



## TRACE ELEMENTAL ANALYSIS OF BLOOD SERUM SAMPLES OF PATIENTS AFFLICTED WITH PARKINSON'S DISEASE

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Received for publication: January 13, 2014; Accepted: February 21, 2014

**Abstract:** Trace elemental analysis was carried out in the blood samples of patients afflicted with Parkinson's disease and controls using ICP-MS technique. 23 elements over a wide Z range from Li to Pb were detected and their concentrations determined. These concentrations levels are analyzed to find a possible correlation between the levels of some of these trace elements in the diseased and the pathology of the disease.

**Keywords:** trace elemental analysis, ICP-MS, Parkinson's disease, blood serum

### INTRODUCTION

Nervous system disorders are a major cause of morbidity in all the societies in general which impair quality of life to a degree rarely affected by other diseases. Parkinson's disease is one of the main neurological disorders affecting the aged. It is an environmentally influenced, neurodegenerative disease that is characterized by tremors, stiffness of limbs and trunk, slowness of voluntary movements called Bradykinesia, postural instability and a distinctive shuffling gait with a stooped position called "Parkinson's gait". More than 1% of the population suffers from Parkinson's disease after reaching 55 years of age [1]

Parkinson's disease was first described in 1817 by the British physician James Parkinson. The etiology and pathology of Parkinson's disease is not clearly understood yet. Most of the movement-related symptoms of Parkinson's disease are caused by a lack of dopamine due to the loss of dopamine-producing cells in the substantia nigra pars compacta of the brain [2]. Dopamine acts as a messenger between two brain areas - the substantia nigra and the corpus striatum - to produce smooth, controlled movements. When the amount of dopamine is too low, communication between the substantia nigra and corpus striatum becomes ineffective, and movement becomes impaired; the greater the loss of dopamine, the worse the movement-related symptoms. Other cells in the brain also degenerate to some degree and may contribute to non-movement related symptoms of Parkinson's disease.

Although it is well known that lack of dopamine causes the motor symptoms of Parkinson's disease, it is

not clear why the dopamine-producing brain cells deteriorate. Genetic and pathological studies have revealed that various dysfunctional cellular processes, inflammation, and stress can all contribute to cell damage. In addition, abnormal clumps called Lewy bodies, which contain the protein alpha-synuclein, are found in many brain cells of individuals with Parkinson's disease [3, 4]. The function of these clumps in regards to Parkinson's disease is not understood. In general, scientists suspect that dopamine loss is due to a combination of genetic and environmental factors.

There have been numerous studies [5-12] linking Parkinson's disease to environmental pesticide and heavy metal exposure. A positive correlation between Parkinson's disease and industrialization has been well documented in the literature implicating pesticides, herbicides and heavy metals as contributory factors to the development of Parkinson's, the "environmental disease". These studies further support a holistic approach to this disease, which focuses on reduction and removal of the individual's overall exposure. In addition, this augments the conventional treatment, which focuses on symptoms and provides more holistic choices for the patient.

Based on epidemiological studies, occupational exposure to specific metals, manganese, copper, lead, iron, mercury, zinc and aluminum appear to be a risk factor for Parkinson's disease. An analysis of the Parkinson's disease mortality rates in Michigan (1986-1988) with relation to heavy metal exposure revealed that counties with an industry in the paper, chemical, iron or copper related-industrial categories had significantly higher Parkinson's death rates than

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counties without these industries [13]. Similarly an increased risk for Parkinson's disease from prolonged occupational exposure to heavy metals was established in Valleyfield, Quebec [14] (1987-1989). Post mortem analysis of brain tissues from patients with Parkinson's disease gives further confirmation to the involvement of heavy metals and this disorder. These previous studies have been done on occupational exposure and Parkinson's disease. However, in a recent study [15] on U.S. urban communities, long-term environmental exposure was found to contribute to the development of this disease. This 2003 study examined 35,000 Parkinson's disease patients who have not changed residence since 1995 and found an increased incidence in Parkinson's disease around urban areas with metal emitting facilities. With all this evidence supporting the environmental link and Parkinson's disease, the approach to treating this disease should also address these potential underlying causal factors.

Metals are ubiquitous and play a critical role in neurobiology. Transition metals are important because they alter the redox state of the physical environment. Biologically, transition metals catalyze redox reactions that are critical to cellular respiration, chemical detoxification, metabolism, and even neurotransmitter synthesis [16]. Many metals such as iron, zinc, copper, and manganese are both nutrients and neurotoxins (at higher levels). Other metals, such as lead and cadmium, are metabolized to these metals, particularly iron. Iron metabolism and genes that regulate iron metabolism may be the key to understanding metal toxicity. With all this evidence supporting the environmental link and Parkinson's disease, the approach to treating this disease should also address these potential underlying causal factors.

