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Comparison of Serum Copper, Zinc, Lead, Aluminium and Iron Levels in Patients with Parkinson's Disease with Healthy Controls in Tertiary Care Hospital

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ABSTRACT

Objective: To compare serum Copper, Zinc, Lead, Aluminium and Iron levels in patients with Parkinson's disease with healthy subjects and the association of these trace elements.

Study Design: Comparative Cross-sectional study.

Place and Duration of Study: Department of Chemical Pathology and Endocrinology at Armed Forces Institute of Pathology (AFIP), Rawalpindi Pakistan, from Nov 2019 to Jul 2020.

Methodology: Out of the total of 129 study participants, 83 were Parkinson's disease patients, and 46 were healthy subjects. Serum Iron level was measured on random access fully automated chemistry analyser advia 1800. In addition, Copper, Zinc, Lead and Aluminium were measured by atomic absorption spectrophotometry. These levels were compared between patients with Parkinson's disease and healthy subjects using the Mann-Whitney U test.

Results: Out of the total 129 subjects, males accounted for 51(61.4%) and females were 32(38.6%) in the diseased Group, while in healthy subjects, males accounted for 22(47.8%) and females for 24(52.2%). Levels of Copper, Zinc and Iron were significantly decreased in patients with Parkinson's disease compared to healthy subjects (p<0.001). However, serum concentrations of Aluminium and Lead showed an increasing trend in patients with Parkinson's disease compared to age-matched healthy subjects (p<0.001).

Conclusion: Decreased levels of serum copper, iron and Zinc were seen in patients with Parkinson's disease, while aluminium and lead levels were raised, showing the potential neurotoxic role of these elements.

Keywords: Neurodegenerative diseases, Parkinson's disease, Trace elements.

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INTRODUCTION

After Alzheimer's disease, Parkinson's disease (PD) is the second most commonly found neurodegenerative disorder. It is characterized by slow movements and other cognitive functions, posture instability, tremors, bradykinesia and rigidity.[1] Main reason behind these symptoms is the progressive loss of neurons producing dopamine present in the substantia nigra of the brain. The overall prevalence of PD is estimated to be 6.5 million and in people over 85 years of age, its prevalence increases to another 4-5%.[2] According to an estimation, presently, one million people have PD in Pakistan, and this number will increase to 1,200,000 by 2030.[3]

The cause and the precise mechanism of neurodegeneration in PD are unknown. However, the suggested mechanisms can involve a cascade of events that include interaction between genetic factors, abnormalities in protein processing, oxidative stress, mitochondrial dysfunction and environmental factors.[4] These include exposure to water from wells, pesticides, head injury, herbicides and chemicals from industries etc. One of the emerging oxidative stress hypotheses postulates that inappropriate production of free radicals leads to neurodegeneration. The familial form of Parkinsonism, though not very common, accounts for about 5-10% of the cases.[5] Pattern of inheritance is autosomal dominant.

Early stages of PD can pose a challenge for clinicians to diagnose because of closely related clinical features of other diseases such as multiple system atrophy, dementia with Lewy bodies, corticobasal syndrome, progressive supranuclear palsy etc. Diagnosis of PD is made basically on clinical neurological examination and medical history. An accurate and early diagnosis of PD has both therapeutic and prognostic significance. Various scales have been established to categorise the severity of disease, such as the Hoehn and Yahr scale, unified PD rating scale etc.[6] Recent advancements in diagnosis have led to the establishment of the role of evaluation of metallic biomarkers in easily accessible fluids such as saliva, urine, serum or CSF in diagnosing PD.[7]

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Several studies have evaluated levels of essential elements such as Aluminium (Al), Zinc (Zn), Calcium (Ca+2), Iron (Fe), and Lead (Pb) in PD. Some have associated imbalances in these elements with the progression of PD.[8] While others show a negative correlation or no difference at all.[9] Scarcity of literature in the Pakistani population prompted us to conduct this study in our population. This study aims to compare the serum Copper, Zinc, Lead, Aluminium and Iron levels in patients with PD with healthy subjects and the association of these trace elements in PD patients.

METHODOLOGY

It was a comparative cross-sectional study carried out at the Department of Chemical Pathology, Endocrinology, and Toxicology Department at AFIP in collaboration with the Neurology Department at Pak Emirates Military Hospital (PEMH), Rawalpindi Pakistan, from November 2019 to July 2020. The study was started after approval from the Institutional Ethical Review Board of AFIP (FC-CHP18-3/READ-IRB/20/466 dated 5th June 2020). The sample size of 129 (patients and suitable controls) was calculated using Open Epi sample size calculator version 3.0, with the prevalence of PD as 0.5%.[10] Alpha was set at 0.05, the power of the test 80% and a confidence level of 95%. The sampling technique used was non-probability convenient sampling.

Inclusion Criteria: All the patients diagnosed with PD (presence of resting tremor, muscle rigidity and bradykinesia) were included in the study. The healthy group consisted of age-matched healthy individuals showing no signs and symptoms or history of systemic disease or comorbid conditions.

