Evaluate the correlation of electrolytes with biochemical parameters in biological samples of Parkinson's disease patients at different stages

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Research

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Abstract

Background: Neurodegeneration is complex and multifaceted leading to many chronic diseases like Parkinson's disorder. Electrolytes imbalance have significant role in the pathophysiology of neurological disorders, which might be serve as a bio-indicator of the neurological problems. In present study, the disturbances of electrolytes (calcium, magnesium, sodium and potassium) and its correlation with biochemical parameters in patients with Parkinson's at different stages with related to referent were studied.

Methods: The biological samples were collected from patients recently diagnosed for mild Parkinson disease (MPD) (n=95) as well as chronic PD (CPD) patients, diagnosed for last 5 to 10 years (n=125).The Precision of digestion method was verified by applying matrixes matched certified reference materials of biological samples. The recovery of electrolytes was obtained in the range of 98.4–99.1 % of certified reference materials. The electrolytes concentrations were measured by acid digested biological samples preliminary analysis through flame atomic absorption spectrometry.

Results: The experimental data indicated the content of Calcium and Magnesium were found to be higher in scalp hair samples of both patients while Sodium and Potassium showed inverse relation ($p<0.05$ for both groups). Electrolytes levels in blood serum and plasma was found to be lower in both types of patient groups than healthy groups ($p<0.01$).

Conclusions: These results suggest a disturbance in the elemental homeostasis during the diseases of PD at different stages. In spite of, additional work is proposed to study the actual correlation among micro elemental level and the grade of diseases in neurological sick person.

Background

The function of electrolytes in neuroscience has been designated that they have role in neurological disorders/diseases. Neurodegenerative diseases affect the central nervous system and peripheral nervous system. Neurodegenerative diseases not only impair biological processes but also have impacts on a patient cognition, and memory [1]. The neuroscience of d-block elements has extended progressively in the earlier periods through findings of their consequence to main neurodegenerative diseases/disorders such as Parkinson's and Alzheimer's [2, 3]. In Parkinson's disease injury of dopaminergic neurons occurs in Substantia Nigra part of midbrain, with a loss of striatal dopamine. This phenomenon creates reducing the organization of the nerve cells, resulted in mental and physical destruction of the sequences and cognitive de-regulation [4]. Electrolyte disruptions are significantly always secondary processes. Effectual administration have need of identification and cure of the underlying most important disorder [5, 6]. Majority of electrolyte disturbances in neurologic patients, except dysnatremia, display equivalent regularity with sick person from further disorder and primarily affected by hospital co-morbidities [7]. Abnormality of electrolyte may influence several organs, tissues and the brain [8–10]. It is known that significant changes in serum calcium levels, especially

hypocalcemic states, can lead to neurological disorders, among them, Parkinsonism [11, 12].Parkinsonism refers to a group of diseases characterized by tremors, gait disorders, stiffness, slowness, muscle pain, paresthesia, generalized fatigue, depression, nervousness and others. Calcium is vital to normal cell physiology and the function of excitable cells [10, 13]. It is known that calcium is necessary for neuromuscular stimulation and also in neurotransmitters [14]. Therefore, altered calcium levels affect the transmission of nerve impulse, which could lead to partial or total damage of dopaminergic receptors [11].Moreover, the Calcium (Ca) concentration in blood serum decreases in PD patients. The suggested reasons for decrease in levels of serum calcium of PD patients are inactivity, reducing sun exposure and Ca and vitamin D consumption [15]. The magnesium (Mg) is a crucial electrolyte for normal physiological activity of human health [16, 17].It was investigated that Parkinson's patients have low Mg in different parts of nervous system such as in white matter, brain stem cortex and, basal ganglia [16, 18]. In Parkinson's the depleted Mg as well as Ca termed as hypo magnesemia and calcemia which is illustrated by tremendously low levels of Mg in blood serum as 0.1–0.3 mM, along with depleted levels of Ca in serum, which creates rigorous complications in muscular and neurological problems involving mental and seizures disorders. [16, 19–21].

The brain accomplishes in an extremely convoluted environment which requires accurate balance of electrolytes. Perturbations of sodium are the electrolyte imbalances that mostly lead to neurologic diseases. Changes in extracellular liquid sodium (Na) levels produce water alters that causes brain to damage, contraction or enlargement [4]. The diagnosis of PD stays fundamentally a clinical one, and it is necessary to recognize the early stages together with symptoms and signs suggesting other causes of Parkinsonism. The exact diagnosis of seizures insignificant to electrolyte abnormalities initiate with complete serum chemistry estimation, including determinations of electrolytes particularly sodium. This diagnostic examination of patients should remain be part of the first stage of workup in younger age group patients with initial seizures.

Imbalances in concentration of potassium (K) hardly create problems in the central nervous system but it is possible that it is associated with weaknesses of muscles and tremors as the main clinical problems. Disorders of electrolytes mostly caused seizures and seizures are mostly found in Parkinson's patients suffers from sodium disorders. The goal of present research work was to assess the electrolytes (Ca, Mg, Na, K) levels in the biological samples (scalp hair, blood serum and blood plasma) of mild and chronic male Parkinson disease (PD) patients, age ranged (55–75). For comparative study, control subjects n = 69 of the same age group, nutritional habits, socioeconomic status and localities were selected as control subjects. In addition, we also correlated this electrolytes concentration with different biochemical parameters between PD and controls.