Oxidative stress phenomena have been related with the onset of neuro degenerative diseases. There is a growing amount of evidence pointing to a role of mitochondrial damage as a source of free radicals involved in oxidative stress. Among the elements that participate in the production of oxygen reactive radicals, transition metals are one of the most important. Several reports have implicated the involvement of redox- active metals with the onset of different neurodegenerative diseases such as AD, PD and ALS [17].

The diagnosis of PD is entirely clinical with no biochemical tests presently available to diagnose PD. Current diagnosis is made by standard neurological examination and medical history. The severity of the disease is categorized as stages based on the overall motor function evaluation using the Unified Parkinson's Disease Rating Scale (UPDRS) or Hoehn

and Yahr scale or Schwab and England Activities of Daily Living Scale. Three major cardinal symptoms of PD are tremor, rigidity and motor dysfunction, which significantly help in the detection of disease. However, the clinical diagnosis fails to identify the PD before significant loss of dopamine neurons occurs [18]. Hence, there is a need for early detection and more effective drugs to stop the progression of nigral degeneration.

There is scope for using some trace element levels as biomarkers in the diagnosis of PD as the imbalances in these levels can be an indication of the onset of this disease. But to arrive at a definitive correlation between the excess or deficiency of the trace elements and the disease, good amount of data should be generated using sophisticated instrumentation. There is a need to generate such data from different geographical regions of the globe. The present study is undertaken with this aim using a highly reliable and accurate analytical technique, ICP-MS.

## MATERIALS AND METHODS

Blood serum samples of 18 recently diagnosed PD patients were collected from areas surrounding Visakhapatnam and Vizianagaram. The patients had mean age  $60.29 \pm 10.40$  years (range 45 -84 years). Blood serum samples were also collected from age matching controls. Syringes free from contamination were used to draw blood from the patients and the blood samples were collected in vacuutainers. These samples were then centrifuged to separate the serum. The separated sera were lyophilized to a powder form. A quantity of around 50-100 mg of each sample was (transferred to Suprasil synthetic quartz vials and heat-sealed. The sample-containing end of the vial was immersed in liquid nitrogen during heat-sealing to prevent the possible loss of volatile elements.

A commercially available microwave digestion system (CEM MARS) equipped with 120mL Teflon PFA vessels and a turntable was used. Each vessel containing the lyophilized sample was taken in triplicate in 100ml digestion flasks and 1ml of sub boiled nitric acid added and vessel was closed by using a Capping station (CEM Corp). Routinely, 12 vessels were subjected to the digestion programme, ramp to temperature  $150^{\circ}$  C for 20 minutes and held for 5 minutes. After cooling, the digested samples were diluted to a final volume of 10mL with distilled water and analyzed with ICP-MS. For each series of 10 samples two analytical blanks were prepared in a similar manner to check any possible contamination. Ultra pure water (18.2 MV) obtained from a water-purification system (Millipore synergy) and ultra pure grade  $\text{HNO}_3$  was used.

Agilent 7700s inductively coupled plasma mass spectroscopy (ICP-MS) system (Agilent Technologies, Tokyo, Japan) was employed to measure the ion profile. Platinum sample cone and skimmer cone were utilized with an orifice diameter of 1.0 and 0.4 mm, respectively. Sample introduction was performed with a micro mist nebulizer combined with a Scott-type double pass spray chamber (Agilent Technologies).

The instrument was tuned to optimal conditions in terms of sensitivity (Li, Y, Co, and Tl) and CeO/Ce and Ce<sub>2+</sub>/Ce by using a tuning solution (Agilent Technologies) containing 1 mg/L of Li, Y, Tl, Ce and Co in 2% HNO<sub>3</sub> (w/v). The instrument was operated in full quantitative mode, and the typical operating conditions used in this study are summarized in Table 1.

Rhodium was used as internal Standard (ISTD). This was added to the each sample maintaining 2mg/L concentration approximately. The instrument was calibrated using multi-element standard solution which contains Aluminum (Al), Arsenic (As), Barium (Ba), Beryllium (Be), Bismuth (Bi), Calcium (Ca), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe), Potassium (K), Lithium (Li), Magnesium (Mg), Manganese (Mn), Molybdenum (Mo), Sodium (Na), Nickel (Ni), Lead (Pb), palladium (Pd), rhenium (Re), Antimony (Sb), Se, silicon (Si), Tin (Sn), strontium (Sr), vanadium (V), titanium (Ti), wolfram (W) and Zn, each at a concentration of 1000 mg/mL . Calibration curves for were plotted for 0, 0.25, 0.5, 1, 5, 10, 100 mg/L concentrations using the certified sample SPEX Certi Prep.