Exclusion Criteria: Patients with a history of severe systemic disease, chronic liver disease, pancreatic disease, malabsorption, chronic kidney disease and those taking antioxidant drugs, e.g. Tocopherol, supplements (Iron, Copper, Zinc) or chelating agents (D-penicillamine) were excluded from both groups.

Informed written consent was taken from the patients or their guardians whose samples were collected and analysed. About 3.0ml of venous blood was taken in an EDTA tube to estimate Lead and in a gel tube for serum Copper, Zinc, Aluminium and iron levels. Serum Iron levels were measured on random access fully automated chemistry analyser Advia 1800 (Siemens, Germany). Copper, Zinc, Lead and Aluminium were measured by atomic absorption spectrophotometry (Agilent Technologies, USA).

Statistical Package for Social Sciences (SPSS) version 21.0 was used for the data analysis. The normality of data was checked by using the Shapiro-Wilk test, which showed a non-parametric distribution of data. The median (IQR) was calculated for numerical variables. Percentage and frequency were calculated for categorical variables. Where appropriate, comparisons between groups were calculated using the Mann-Whitney U and chi-square tests. Correlations between various trace elements with PD were analysed using spearman's correlation, considering a *p*-value of ≤0.05 to be significant.

RESULTS

Out of 129 study participants, 83(64.3%) were diagnosed cases with PD, and 46(35.7%) were healthy subjects. Males accounted for 51(61.4%), and females were 32(38.6%) in PD patients and healthy subjects; males accounted for 22(47.8%) and females for 24(52.2%). The median (IQR) age of the patients was 64(58.0–66.0) years. Among trace elements median (IQR) of serum Copper was 7.9(6.1-15.4)umol/L, Zinc 5.9(4.6-14.1)umol/L, Aluminium 5.8(4.1-6.1)ug/dL, Fe 6.4(4.6-12.6)umol/L and Laed 5.7(3.9-6.2)ug/dL.

Mann Whitney U test was applied to seek the difference between the median serum levels of various trace elements in patients with PD and healthy subjects, which showed a significant difference, as illustrated in Table-I.

Table-I: Serum Levels of Trace Elements in Patients with Parkinson's Disease and Healthy Subjects (n=129)

	Median (IQR: 25th –75th Percentiles)		*p*-value
	Healthy Individuals (n=46)	Parkinson's Disease (n=83)	
Copper (Cu) umol/L	16.4(15.1-17.7)	6.4(4.9 – 7.9)	<0.001
Zinc (Zn) umol/L	15.0(13.7- 16.4)	4.9(3.6- 5.9)	<0.001
Aluminium (Al) ug/dL	3.6(3.5- 4.2)	6.0(5.8- 6.1)	<0.001
Iron (Fe) umol/L	18.1(12.3 -19.8)	5.3(4.2-6.4)	<0.001
Lead (Pb) ug/dL	3.7(3.2- 4.0)	6.0(5.7-6.3)	<0.001

Copper, Zinc and Iron levels were significantly decreased in patients with PD compared to healthy subjects ($p< 0.001$). However, Aluminium and Laed serum concentrations showed an increasing trend in patients with PD compared to age-matched healthy subjects ($p< 0.001$).

The association was assessed after dividing Copper, Zinc, Aluminium, Iron and Lead levels into two groups according to their median, which showed a

strong association between diseased and healthy individuals (p<0.001) (Table-II).

Table-II: Association of Serum Copper, Zinc, Aluminum, Iron and Lead Levels in Parkinson's Disease and Healthy Subjects (n=129)

Groups	Parkinson's Disease (n=83)	Healthy Individuals (n=46)	*p*-value
Copper (Cu) umol/L			
Less than median (<7.9)	62(74.7%)	1(2.2%)	<0.001
Equal or more than median (>7.9)	21(25.3%)	45(97.8%)	
Zinc (Zn) umol/L			
Less than median (<5.9)	46(55.4%)	2(4.3%)	<0.001
Equal or more than median (>5.9)	37(44.6%)	44(95.7%)	
Aluminium (Al) ug/dL			
Less than median (<5.8)	34(41.0%)	45(97.8%)	<0.001
Equal or more than median (>5.8)	49(59.0%)	1(2.2%)	
Iron (Fe) umol/L			
Less than median (<6.4)	68(82.0%)	2(4.3%)	<0.001
Equal or more than median (>6.4)	15(18.0%)	44(95.7%)	
Lead (Pb) ug/dL			
Less than median (<5.7)	13(15.7%)	45(97.8%)	<0.001
Equal or more than median (>5.7)	70(84.3%)	1(2.2%)	

Spearman's correlation was used to determine the relationship between the serum concentrations of these elements in PD patients, as illustrated in Table-III. A strong negative correlation was seen in serum concentrations of Copper (r=-0.830, p<0.001), Zinc (r=-0.83, p<0.001) and Iron levels with PD (r=-0.830, p< 0.001). Whereas a strong positive correlation was observed for Aluminium and Lead levels with PD patients (r=0.832, p<0.001) and (r=0.830, p<0.001) respectively.