Materials And Methods

Instrumentation

Domestic microwave oven Pel (Osaka, Japan), with maximum heating power of 900 W, was used to oxidize organic matrixes and all the understudy electrolytes analysed through flame atomic absorption spectrometry (FAAS) which was connected with air–acetylene flame. The instrumental conditions are showed in Table 1.Centrifugation was proceed to separate the serum and plasma from blood samples, through a WIROWKA Laboratory in a type WE-1, nr-6933 centrifuge speed range 0–6000 rpm, time 0–60 min, 220/50 HZ (MechanikaPhecyzyjna, Poland).For preparation and storage of standard/ sample solutions Acid-washed polytetrafluoroethylene (PTFE) flasks and vessels were used22.

Table 1
Measurement conditions for flame Atomic Absorption Spectrometer 700

Parameters	Calcium	Magnesium	Sodium	Potassium
Wave length (nm)	422.7	285.2	589	766.5
Slit width (nm)	0.7	0.7	0.2	0.7
Lamp current (mA)	7.5	7.5	10.0	10.0
Burner height (mm)	12.5	7.5	7.5	12.5
Oxidant (air; l/min)	17.0	17.0	17.0	17.0
Fuel (acetylene; l/min)	2.0	2.0	2.0	2.0

Reagents and Standard Solutions

Ultra-pure water took from an ELGA lab water system (Bucks, UK) was used all over research study. H_2O_2 (30%) and concentrated acid HNO_3 (65%) were obtained from Merck (Darmstadt, Germany) and trace metal contamination was inspected by it. Standard solutions of all electrolytes (Ca, Mg, Na, K) were made by dilution 1,000 ppm certified standard solutions, Fluka Kamica (Bush, Switzerland).Step wise dilution of stock standard solution with 0.2 mol/L nitric acid was performed instantly for making dilute standard solutions earlier to their usage. Polyethylene bottles were used to stored prepared samples and all sample bottles were kept in refrigerator at 4 °C. Certified reference materials (CRMs) of human hair BCR 397 (Brussels, Belgium), human serum ERM-DA252a (Teddington, Middlesex, UK) human blood plasma (Teddington, Middlesex, UK) were used for checking the validation of our methodology. All the glassware used in laboratory work were previously soaked in 5 mol/l nitric acid for 24 h and then rinsed through Milli-Q water, dried and then stored in a class 100 laminar flow hoods.

Study population

The studied population consisted of the mild PD patient having age group (50–65 years) n = 95registered as recently diagnosed (Mild PD) as well as patient with chronic PD having age group (66–75 years) n = 125 which were already registered for < 5 to 10 years, in neurological ward of Liaquat University of

medical and health sciences and National hospital. Most of the patients included recently diagnosed and previously confirmed diagnosed PD patients are attending the OPD regularly and checked by related neurologist. Further 69 randomly selected, age and gender matched subjects particularly the relatives of the patients, who had not suffered from any signs and symptoms of PD or other neurological disorders (Table 2). Before collection of biological samples control group have visited for regular medical inspection. Physical examinations were carried out in the neurological ward of Sir Cowasji Jehangir Institute of Psychiatry and civil hospital Hyderabad, to analyze participant's biochemical parameters which were recorded Table 3.The study procedure was permitted by the local ethics committee of HEC of Pakistan. A questionnaire was also employed to them in order to gather particulars regarding physical data and consent. Consultations were accomplished to examine the disease history, related co morbidities, nutritional intake and compliance with recommended pharmacological treatments.

Table 2
Number of Subjects as referents and Parkinson's disease Subjects

Referent	PD patient (mild PD)	Referent	PD patient (chronic PD)
50–65 years		66–75 years	
n = 43	n = 95	n = 26	n = 125
Referents, n=69; PD patients initially diagnosed (mildPD), n=95; chronic PD (diagnosed 5 to 10 years), n=125			

Table 3
Clinical and biochemical characteristics of referents and MPD and CPD patients

Parameters	Normal range	55–65		66–75	
		Referents	MPD	Referents	CPD
BMI (kg/m^2)	------	24.9 ± 0.42	23.3 ± 0.65	23.5 ± 0.50	21.8 ± 0.35
SBP (mmHg)	------	127.9 ± 4.60	145.3 ± 5.82	129.8 ± 7.50	163.5 ± 5.95
DBP (mmHg)	------	83.6 ± 6.15	95.5 ± 7.35	85.2 ± 6.15	109 ± 8.05
Heart rate /min	72–80	76.9 ± 7.55	87.9 ± 6.60	78.5 ± 5.05	92.7 ± 5.38
Hb(g/dl)	13.5–17.5	14.0 ± 0.12	13.0 ± 0.15	12.3 ± 1.34	10.6 ± 0.45
RBC (× 10^{12}/l)	4.28–5.81	4.92 ± 0.16	4.15 ± 0.33	4.10 ± 0.32	3.05 ± 0.39
Vitamin D (ng/ml)	25–30	24.8 ± 0.63	8.65 ± 1.62	22.5 ± 0.40	6.38 ± 1.62

Hb, hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure; RBC, red blood cells; BMI, body mass index

Biological sampling

Heparinized lithium vacutainer® tubes (Becton Dickinson) 7 mm were used for the collection of venous blood samples (5 mL). For elemental analysis, intravenous blood specimen approximately 2 mL was kept at − 20 °C. Participant's blood samples (2–3 mL) were sent to pathological laboratories of different hospitals for biochemical reports using standard methods. Whereas remaining 2 mL was used for separating the sera and plasma. The blood was left to coagulate for 15–30 minutes at normal room temperature. As the blood had coagulated absolutely, it was centrifuged for about 5–10 minutes at 3200 rpm. The supernatant fluid was then separated using a Pasteur pipette, labeled serum accordingly, whilst, the buffy coat layer, consisting of platelets and white blood cells called plasma, stocked at -20 °C till analysis.

Sampling of Scalp hair

Approximately (0.5 gm) scalp hair samples were taken form 5 cm root of scalp. For each participant, hair specimen were put into individually in sealed plastic bags and labeled with different identification numbers and questionnaire of the respondent. The sample pretreatment and washing procedure of hair sample are same as reported our previous study.

