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Comparative Spectroscopic determination of Heavy Metals Profile of kidney Patients before and after Dialysis and Healthy voluntaries

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ABSTRACT

Present study was conducted to quantify and compare the levels of heavy metals in plasma sample of kidney patient before and after dialysis belonging to different areas of Sargodha region. In this study, the level of copper, zinc, cadmium, iron, manganese, lead, chromium and cobalt in plasma sample of Kidney patients has been investigated using atomic absorption spectrophotometer. Heavy metal profile in both normal and diseased subject was determined using dialysis time period and age factor parameters. The mean value of Zn and Fe was found lower in kidney patient as compared to normal subject and reverse was found in case of Pb, Mn, Co and Cr. The heavy metals composition in male patients were found somewhat higher but non-significantly ($P>0.05$). Age factor revealed that those patients whose age was >30 years have greater values of metals as compared to <30 years patients. Similarly, the patient on dialysis with <2 years have low concentration of metals as compared to >2 years.

INTRODUCTION

Heavy metals are five times denser than water and cannot be metabolized by the body system (Duffus, 2002; Housecroft and Sharpe, 2008; Naumann, *et al.,* 2007). Exhausts of heavy metals from different industrial processes and vehicles in residential area, is a growing problem throughout the world due to their toxicity (Chander, *et al.,* 2001). Heavy metals have no constructive function or nutritional values in the body and are toxic (Eman *et al.,* 2011; Clemens 2006). It is well established fact that exposure to heavy metals may trigger the initiation of several autoimmune diseases such as diabetes mellitus (Hemdan *et al.,* 2007; Bertrand and Poirier 2005; Schützendübel, and Polle 2002).

Metals such as mercury, chromium, lead, manganese and cadmium are known to affect the immune system of members of kingdom animal (Järup 2003; Wirth and Mijal 2010; Iavicoli 2009). Anyhow some heavy metals when present within permissible limits in the body play important role Chung et al.,1995; Eliasson *et al.,* 1995). Metals are essential part of many enzymes as co-enzyme, various physiological processes in the body and tissue growth regulation (Di-Silvestro, 2000). Balanced amount of the metals plays significant role in proper growth, development & maintenance of body tissues (Rodushkin *et al.,* 2000).

Heavy metals become toxic, if their level increased in the body from allowed concentration (Afridi *et al.,* 2006). Copper is an essential nutrient which helps in the production of haemoglobin, collagen, myolin and melanin while high intake of copper cause kidney disorder (Sugawara *et al.,* 2002). Zinc is essential of enzyme activity but the low intake of zinc can cause chronic renal disease (Daisuke *et al.,* 2008).

Non-metabolized heavy metals accumulation in the body start serious damaging but excretion of appreciable concentration of these metals through kidneys play vital role to reduce risk factors related to its accumulation.

Malfunctioning of kidney may cause failure to excrete heavy metal from blood. Consequently all these metals come back to different body parts and accumulate there that results serious diseases. This study was mainly carried out in order to explore the profile of common heavy metals (Cu, Zn, Cd, Fe, Mn, Pd, Cr and Co) in patients of renal disorder in comparison with non renal disordered subjects.

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Table. 1: Parameters used for measurement on flame Atomic Absorption Spectrophotometer.

Metals	Wave Length (nm)	Slit width (nm)	Lamp mode	Lamp current low (mA)	Lamp Current high	Burner height	Burner Angel Oº C	Flame type (Fuel + oxidant)	Fuel gas L/min	Support gas (L/min)
Cd	228.8	0.7	BDC-D2	8	0 mA	7 mm	0º C	Air + C_2H_2	1.8 L/min	15 L/min
Co	240.7	0.2	BDC-D2	12	0 mA	7 mm	0ºC	Air + C_2H_2	1.6 L/min	15 L/min
Fe	248.3	0.2	BDC-D2	12	0 mA	7mm	0ºC	Air + C_2H_2	2.2 L/min	15 L/min
Cu	324.8	0.7	BDC-D2	6	0 mA	7 mm	0ºC	Air + C_2H_2	1.8 L/min	15 L/min
Mn	279.5	0.2	BDC-D2	10	0 mA	7mm	0ºC	Air + C_2H_2	2 L/min	15 L/min
Pb	283.3	0.7	BDC-D2	10	0 mA	7mm	0ºC	Air + C_2H_2	2 L/min	15 L/min
Zn	213.9	0.7	BDC-D2	8	0 mA	7 mm	0ºC	Air + C_2H_2	2 L/min	15 L/min
Cr	357.9	o.7	BDC-D2	10	0 mA	7 mm	0ºC	Air + C_2H_2	2.8 L/min	15 L/min