## RESULTS AND DISCUSSION

Twenty-three elements over a wide Z range from Lithium to Lead were identified and their concentrations determined. The concentrations of different trace elements in the samples of persons afflicted with PD and controls along with the corresponding standard deviations are furnished (Table-2). The p values calculated for each element are also furnished in the same table.

It can be seen that the concentration levels of Li, Al, V, Br, Mn, Fe, Co, Ni, As, Se, Ag, Cd, Hg and Pb are higher in samples of the PD afflicted patients compared to controls ( $p < 0.05$ )

The levels of Cu, Zn, Cs are also high but the difference is statistically insignificant as p-values are greater than 0.05. The concentration levels of Ga and Ba are lower in the samples of the afflicted persons compared to controls ( $p < 0.05$ )

The concentration level of Aluminum was reported to be lower in the blood, serum, urine and

CSF samples of PD afflicted patients compared to controls in a previous study using ICP-AES by G. Forte et al., [19]. But in a study conducted by G. Forte using ICPAES and SF ICPMS, and similarly in another study by Muralidhar et al., [20] conducted using ICP AES technique, Aluminum levels in the serum sample were reported to be higher. Thus there is a clear contradiction between the findings of these previous studies, which needs to be resolved. Aluminum (Al) levels in the serum samples of PD patients are significantly higher compared to the controls in the present study, lending support to the findings of Forte et al., and Muralidhar et al.,

**Table 1:** ICP-MS operating parameters

Parameter	Value
Plasma conditions	Forward power 1550W
Plasma gas flow	15.0L/min
Carrier gas flow	0.75 L/min
Dilution gas flow	1 L/min
He gas flow	4.5 mL min <sup>-1</sup>
QP bias	-15V
Oct bias	-18V
Cell entrance	-40 V
Cell exit	-60 V
Deflect	-0.8 V
Plate bias	-60 V
Nebulizer type	Miramist
Sample uptake rate	1.5 mL/min

Aluminum is a major player in Parkinson's disease. There is a tenfold increase in Parkinson's disease in areas of the world where they mine bauxite or aluminum and in city water systems that precipitate out the flocculants from the water supply with alum [21]. When people were first dialyzed in Denver, Colorado, there was excess aluminum in the water used, and this produced a condition similar in some respects to Parkinson's disease and early Alzheimer's disease [21]. This was called Denver Dementia. When the aluminum was removed from the dialysate, the Parkinson's disease and Alzheimer's disease type symptoms disappeared. Aluminum is a neuro toxin involved in A $\beta$  aggregation neuronal apoptosis and memory loss in animal modal [22,23]. Higher levels of Al were found to increase superoxide dismutase (SOD) activity to protect the cell from oxidative stress [24] this increased SOD was previously reported in PD [25]. Also higher levels of Al affect the functionality of mitochondria and endoplasmic reticulum, which activates caspase-12 [26] that leads to endoplasmic mediated cell death [27]. Thus there is a correlation between the higher levels of Al in the serum samples of PD patients and the pathology of PD.

Copper (Cu) plays a key role in cell metabolism as transition metal acting as a cofactor in many detoxifying enzymes and proteins (SOD, ceruloplasmine, metallothioneine etc.), but a modified brain homeostasis of this element together with oxidative stress and mitochondrial alterations could

lead to cell death and, thus, to the PD [28]. Copper binding proteins that form bio inorganic complexes are able to display anti oxidant properties which would impact on neuronal functions or in the triggering of neuro degenerative process (amyloid precursor protient (APP) and prion protein (PrP)[29].