Microwave assisted acid digestion (MAD)

Duplicate six specimen of both certified reference materials, BCR 397 human hair (0.2 g), human serum ERM-DA252a and second copy of samples of (0.2 g) scalp hair, (0.5 mL) plasma and serum were taken independently in 25 mL (PTFE) polytetrafluoroethylene flasks. After that 3 mL of concentrated 2:1 v/v (HNO_3 - H_2O_2) which was freshly prepared were added it was then reserved at room temperature for 10 minutes. After that put the flasks in wrapped PTFE container and heated at 80 ºC of total power (900W) for three or four minutes. Upto 10 mL Concentrated nitric acid solution (0.2 mol /L) was used for dilution of digested biological samples.

Biochemical test

Demographic data such as sex, age, body mass index (BMI), systolic/ diastolic blood Pressure, Vitamin D (ng/ml), hemoglobin (Hb), red blood cells (RBC) and Heart rate /min were measured in OPD and concerned pathological laboratories of hospitals for all patients. The body mass index (BMI) of each patient and healthy participant was calculated by dividing the height value by its square (kg/m^2).Table 3

Statistical analysis

For statistical analysis different software was used for data processing of our study such as Software packages, XLState (Addinsoft, NY, USA), Excel 2003 (Microsoft Office ®), and Minitab 13.2 (Minitab Inc., State College, PA). The analysis of variance was used to assess the outcomes of alterations between the

contents of (Ca, Mg, Na and k) in the biological specimen of PD sufferers and control groups, determined by the unpaired two-sample t-test. A ($p > 0.05$) was measured substantial alteration. Student's t-test was carried out for the assessment of the substantial variation of all electrolytes in obtained and certified reference values.

Analytical figures of merit

Calibration curve reached from the detection limit up to 10 µg /for the concentration range of Ca, Mg, Na and K. The limit of quantification (LOQ) and detection (LOD) were found as and respectively, where is the standard deviation of 10 readings of blank ($n = 10$) and m is the slope of the linear section of the calibration graphs. The microwave acid digestion requires only 2–3 min to digest the samples. Accuracy and validation of methodology was conformed through certified samples of blood serum, plasma and scalp hair (Table 4). From the certified values, the difference for the mean values of Ca, Mg, Na and K were obtained to be less than 1–2%. <2% of the coefficient of variation was observed and by comparing both procedures, non-significant differences ($p > 0.05$) was achieved.

Table 4
Determination of Ca, Mg, Na and K in certified samples by MWD (n = 10)

Elements	Certified values	Microwave digestion method MWD	T value[a]	% recovery[b]
Certified sample of human blood serum (mg/L)				
Calcium	58 ± 1.9	56.3 ± 0.65(1.15)	0.00376	97.7
Magnesium	8.1 ± 0.36	8.01 ± 0.104(1.30)	0.0003	99.4
Sodium	2400 ± 120	2310 ± 165(7.14)	0.321	98.7
Potassium	67 ± 1.90	66.2 ± 1.09(1.65)	0.628	99.4
Certified sample of human blood plasma (mg/L)				
Calcium	-	-	-	-
Magnesium	30.0 ± 7.0	29.6 ± 0.94(7.10)	0.889	98.7
Sodium	-	-	-	-
Potassium	-	-	-	-
Certified sample of human hair (µg/g)				
Calcium	1560.0 ± 40[c]	1546.0 ± 45.6(2.95)	0.889	99.7
Magnesium	200 ± 5[c]	198.2 ± 12.3(6.2)	0.838	99.6
Sodium	1.08 ± 0.007	1.06 ± 0.06(5.66)	0.855	99.1
Potassium	8.6 ± 0.03	8.53 ± 0.7(8.21)	0.941	99.8

Values in () are RSD

[a] Paired t test between Certified values and MWD, df=5, T (critical) at 95% CI=2.262, p<0.005

[b] %recovery was calculated according to: [MDM]/[certified value] × 100

[c] Informative value

Result

The macro and micro minerals are needed for progression and physiology of the organism. The current research was performed to find out the different amount (concentrations) of Ca, Mg, Na and K in the specimen (scalp hair, blood serum and blood plasma) of male referents and Parkinson disease (PD) patients (Table 5).

Table 5
Electrolytes concentrations (µg/g) in the scalp hair and (mg/l) in blood serum of the male referents and PD patients

Age groups	Elements	Referents	Mild PD	p-value	Referents	Chronic PD	p-value
		(52–65) years			**(66–75) years**		
Scalp hair	**Ca**	350 ± 34.5 (317–387)	435 ± 36.7 (399–472)	0.010	242 ± 44.1 (218–265)	324 ± 31.7 (302–342)	0.001
	Mg	43.4 ± 9.52 (33.5–50.5)	47.9 ± 7.93 (40.2–53.5)	0.062	38.7 ± 6.02 (30–45)	50.3 ± 6.54 (46.5–53.4)	0.006
	Na	310 ± 21.9 (285–330)	267 ± 16.4 (250–280)	0.008	410.2 ± 23.2 (385–432)	252 ± 15.2 (237–266)	0.001
	K	64.87 ± 12.7 (50–76)	31.2 ± 4.50 (26–37)	0.001	87.5 ± 13.2 (73–99)	32.8 ± 3.15 (28–37)	0.001
Blood Serum	**Ca**	88.9 ± 5.10 (82–94)	73.4 ± 5.02 (68–77)	0.001	84.3 ± 7.09 (80.6–87.9)	77.1 ± 5.62 (73.9–80.4)	0.001
	Mg	18.2 ± 1.42 (16.7–19.6)	15.5 ± 0.34 (15.16–15.84)	0.001	16.6 ± 1.25 (15.9–17.2)	15.0 ± 1.10 (14.5–15.5)	0.001
	Na	3194 ± 57.5 (3135–3252)	2655 ± 47.6 (2608–2700)	0.001	3160 ± 60.2 (3100–3220)	2570 ± 50.6 (2530–2610)	0.001
	K	174 ± 17.5 (155–190)	132.5 ± 13.5 (145 – 120)	0.001	158 ± 12.5 (170 – 145)	78.9 ± 9.86 (73.9–84.0)	0.001
Blood Plasma	**Ca**	97.5 ± 7.10 (90–105)	77.5 ± 9.02 (66.5–87.2)	0.001	90.3 ± 9.20 (84.3–96.9)	71.9 ± 5.90 (65.9–75.8)	0.001