Table. 2: Heavy metal concentration (µg/mL for plasma samples) of Kidney patients before dialysis of male subjects with age <30 years and >30years.

Patient's Status	Cd	Cr	Co	Cu	Mn	Pb	Zn	Fe
Before Dialysis Male<30 (N=15)	0.82±0.04	28.8±12.2	18.0±2.21	7.78±1.00	32.2±13.2	18.7±3.08	1.91±0.03	1.43±0.65
Before Dialysis Male>30(N=15)	0.13±0.04	12.7±4.12	11.0±2.51	2.78±0.71	14.1±4.62	15.7±2.08	0.21±0.03	1.21±0.65
Control Subjects (N=50)	0.25±0.09	1.93±0.41	6.34±0.76	9.97±0.21	2.48±1.05	0.41±0.02	56.7±12.1	60.9±5.2
P-value	P>0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P>0.05

t-Test Analysis (p<0.05=Significant Difference, p>0.05=Non-Significant difference).

Table. 3: Heavy metal concentration (µg/mL for plasma samples) of Kidney patients after dialysis of male subjects with age <30 years and >30years.

Patient's Status	Cd	Cr	Co	Cu	Mn	Pb	Zn	Fe
After Dialysis Male<30 (N=15)	0.15±0.02	9.81±2.81	8.30±1.97	6.80±1.04	43.5±21.9	28.2±11.4	2.17±0.07	2.47±0.96
After Dialysis Male>30(N=15)	0.11±0.03	4.41±11.6	3.12±0.67	5.53±1.01	25.5±12.1	23.5±9.41	1.01±0.03	1.77±0.67
Control Subjects (N=50)	0.25±0.09	1.93±0.41	6.34±0.76	9.97±0.21	2.48±1.05	0.41±0.02	56.7±12.1	60.9±18.2
P-value	p>0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

t-Test Analysis(P<0.05=significant difference, P>0.05=non significant)

Table. 4: Heavy metal concentration (µg/mL for plasma samples) of Kidney patients before dialysis of female subjects with age <30 years and >30years.

Patient's Status	Cd	Cr	Co	Cu	Mn	Pb	Zn	Fe
Before Dialysis Female<30 (N=10)	0.96±0.01	5.91±0.17	3.10±0.91	1.50±0.41	32.7±11.2	14.7±4.01	0.60±0.27	1.66±0.25
Before Dialysis Female>30 (N=10)	0.41±0.07	4.07±0.12	2.02±0.08	0.52±0.07	28.4±10.1	10.9±141	0.51±0.18	1.95±0.21
Control Subjects(N=50)	0.25±0.09	1.93±0.41	6.34±0.76	9.97±0.21	2.48±1.05	0.41±0.02	56.7±12.1	60.9±18.2
P-value	P>0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P>0.05	P>0.05

t-Test Analysis(P<0.05=significant difference, P>0.05=non significant)

Table. 5: Heavy metal concentration (µg/mL for plasma samples) of Kidney patients after dialysis of female subjects with age <30 years and >30years

	Cd	Cr	Co	Cu	Mn	Pb	Zn	Fe
After Dialysis Females<30 (N=10)	0.16±0.03	8.79±2.10	3.82±1.02	1.79±0.75	41.5±20.2	20.2±11.9	0.81±0.04	2.37±0.65
After dialysis Female>30 (N=10)	0.11±0.02	6.21±0.19	3.38±0.92	0.62±0.11	26.5±14.1	17.6±6.41	0.76±0.02	1.95±±0.21
Control subjects (N=50)	0.25±0.09	1.93±0.41	6.34±0.76	9.97±0.21	2.48±1.05	0.41±0.02	56.7±12.1	60.9±18.2
P-value	P>0.05	P<0.05	p>0.05	P<0.05	P<0.05	P<0.05	P>0.05	P<0.05

t-Test Analysis(P<0.05=significant difference, P>0.05=non significant difference)