**Table 2:** Concentrations of various elements in the blood serum samples (ppb)

Element	Parkinson		Controls		p-value
	Avg. ± Stdv.		Avg. ± Stdv.		
Li	87.54	± 9.72	68.08	± 9.40	0.009345
Be	2.91	± 1.23	1.73	± 1.17	0.009345
Al	29804.72	± 10247.04	5912.67	± 861.94	0.005062
V	117.44	± 23.91	39.94	± 10.60	0.005062
Cr	9569.16	± 2148.58	5330.85	± 995.30	0.005062
Mn	2326.90	± 777.33	490.34	± 142.27	0.005062
Fe	81977.63	± 19245.68	34591.22	± 5765.25	0.005062
Co	83.73	± 14.42	51.62	± 11.03	0.006911
Ni	3897.06	± 688.45	2340.34	± 432.41	0.006911
Cu	8568.70	± 1596.64	7671.74	± 1243.65	0.059337
Zn	5463.45	± 1438.51	4155.83	± 792.05	0.092602
Ga	209.68	± 59.75	230.19	± 76.61	0.386271
As	48.86	± 24.77	48.12	± 18.60	0.074463
Se	361.48	± 61.84	314.40	± 47.76	0.507625
Rb	4455.58	± 1262.81	3545.80	± 1026.38	0.332880
Sr	855.76	± 226.17	958.66	± 188.53	0.059337
Ag	597.37	± 527.44	52.19	± 14.32	0.005062
Cd	11.42	± 4.28	3.67	± 1.25	0.005062
Cs	8.10	± 3.27	6.01	± 1.61	0.046854
Ba	1532.98	± 291.96	1760.39	± 400.61	0.059337
Hg	425.38	± 108.09	102.54	± 67.22	0.005062
Tl	1.31	± 0.63	1.15	± 0.73	0.202623
Pb	536.83	± 186.87	150.17	± 51.04	0.006911

Higher serum Cu levels though not statistically significant (p value 0.059) was found in PD patients compared to controls in the present study. In a previous study by Muralidhar L. Hegde et al., [30] higher serum Cu levels in PD patients was reported. Similarly higher Cu levels were found in the whole blood samples of PD patients in a study by B. Bocca et al., [31]. Thus there is a correlation between the higher copper levels and in PD patients and the pathology of the disease.

There is evidence to suggest that Arsenic (As) can affect the peripheral as well as the central nervous system and it has been suggested that arsenic could play a significant role in causing neurological diseases [32]. In addition, animal studies have shown that Arsenic can cross the blood brain barrier, accumulate in different regions of the brain including the striatum [33], alter neurotransmitter synthesis and release, and decrease locomotor activities [34] thus causing neurological diseases. Rodrigue et al., [35] exposed male Sprague-Dawley rats to As and observed decreased locomotor activity and behavioral disorders.

The development of the central nervous system in neonatal rats is affected by As and As has been shown to cause neuronal death in adult rat brain by Chatopadhyaya et al., [36]. They hypothesized that the

oxido reductive system is involved in the As toxicity. In a study using primary cultures of rat cerebellar neurons Namgung and Xia [37] observed that As reduced neuronal viability and induced nuclear fragmentation and condensation as well as DNA degradation.

These results indicate that Arsenic affects neuro behavioral parameters of the developed as well as the developing brain. Increased level of lipid peroxidation and consequent damage to the cell membrane due to Arsenic and its compounds was reported by a number of investigators. Okada and Yamanaka (1994)[38] using lung cells showed that the methyl derivatives of Arsenic led to production of free radicals, damage of DNA and the consequent toxicity.

Serum Arsenic levels were found to be higher in PD patients compared to controls in the present study. It may be pointed out that in none of the previous studies on trace elemental analysis of PD patients mentioned in the discussion so far, arsenic levels are reported. The foregoing discussion shows that there is a correlation between higher levels of As and pathology of PD.

In the present study Manganese (Mn) concentration levels was found to be higher when compared to controls. Manganese is an essential

mineral that is found at low levels in food, water, and the air. Under certain high-dose exposure conditions, elevations in tissue manganese levels can occur. Excessive manganese accumulation can result in adverse neurological, reproductive, and respiratory effects in both laboratory animals and humans [39]. In humans, manganese-induced neurotoxicity (manganism) is the overriding concern, since affected individuals develop a motor dysfunction syndrome that is recognized as a form of Parkinsonism. Mn is toxic if orally consumed beyond dietary requirement or absorbed as particulate matter through inhalation. Excessive Mn exposure, observed primarily in occupational settings, has been associated with a Parkinsonian syndrome featuring muscular rigidity and dystopia, slowed movement, spasmodic movements and variable forms of tremors.

Elder et al., [40] has shown that Mn reaches brain tissue by crossing the blood-brain barrier after absorption from particulate matter deposited in the lungs. Trans et al., [41-45] demonstrated that increased dietary Mn supplements fed to lactating dams were associated with decreased striatal dopamine levels as well as significant increase in passive avoidance errors.

Excessive Mn intake from environmental and occupational settings is associated with several negative health outcomes including lethargy, tremor, psychological and neurological disorders resembling both schizophrenia and PD [46].