PD, Parkinson's disease; Ca, calcium; Mg, magnesium; Na, sodium; K, potassium

Mg	23.5 ± 3.5 (20.9–30.2)	15.3 ± 0.51 (14.9–15.7)	0.001	20.2 ± 4.2 (16.9–23.8)	10.7 ± 0.33 (10.5-10.95)	0.001
Na	3015 ± 120 (2895–3137)	2890 ± 95.3 (2794–2987)	0.072	3165 ± 67.8 (3095–3230)	3085 ± 83.6 (3000–3168)	0.060
K	197 ± 13.7 (182–210)	115 ± 6.35 (109–120)	0.001	176 ± 16.7 (160–192)	107 ± 7.29 (103.7–112)	0.001

PD, Parkinson's disease; Ca, calcium; Mg, magnesium; Na, sodium; K, potassium

This study consists of chronic Parkinson disease (CPD) patients (66–75 years) which were diagnosed for last 5 to 10 years and initially diagnosed mild Parkinson disease (MPD) patients, age ranged 50 to 65 years. Among selected 95 MPD patients, 50% patients initially have depression, 30% have palpitation, whilst 20% suffer from insomnia as well as other two physiological disorders. Whereas the CPD patients suffered from somatic and psychiatric co-morbidities, such as pain in body (52.1%), palpitations (70.0%), poor appetite (80.2%), fatigue (67.7%), insomnia (56%), and psychomotor retardation (64.6%)

The Ca content in scalp hair samples of male healthy donors and MPD patients age ranged 50 to 65 years, were found at 95% confidence intervals with median values, 347 [CI:298,386] and 437[CI:315,519]μg/g respectively, which describes 20% Ca higher in scalp hair of MPD than the referent subjects. Whilst in higher age group (66–75) years, the Ca concentrations in scalp hair samples of male referent and CPD patients were found to 222[CI:162,330]μg/g and 320[CI:238,404] μg/g, which shows about 27% Ca concentration was greater in scalp hair of CPD than the age matched referent subjects (Fig. 1).

The concentration of Ca in blood serum samples of male referent and MPD patients age ranged (50–65) years was found to be 88 [CI: 76.3, 103] and 72.5 [CI: 63.2, 88.3] mg/l, Whereas in higher age group, the level of Ca in blood serum samples of referent and CPD patients were found as 85.1[CI: 69.8, 103] and 77.9[CI: 65.1, 89.3] mg/l, respectively. It was found that the levels of Ca were found to be lower in MPD and CPD patients 9.2–16.9%, respectively than age matched non-diseased male subjects (Fig. 2).

The Ca concentration in blood plasma samples of referent and MPD patients was observed to be [CI:79.2,116] and [CI:48.9,92.8] mg/l, whilst the concentration of Ca in blood plasma samples of male referent and CPD patients was found to be[CI: 72.7,102] and [CI: 60.2,85.0]mg/l, correspondingly. It was noted that the levels of Ca were found to be 22.1% to 17.6% lower in blood plasma of MPD and CPD patients, respectively than age matched referent subjects (Fig. 3).

The median Mg contents in scalp hair samples of both male non disease subjects and MPD patients, were detected to be 40.2[CI: 22.6, 66.0] and 48.9[CI: 25.6,71.5]µg/g, Whilst in the higher age group, Mg contents in scalp hair samples of male non disease subjects and CPD patients were 38.9[CI: 29.7, 52.4] and 50.4[CI: 35.6,68.2]µg/g,the resulted data indicated that MPD patients have > 10% Mg than age matched non diseased subjects. Although the concentration of Mg was 20% greater in scalp hair of CPD patients than age matched referents (Fig. 1).

It was observed that the levels of Mg were found to be 14.2% and 11.3%lower in blood serum samples of MPD and CPD patients, respectively than age matched referent subjects of both age groups (Fig. 2).It was also observed that 33.9% and 46% Mg concentrations were observed to be least in plasma samples of mild and CPD patients, respectively than age matched non-diseased male subjects (Fig. 3).

The median Na contents in scalp hair samples of male non disease subjects and MPD patients were found to be 309[CI: 263,345] and 268[CI: 214,304] µg/g respectively, in the higher age group of male referent and CPD patients were408 [CI: 352,456] and 251[CI: 223,288] µg/g. The resulted data indicated that MPD patients have < 15% Na in scalp hair than referents, whereas the concentration of Nain scalp hair of CPD patients was < 38.4% lower than age matched controls (Fig. 1).

