However, several studies [30,40] found decreased levels of Cu and increased levels of Fe and Zn in the substantia nigra, lateral putamen and caudate nucleus in PD brain. The authors related the increased Zn concentration to an attempt of protection against oxidative stress arising from the increased Fe level. An inverse relation between Fe and Cu (in liver) [41] and a direct relation between Fe and S as well as Zn has been found [28]. Thus the increased Zn levels and decreased Cu levels in PD brain may be causally related to the increase in Fe concentration. CSF Zn levels were found to be decreased in a study conducted by Jimenez-Jimenez et al. [15]. However, it is not clear, whether the source for increased Fe and Zn levels in the brain is serum or CSF. Furthermore, there is a need to understand the primary factor triggering the element imbalance in the body and its consequences. Trace metals play an important role in neuronal functions. The levels of trace metals in serum may be related to the levels in brain with reference to essential elements, but not regarding non-essential elements or metals causing toxic effects. The essential metals are able cross the blood brain barrier (BBB) by selective uptake mechanisms. However, non-essential or harmful metals can also cross the BBB by replacing essential metals in carrier proteins like transferrin.

How or why a specific increase in the total Fe content of substantia nigra should occur in PD is not understood. According to Lenders et al. [42], the Fe uptake across the BBB into the brain is significantly higher in PD patients than in matched controls (PET study). They suggested that this elevated Fe content in brain could be related to an increased transferrin receptor formation in PD. Fe is transported from blood to brain by the carrier protein transferrin. Fe and transferrin are transported through the BBB by means of a transferrin receptor mediated transcytosis [43–45]. In another study it has also been argued that the increased Fe levels correlated with the severity of neuropathological changes in PD are presumably due to an increased transport through the BBB [7]. Thus, two likely pathways for an increased Fe and Al uptake in dopaminergic neurons of substantia nigra may be the increase in transferrin receptor protein in PD brain and the non-specific pathological influx from other regions of the brain [30]. Al is known to be co-transported with the Fe-transferrin complex in neurological disorders [34]. In normal brain, Fe and Al compete for the transport across the BBB [27] while

in Alzheimer's disease, Fe and Al co-transport [34]. This differential mechanism is puzzling.

Fe exists in the brain in different complex forms, not all of them being capable of catalyzing oxidative stress. The majority of Fe is bound to ferritin and thus inactivated under normal physiological conditions. The biosynthesis of ferritin is controlled by the availability of Fe [46]. Glial Fe is mainly stored as ferric iron in ferritin, while neuronal Fe is predominantly bound to neuromelanin [7]. The potential toxicity of the increased Fe load in substantia nigra in PD is therefore determined by the extent of the binding of Fe to ferritin and other moieties. In PD, the increased total Fe level in substantia nigra was not associated with a compensatory increase in ferritin; on the contrary, the brain ferritin immunoreactivity was decreased [47]. Hence the increased Fe load in PD may exceed the storage capacity of available ferritin, leading to an excess of reactive Fe, driving free radical generation [30]. This hypothesis is supported by an increase in basal lipid peroxidation found in substantia nigra in PD [48]. Thus, an iron overload and an imbalance in other redox metal levels may induce the progressive degeneration of nigrostriatal neurons by facilitating the formation of reactive intermediates, including reactive oxygen species, and of cytotoxic protein aggregates [7,49].

We believe that—regardless whether metals are primary risk factors or imbalances are consequences of pathological mechanisms—a moderate change in a single metal ion concentration will upset the whole element homeostasis, resulting in significant imbalances in element levels in the whole system (serum, CSF and brain). The effect of an increase or decrease in a single element concentration is not restricted to this element alone, but the total element distribution pattern in the system will be affected. The results of the comparison of trace elements in serum of PD patients and control subjects in the present study showed that a disturbance in element homeostasis and inter-element relations occurs in serum during progression of PD.

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