The most common form of toxicity is the result of chronic inhalation of large amounts of air borne Mn in miners, steel mills and some chemical industries. The major signs of Mn toxicity in animals are depressed growth, depressed appetite, impaired iron metabolism and altered brain function. Signs of toxicity in Chilean Mn miners were first manifested in the form of severe psychiatric abnormalities, including hyperirritability violent acts and hallucinations; these changes were called Mn madness [47]. As the disease progressed, there was a permanent crippling neurological disorder of the extra pyramidal system with morphological lesions similar to those of PD.

In the present study, the serum manganese levels are significantly higher in PD patients compared to those in controls. This is in tune with the finding of higher Mn levels in the whole blood samples of PD patients in a study by Fukushima et al., [48].

In the present study, serum Cadmium (Cd) levels were found to be higher in PD patients compared to controls. This is contrary to the findings of some previous studies. Bocca et al., [49] who conducted a study of serum and whole blood samples of PD

patients using SF ICP-MS reported a lower level of Cadmium. Similarly Forte et al., [50] also reported lower concentration levels in the serum and whole blood samples of PD patients.

But in a study on neurotoxicity of Cd in the hair samples of children, higher concentrations were reported in children with mental retardation and learning difficulties or dyslexia [51,52]. In another study[53] higher levels of Cd were correlated with children's performance on visual motor tasks.

In the present study, serum Zinc (Zn) levels were found to be higher in PD patients compared to controls. A similar finding was reported by Giovanni Forte et al., [54] who conducted a study on trace elemental distribution of hair samples of PD patients. Bocca et al., [55] also reported higher levels of Zn in the blood samples of PD patients. However it should be mentioned here that lower serum Zn levels were reported by Alimonti et al [56] in PD patients.

Excess Zinc, like excess Fe and Cu is a common finding in neurodegenerative diseases. Excess Zn is involved in the neuronal injury observed in cerebral ischemia, epilepsy and brain trauma [57]. The mechanism by which Zn exerts its neuro toxicity includes mitochondrial production of reactive oxygen species and the disruption of metabolic enzymes, ultimately leading to activation of apoptotic processes [58]

In the present study, serum Lead (Pb) levels were found to be higher in PD patients compared to controls. Higher concentrations of Lead are reported by Bocca et al., [56] also in their study of trace elements in the blood samples of PD patients.

All though controversies still exist regarding the levels at which Pb toxicity manifests itself clinically, there is wide spread acceptance that Pb is neuro toxic. Neurological disorders produced by the exposure to Pb are well reported and a relationship between long term exposure to this metal and development and acceleration of PD was also suggested [59].

Serum iron levels of PD patients were found to be higher when compared to controls in the present study. A similar trend was reported in serum, whole blood & urine samples of PD patients in an investigation by Bocca et al., using ICP-AES and ICP-MS. Higher serum iron levels in PD patients were also found in a study by Fukushima et al., Higher Fe concentration levels in serum, urine and whole blood samples of PD patients by Forte et al who used the ICP-AES and ICP-MS.

Iron is an essential trace element however it can generate highly toxic hydroxyl radicals by oxidation of iron (II) to iron (III) and decomposition of H<sub>2</sub>O<sub>2</sub> via the Fenton reaction. Thus high iron levels found in the samples of PD patients are an indication of enhanced oxidative stress. This hypothesis is substantiated further by iron-animal model [60-64]. In some previous studies accumulation of iron in the brain was linked to neuro degenerative disorders and mutations in genes responsible for encoding proteins of iron metabolism [65]

In the present study, serum Mercury levels were found to be higher in PD patients compared to controls. A similar trend of higher serum Mercury levels in PD patients was reported by Kristin Gellein et al [66]. It is extensively reported that methyl mercury supports neuro developmental toxicity [67].

### CONCLUSIONS

Trace elemental distribution in PD patients and age-matched controls were determined and compared. Higher levels of Mn, Cu, Pb, Fe and Hg observed in PD patients in the present study are in tune with the findings of previous studies. In addition to these, higher levels of As are also now linked to PD. Regarding Al and Zn, in which cases contradictory results were reported in some previous studies, the present study supports the findings of higher levels and helps to resolve the contradiction. Higher Cd levels are observed in the present work contrary to lower levels reported. On the basis of their role in human physiological processes established in previous studies, a modest attempt is made to correlate the higher levels of some of the trace elements detected, to the pathological features of PD. However, any definitive conclusions regarding their role can be made only after some more studies, and they can then be regarded as biomarkers in the diagnosis of PD.

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**Source of support:** Nil

**Conflict of interest:** None Declared