The Na level in blood serum samples of control and MPD patients was obtained to be 3201[CI: 3048, 3365] and 2655[CI: 2560,2778] mg/l, correspondingly, the concentration of Na in blood serum samples of non-disease subjects is found to be greater [CI: 3039, 3257] mg/l, as compare to CPD patients [CI: 2429, 2670] mg/l (Fig. 2).It was observed that 16% and 18% Na levels were obtained to be lower in MPD and CPD patients, respectively than same age grouped referent male subjects. Whereas 4.6% and 2.5% Na were found to be lower in blood plasma samples of MPD and CPD patients, respectively than referent subjects. (Fig. 3)

The median K contents in scalp hair samples of male referent and MPD patients, were observed to be 68.1[CI: 42.4, 92.3] and 31.5[CI:20.4,46.7]µg/g respectively, which indicates 52% K was higher in scalp hair of MPD than referents. Whilst in the higher age group, the K contents in scalp hair samples of male referent and CPD patients were 85.77[CI:64.3,136] and32.7[CI:25.3,41.6] µg/g, which shows 62% K higher in scalp hair of CPD than referents (Fig. 1).

It was observed that the concentration of K were obtained to be 24% and 52% lower in blood serum samples of mild and CPD patients, respectively than age matched referent subjects of both age groups (Fig. 2). It was also observed that 41.2% and 38% K concentrations were obtained to be lower in blood plasma samples of mild and CPD patients, respectively than age matched non-diseased male subjects (Fig. 3).

The unpaired student t-test between male mild/ chronic PD patients and non-disease healthy subjects at different degrees of freedom was calculated at different probabilities. Our calculated t value exceeds that of tcritical value at 95% confidence intervals, which showed that the difference among mean

concentration of electrolytes in health subjects and mild/ chronic PD patients showed significant differences ($p < 0.019$)23.

Discussion

This research study gives information on electrolytes in biological (scalp hair, serum and plasma) samples, gained from two classes of PD (mild & chronic) sufferers and those of non-diseases subjects of age groups (55–65 and 66–75) years and we showed the function of electrolytes in neurological disorder patients. We also correlated the biochemical parameters of both types of PD patients with electrolytes.

The electrolytes (Ca and Mg) concentrations were obtained to be significantly higher in the scalp hair samples of mild/chronic PD patients than controls. The mean values of Ca content in scalp hair samples of males PD patients were higher than in referents, while the difference was significant ($p < 0.01$) (Table 4).The mean amount of Mg contents in scalp hair samples of both types of MPD and CPD patients were greater than control subjects, but in MPD group, the difference was not statistically significant ($p > 0.05$) while in CPD group, the statistically significant was observed ($p < 0.05$). When comparing MPD and CPD patients, the Ca mean concentrations were a little higher in MPD patients than CPD patients. Whereas the Mg content was slightly lower in MPD patients than CPD group ($p < 0.05$) (Table 4) A reverse link was monitored with Na and K, which displayed a significantly lower concentrations in both types of PD groups as compared control groups levels ($p < 0.05$ for both).

Parkinson's disease (PD), the most common neurodegenerative disorder after Alzheimer's disease (AD), stays hard to diagnose clinically due to having common characteristics symptoms with other neurological diseases [24–26].

PD is a multiorgan disease functionalized by progressive deterioration of the dopaminergic neuronal system, many other organs and neuronal systems responsible for the foundation of motor symptoms like, akinesis, rigidity, tremor and postural instability – and a variety of non-motor and neuropsychological problems that influence the patient's life style [27]. The resulted data indicated higher contents of Ca, Mg in cystosol caused neurotoxicity and also promoting free radical's generation. Usually this observable fact is limited by a voltage-dependent magnesium (Mg) blockade.

We assessed the most common yet the major electrolyte (Serum Calcium) which help in neuronal development and function, maintaining the Resting Membrane Potential, and cause the generation of Action Potential across the excitable tissue along the course of Nerve, nerve transmission, intracellular signaling, and hormonal secretion. The Calcium homeostasis is affected near the beginning of the pathological process and consequence in major neuronal demise [1, 28]. Human body holds about 24 g Mg, of which 99% is accumulated in bone, other soft tissues and muscle. The Mg is important to the function of fundamentally every organ in the human body. Furthermore, insufficiency of Mg is related with a broad series of diseases, and Mg supplementation is considered as potential treatment in many of them16. Deficiency of blood serum magnesium concentration caused different neurological diseases

such as depression, migraine and Parkinson's. Overall magnesium plays important role in the maintainece and releasing of neuropeptide which may have serious consequences in neuronal disease.

Hyponatremia and hypernatremia are frequent in the critically ill patient and associated with significant morbidity and mortality. A rapid change in serum sodium concentration can result in brain damage and death resulting from brain edema or osmotic demyelination However, it is well known that a rapid decrease in serum sodium causes brain edema, and delayed treatment can result in brain damage and death. On the other hand, sodium and potassium regulation effect on serum sodium, which are found by the difference between the input and output of Na + and K+. similarly, water balance is review the serum sodium (input of water minus output, e.g., urine), for that reason serum Na always should be corrected for hyperglycemia (SNa decreases approximately 0.4 mmol/L per mmol/L raise in plasma glucose concentration above 5 mmol/L) [29]. Hypokalemia is generally visible as a mild form associated with hypochloremic alkalosis and largely combined with pharmaceutical treatment such as diuretics, intravenous fluid, and iatrogenic hyperventilation [7, 16].

The electrolytes levels in blood serum were found to be lower in both types of PD patient groups than healthy groups, the difference was significant ($p < 0.01$). When comparing MPD patients with CPD patients group, all the electrolyte level was found to be slightly higher in MPD patients than CPD patients ($p < 0.01$). It was observed that electrolytes concentration in blood plasma was found lower in both groups of patients ($p < 0.01$).