Table-III: Correlation between Serum Concentrations of Various Trace Elements in Patients with Parkinson's Disease (n=129)

	r-value (Parkinson's Disease)	*p*-value
Copper (Cu) umol/L	- 0.830	<0.001
Zinc (Zn) umol/L	- 0.831	<0.001
Aluminium (Al) ug/dL	0.832	<0.001
Iron (Fe) umol/L	- 0.830	<0.001
Lead (Pb) ug/dL	0.830	<0.001

DISCUSSION

Derangements in the concentration of trace elements reflect the alteration of underlying molecular mechanisms. One of the emerging trends in diagnostic modalities is metabolomics, in which analysis of the concentration of various elements in fluids such as urine, serum, CSF and plasma is carried out.[11] Analysis of various trace elements and their alterations in PD have been investigated in various studies. Diversity in the results could be due to population variation, the limited size of the study sample and the utilization of various fluids/mediums.

Copper plays an important role in the pathogenesis of PD. It plays a dual role in increased oxidative stress production and its reduction via acting as a cofactor for Copper-Zinc superoxide dismutase, an important antioxidant enzyme. In our study, the concentration of Copper was significantly less in PD 6.4 (4.9–7.9)umol/L than in the Healthy Group 16.4(15.1-17.7)umol/L. Similar results were published by Ilyechova *et al*.[12] in a study that showed that the development of PD was correlated with low serum Copper levels, and this mainly affected the non-motor symptoms of PD. However, a meta-analysis conducted by Mariana *et al*.[13] revealed no significant differences in serum, plasma and CSF Copper levels in patients with PD and healthy controls.

Our study revealed that Fe levels were decreased in the serum of PD patients 53(4.2-6.4)umol/L as compared to healthy controls 18.1(12.3-19.8)umol/L, who depicted increased serum Fe levels. Previous studies have also depicted similar results with altered Fe haemostasis in patients who developed the disease, i.e. showing decreased iron levels.[14] Iron accumulation has been observed in different brain parts, especially in the Substantia Nigra. Thus, a low level of iron in the circulating blood is considered a risk factor for the development of PD. In addition, iron is a cofactor of the tyrosine hydroxylase, which is involved in synthesising neurotransmitters. Therefore, its decreased levels lead to functional impairment of neurons.

Zinc is one of the essential elements required for the development and normal functionality of CNS. It is mainly concentrated in the hippocampus and cerebral cortex area. Thus, abnormalities in Zinc haemostasis lead to behavioural changes, loss of memory and learning disabilities. Our study revealed that patients with PD have decreased Zinc concentration compared to healthy individuals (p<0.001). Similar results were documented in a meta-analysis by Du *et al*.[15] However, Ajsuvakova *et al*.[16] showed no difference in group serum levels of Copper, Zinc and Iron in patients with PD compared to the control group. Contrary to the findings mentioned above, studies conducted by Hedge *et al*.[17] and Brewer *et al*.[18] have reported a

subclinical decrease in the concentration of Zinc in serum and CSF.

Aluminium and Lead levels were raised in patients with PD compared to healthy controls in our study sample. Al accumulation usually occurs in the neurofibrillary networks in patients with PD. This shows the relationship between exposure to cytotoxic elements and its association with the development of PD. Similarly, Sanyal *et al*.[19] conducted a study showing increased levels of Aluminium, Lead and decreased levels of Copper and Iron in the serum of patients with PD compared to the control group. However, an increase in Al concentration in all the fluids was noted in a study by Forte *et al*.[20] Jiménez-Jiménez *et al*.[21] observed a substantial decrease in the rate of Zn in the CSF relative to the matched controls, implying that this decrease might be linked to the risk of PD development.

Imbalances in the levels of trace elements like Copper, Iron, Zinc, Aluminium and Lead and their effects on the disturbances in neurological function are evident in our study. The possible role of these metallic biomarkers in diagnosing PD cannot be ruled out. The diagnostic emphasis on the concentration of trace elements in patients with PD cannot be overlooked in light of all the recent research. A decrease in the levels of Copper, Zinc and Iron in serum can be considered a potential tool for diagnosing neurodegenerative disorders, but further research is needed with a larger sample size in order to differentiate the levels of these trace elements in PD and other neurodegenerative diseases such as Alzheimer's, dementia etc.

CONCLUSION

Decreased serum Copper, Iron and Zinc levels are seen in patients with PD. Whereas Aluminium and Lead concentration are raised, showing the potential neurotoxic role of these elements. Evaluation of metallic biomarkers in serum, CSF, urine or plasma could be a step forward in diagnosing and differentiating various neurodegenerative diseases, especially PD.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

TA: Drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

MA & ZHH: Study design, data analysis, critical review, drafting the manuscript, critical review, approval of the final version to be published.

MUM & SIK: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

AA: Data acquisition, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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