The correlation (r) between electrolytes (Ca, Mg, Na, K) concentration in biological samples (scalp hair, blood serum and blood plasma) VS biochemical parameters (SBP, DBP, Hb, RBCs, vitamin D) of referents and PD patients subjects of age range (55–75 years) was observed from resulted data that correlation (r) of calcium in biological samples concentration Vs biochemical parameter of referents shows ($r = 0.72–0.93$) while in the PD patients the correlation were found to be in the range of ($r = 0.37–0.87$) respectively, in case of magnesium the correlation (r) values in biological samples concentration Vs biochemical parameter of referents shows ($r = 0.49–0.85$) while in the PD patients the correlation were found to be ($r = 0.40–0.88$) of both age groups. In Na the correlation (r) values in biological samples concentration Vs biochemical parameter of referents shows ($r = 0.47–0.88$) while in the PD patients the correlation were found to be in the range of ($r = 0.24–0.57$) of both age groups and it was observed from the obtained result that in K the correlation (r) values in biological samples concentration Vs biochemical parameter of referents shows ($r = 0.42–0.85$) while in the PD patients the correlation were found to be in the range of ($r = 0.31–0.54$) of both age groups (Table 6). This indicated that the imbalances in concentration of electrolytes are highly associated with biochemical parameters and the resulted data showed strong correlation with calcium and magnesium in both categorized.

Table 6
Linear regression and Pearson's coefficient for electrolytes versus different biochemical parameters in referents and PD patients

Age groups	Elements	biochemical parameters	Referents	Mild PD	Referents	Chronic PD
			(52–65) years		(66–75) years	
Scalp hair	Ca	SBP	y = 7.765x − 644.5 r = 0.84	y = 5.965x − 434.2 r = 0.86	y = 4.619x − 374.0 r = 0.82	y = 4.197x − 366.4 r = 0.87
		DBP	y = 4.55x − 31.27 r = 0.81	y = 4.03x + 48.71 r = 0.88	y = 5.391x − 235.0 r = 0.88	y = 3.826x − 93.61 r = 0.87
		Hb	y = -285.5x + 4340 r = 0.86	y = 205.1x − 2234 r = 0.83	y = 21.71x − 28.10 r = 0.83	y = 57.91x − 291.3 r = 0.85
		RBC	y = 201.6x − 643.7 r = 0.79	y = 78.84x + 107.8 r = 0.78	y = 78.84x + 107.8 r = 0.77	y = 89.56x − 133.3 r = 0.82
		Vitamin D	y = 109.0x − 2225 r = 0.72	y = 21.89x + 241.4 r = 0.84	y = 140.9x − 2945 r = 0.93	y = 13.45x + 235.0 r = 0.75
	Mg	SBP	y = -1.964x + 293.1 r = 0.77	y = -1.407x + 253.4 r = 0.85	y = -0.517x + 106.4 r = 0.75	y = -0.743x + 171.4 r = 0.85
		DBP	y = 1.170x − 54.69 r = 0.76	y = 0.942x − 42.05 r = 0.76	y = 0.620x − 15.43 r = 0.83	y = -0.673x + 122.5 r = 0.85
		Hb	y = 76.23x − 1024 r = 0.84	y = -47.30x + 663.7 r = 0.81	y = -2.658x + 70.52 r = 0.81	y = 9.819x − 53.72 r = 0.80
		RBC	y = 42.90x − 168.2 r = 0.61	y = 20.25x − 35.69 r = 0.84	y = -10.71x + 82.38 r = 0.81	y = -12.63x + 87.44 r = 0.88
		Vitamin D	y=-15.34x + 422.8 r = 0.81	y = 5.317x + 1.344 r = 0.87	y = 14.69x − 292.8 r = 0.79	y = 3.235x + 29.98 r = 0.86

Age groups	Elements	biochemical parameters	Referents	Mild PD	Referents	Chronic PD
	Na	SBP	y = 2.752x − 42 r = 0.65	y = 1.271x + 80.59 r = 0.44	y = 1.912x + 158.2 r = 0.63	y = -1.148x + 439.8 r = 0.52
		DBP	y = 1.722x + 166.2 r = 0.67	y = -0.902x + 352.4 r = 0.47	y = 2.119x + 225.4 r = 0.64	y = -0.951x + 354.7 r = 0.47
		Hb	y = 101.5x − 1110 r = 0.47	y = -53.57x + 963.0 r = 0.51	y = -8.945x + 517.3 r = 0.62	y = -16.19x + 422.9 r = 0.51
		RBC	y = -79.00x + 697.1 r = 0.68	y = 22.55x + 172.3 r = 0.53	y = -37.91x + 564.9 r = 0.64	y = 16.71x + 202.3 r = 0.46
		Vitamin D	y = 21.54x − 224.7 r = 0.66	y = 5.246x + 219.5 r = 0.48	y = -51.73x + 1576 r = 0.63	y = 4.794x + 222.6 r = 0.25
	K	SBP	y = -1.166x + 215.4 r = 0.43	y = 0.331x − 16.76 r = 0.39	y = 0.977x − 40.84 r = 0.44	y = 0.182x + 3.027 r = 0.37
		DBP	y = 0.725x + 6.310 r = 0.45	y = -0.237x + 54.28 r = 0.43	y = 1.132x − 10.75 r = 0.47	y = -0.156x + 49.71 r = 0.35
		Hb	y = -48.89x + 750.4 r = 0.51	y = 12.08x − 125.8 r = 0.41	y = -4.434x + 141.0 r = 0.42	y = 2.782x + 3.453 r = 0.41
		RBC	y = -36.3x + 244.7 r = 0.49	y = -4.981x + 52.12 0.41	y = 18.15x + 13.33 r = 0.43	y = -2.825x + 41.21 r = 0.35
		Vitamin D	y = -8.571x + 278.9 r = 0.43	y = 1.241x + 20.49 r = 0.40	y = 9.015x − 156.9 r = 0.45	y = -24.51x + 640.4 r = 0.42
Blood serum	**Ca**	SBP	y = 0.732x − 5.200 r = 0.54	y = -0.498x + 145.8 r = 0.57	y = -0.568x + 159.8 r = 0.56	y = -0.48x + 155.9 r = 0.61

Age groups	Elements	biochemical parameters	Referents	Mild PD	Referents	Chronic PD
		DBP	y = 0.493x + 47.35 r = 0.59	y = 0.493x + 47.35 r = 0.59	y = -0.623x + 139.2 r = 0.56	y = 0.415x + 32.61 r = 0.58
		Hb	y = 26.04x − 276.0 r = 0.53	y = -17.97x + 307.1 r = 0.57	y = 2.708x + 52.43 r = 0.55	y = 6.642x + 7.359 r = 0.59
		RBC	y = 22.23x − 20.85 r = 0.59	y = 7.511x + 42.11 r = 0.58	y = -11.02x + 130.1 r = 0.55	y = -7.657x + 100.2 r = 0.59
		Vitamin D	y = -6.763x + 255.9 r = 0.67	y = -1.866x + 89.76 r = 0.57	y = 15.93x − 274.3 r = 0.57	y = 0.738x + 1.643 r = 0.73
	Mg	SBP	y = 0.790x + 3.796 r = 0.79	y = 0.027x + 11.54 r = 0.47	y = -0.090x + 28.64 r = 0.61	y = -0.054x + 23.78 r = 0.40
		DBP	y = -0.153x + 30.83 r = 0.75	y = -0.022x + 17.66 r = 0.57	y = -0.119x + 27.07 r = 0.74	y = 0.065x + 7.750 r = 0.53
		Hb	y = -8.864x + 142.1 r = 0.74	y = -0.931x + 27.66 r = 0.45	y = 0.467x + 11.07 r = 0.66	y = 1.048x + 3.754 r = 0.54
		RBCs	y = -6.183x + 48.47 r = 0.68	y = 0.419x + 13.79 r = 0.49	y = -2.443x + 26.71 r = 0.85	y = -1.224x + 18.46 r = 0.54
		Vitamin D	y = -1.932x + 66.00 r = 0.78	y = -0.116x + 16.56 r = 0.53	y = 2.866x − 47.93 r = 0.72	y = 0.279x + 13.10 r = 0.47
	Na	SBP	y = 41.83x + 2442 r = 0.88	y = -4.198x + 3269 r = 0.52	y = -7.283x + 4107 r = 0.86	y = 1.731x + 2281 r = 0.24
		DBP	y = -6.118x + 3708 r = 0.63	y = 3.000x + 2369 r = 0.56	y = 6.612x + 2575 r = 0.73	y = -3.446x + 2936 r = 0.53

Age groups	Elements	biochemical parameters	Referents	Mild PD	Referents	Chronic PD
		Hb	y = 392.1x − 2284 r = 0.69	y = -153.0x + 4648 r = 0.53	y = -32.07x + 3536 r = 0.64	y = -57.58x + 3171 r = 0.56
		RBCs	y = 317.3x + 1642 r = 0.73	y = 60.32x + 2407 r = 0.51	y = -135.6x + 3705 r = 0.83	y = 59.53x + 2387 r = 0.51
		Vitamin D	y = -82.56x + 5246 r = 0.70	y = -18.65x + 2822 r = 0.61	y = -63.73x + 1846 r = 0.78	y = -4.859x + 281.9 r = 0.51
	K	SBP	y = -9.684x + 349.5 r = 0.78	y = -0.802x + 247.8 r = 0.31	y = -1.177x + 316.0 r = 0.77	y = -0.670x + 186.0 r = 0.48
		DBP	y = 2.114x − 1.310 r = 0.84	y = 0.791x + 55.00 r = 0.45	y = 1.337x + 45.03 r = 0.81	y = -0.483x + 128.7 r = 0.38
		Hb	y = 105.4x − 1301 r = 0.71	y = -42.55x + 684.6 r = 0.45	y = -5.563x + 228.2 r = 0.76	y = 9.013x − 18.57 r = 0.46
		RBC	y = -85.59x + 594.3 r = 0.755	y = -21.12x + 218.5 r = 0.54	y = 19.34x + 82.02 r = 0.66	y = 11.22x + 43.13 r = 0.49
		Vitamin D	y = 23.63x − 411.6 r = 0.77	y = 3.332x + 101.4 r = 0.39	y = -28.76x + 809.9 r = 0.71	y = -2.845x + 93.99 r = 0.47
Blood plasma	Ca	SBP	y = -0.971x + 221.3 r = 0.59	y = 0.890x − 54.01 r = 0.57	y = -0.657x + 174.7 r = 0.61	y = 0.481x − 5.964 r = 0.37
		DBP	y = 0.649x + 43.40 r = 0.63	y = 0.622x + 16.09 r = 0.61	y = 0.672x + 29.78 r = 0.57	y = -0.403x + 116.3 r = 0.57
		Hb	y = 33.90x − 376.9 r = 0.56	y = 32.33x − 344.9 r = 0.58	y = 3.233x + 49.29 r = 0.621	y = -6.928x + 145.8 r = 0.62

Age groups	Elements	biochemical parameters	Referents	Mild PD	Referents	Chronic PD
		RBCs	y = 26.21x − 31.43 r = 0.564	y = -13.59x + 132.1 r = 0.590	y = -11.94x + 137.1 r = 0.57	y = -7.043x + 93.66 r = 0.55
		Vitamin D	y = -7.430x + 281.4 r = 0.59	y = -3.673x + 108.2 r = 0.392	y = 26.61x − 511.7 r = 0.59	y = -1.251x + 80.39 r = 0.37
	Mg	SBP	y = -1.020x + 227.6 r = 0.61	y = 0.035x + 10.03 r = 0.44	y = -0.426x + 75.99 r = 0.75	y = 0.023x + 6.854 r = 0.44
		DBP	y = 0.248x + 2.494 r = 0.49	y = 0.023x + 13.00 r = 0.44	y = -0.436x + 57.81 r = 0.70	y = -0.023x + 13.27 r = 0.48
		Hb	y = -20.21x + 305.8 r = 0.68	y = 1.468x − 3.872 r = 0.51	y = -1.963x + 43.55 r = 0.72	y = -0.431x + 15.28 r = 0.57
		RBCs	y = -17.20x + 107.5 r = 0.75	y = 0.562x + 12.90 r = 0.47	y = 9.205x − 17.79 r = 0.83	y = 0.417x + 9.497 r = 0.48
		Vitamin D	y = -4.701x + 139.6 r = 0.76	y = 0.157x + 13.84 r = 0.52	y = 12.41x − 260.0 r = 0.80	y = -0.115x + 11.44 r = 0.50
	Na	SBP	y = -20.70x + 5679 r = 0.75	y = -8.973x + 4201 r = 0.52	y = -6.313x + 3991 r = 0.73	y = -5.771x + 4024 r = 0.51
		DBP	y = -14.23x + 4218 r = 0.85	y = 5.785x + 2339 r = 0.54	y = 7.001x + 2552 r = 0.74	y = 5.552x + 2481 r = 0.54
		Hb	y = 708.5x − 6874 r = 0.72	y = -346.1x + 7398 r = 0.57	y = 33.32x + 2759 r = 0.80	y = -89.06x + 4020 r = 0.56
		RBCs	y = -571.7x + 5844 r = 0.76	y = 135.2x + 2333 r = 0.54	y = -128.7x + 3688 r = 0.59	y = 98.74x + 2787 r = 0.53

Age groups	Elements	biochemical parameters	Referents	Mild PD	Referents	Chronic PD
		Vitamin D	y = 85.27x + 1086 r = 0.72	y = 35.71x + 2578 r = 0.56	y = 185.1x – 1013 r = 0.79	y = 26.08x + 2921 r = 0.53
	K	SBP	y = -2.138x + 469.4 r = 0.78	y = -0.474x + 184.6 r = 0.42	y = -1.606x + 385.9 r = 0.73	y = 0.473x + 29.33 r = 0.43
		DBP	y = 1.412x + 79.39 r = 0.85	y = 0.345x + 82.28 r = 0.46	y = -1.751x + 326.9 r = 0.73	y = 0.451x + 57.97 r = 0.45
		Hb	y = 86.60x – 1014 r = 0.84	y = -17.61x + 344.6 r = 0.44	y = -8.434x + 276.3 r = 0.65	y = -6.184x + 172 r = 0.40
		RBC	y = -53.59x + 459.7 r = 0.71	y = 6.962x + 86.54 r = 0.42	y = -34.19x + 314.7 r = 0.81	y = -8.931x + 133.3 r = 0.49
		Vitamin D	y = -14.81x + 563.8 r = 0.73	y = -1.559x + 129.2 r = 0.37	y = -44.31x + 1173 r = 0.75	y = -1.862x + 118.1 r = 0.39

Conclusion

Our study results exposed that efficiently increase levels of Ca and Mg and lower concentrations of Na and K in scalp hair samples, whilst lower electrolytes values in blood serum and plasma correlated well with the consequences of mild and chronic PD. Apparent imbalance in electrolytes content, as an outcome or reason of the neuropathology, have been viewed. Even though these imbalances of electrolytes cannot be elucidated through an easy model and it is not possible to remove elemental functions and its role because there are number of elements which can act as biomarkers in this category of Neurodegeneration. Further it may be possible that occurrence of oxidative damage in the Parkinson's patients gives the idea about oxidative injury may be plays its role in neurodegenerative disorders. Although disturbances in electrolytes concentration are not considered to caused pathogenetic diseases observation revealed that they are worked as risk factors. At last, we can concluded that there is lots of epidemiologic evidence related about functions of trace elements which creates neurological disorders risk, additional studies are required to illustrate further the mechanisms underlying metal toxicity.

More studies are necessary to elucidate the main function of serum electrolyte concentrations in vascular events. This study helps in exploring serum electrolyte thresholds in which interventions for falling dietary sodium and rising dietary potassium intake may help the population at serious hazard. This research helps in exploring serum and plasma electrolytes thresholds in which interventions for reducing dietary sodium and increasing dietary potassium intake may essential the population at risk. It is concluded that electrolytes supplementation may be beneficial for patients suffering from Parkinson's disease. Intravenous electrolytes supplementation may be more efficient, but this cure has the disadvantage that it needs regular hospital concerns.

List Of Abbreviations

Alzheimer's disease :AD

Body mass index :BMI

Calcium :Ca

Certified reference materials :CRMs

Chronic Parkinson disease :CPD

Hemoglobin :Hb

Limit of detection :LOD

Limit of quantification :LOQ

Magnesium Mg

Mild Parkinson disease :MPD

Parkinson's disease :PD

Polytetrafluoroethylene : PTFE

Potassium K

Red blood cells :RBC

Sodium : Na

Declarations

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Authors' contributions HIA and TGK were responsible for the conception and design of the study. KR, HIA and FNT were responsible for acquisition of the data. KR, HIA, and JAB were responsible for analysis, and interpretation of data, drafting the manuscript and revising it critically for important intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials The data that support the findings of this study are available from the

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Ethics approval and consent to participate The study procedure was permitted by the local ethics committee of HEC of Pakistan.

Consent for publication Not applicable.

Competing interests None

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