MATERIAL AND METHODS

All chemicals were purchased from Aldrich and Fluka chemicals (Pvt) Ltd. and used as received without prior treatment. The blood sample was collected from patients who have suffered seriously in renal disorder from different hospitals and healthy subjects according to the medical health committee suggestions. Blood sample (5 mL) was collected in Na2EDTA glass vials from each subject by sterilized syringe and stored in refrigerator at 4 ^oC till further analysis.

Spectrophotometeric Analysis

Plasma from each blood sample was taken by centrifugation at 1000 r.p.m. and 4 oC. Plasma sample 1 mL of each subject was taken in digestion flask followed by the addition of 60% HNO3 (1 mL) for digestion process. Digestion was performed on hot plate at 180 ℃ for 2-3 min. up to 15 mL with doubly distilled de-ionized water and stored in sample vials for etermining the concentration of the metal ion profile using flame atomic absorption spectrophotometer.

RESULTS

Atomic absorption parameters

The analysis of the blood samples were carried out using the state of the art technique, Atomic Absorption Spectrometer. The parameter of AAS selected for this study shown in Table 1.

Determination of heavy metal profile

The heavy metal profile of volunteers were analysed in four groups such as kidney patients male before dialysis, kidney patients male after dialysis, kidney patients female before dialysis and kidney patients female after dialysis. All the patients that were considered for this study was also broadly categories into two

groups with respect to age i.e. less than 30 and more than 30 years. The metal profile for all four categories is shown in Table 1-4.

DISCUSSION

Balanced concentration of different metals in body has a living impact on the systematic functions of human body. Most of the metals in human body act as co-enzymes and some others play its role in neuron transmission and blood pressure regulation. In this study a systematic approach was undertaken to investigate the role of heavy metals in the process of different diseases such as renal disease. It was very difficult to explore a correlation between heavy metal concentration and particular disease but appearance of some what similar results in different subjects of same diseased attributed the way to find a correlation. Human exposure and ingress of heavy metals is higher due to enhanced industrialization, uncontrolled urbanization, illegal adulteration human food stuff, non-branded local medicines, cosmatics and low grading cooking utensils [ref]. The imbalance concentration of certain heavy metals causes kidney diseases. It is an effort to determine a correlation of heavy metal profile in blood of renal patients. This study shows very appreciable results. Profile of heavy metals (Cu, Zn, Cd, Fe, Mn, Pb, Cr& Co) composition was determined using atomic absorption spectrophotometer. Almost same parameters were used except at different wavelengths as shown in table 1.

The two major factors that were studied are age group and time period of dialysis have been considered during the research. The study revealed that before dialysis the concentration of certain metals like Cr, Cd, Co, Mn and Pb significantly increased in kidney patients as compared to the normal subjects while the metals like Cu, Zn and Fe decreased to its normal values as shown in table-2. No appreciable change was found in metal concentrations within the same group of kidney patients after dialysis. The only change that was noted, the concentration of the Cd was decreased after dialysis as shown in table-3. Table 4 shows the metal profile of female patients with same age group as selected for male patients before dialysis. The data obtained shows increase in metal concentration of Cr, Cd, Mn and Pb while the concentration of the Co was decreased to significant level. But if we see the concentration of other heavy metal such as Cu, Zn and Fe which take part in enzymatic action of the body, going to decrease in kidney patients. The similar findings were also observed in male patients. After Dialysis of female patients, no significant change in elevation of metal profile was observed. However, significant difference between normal and dialysis patient were seen. One thing that was noticeable is that the increase in metal concentration of Co, Cd, Mn, Pd and Cr is less in female kidney patients as compared to the male patients. The concentration of Fe, Cu and Zn, however, decreased appreciably in both group of patients.

CONCLUSION

The data obtained in this research work has created a strong association between heavy metal concentrations and kidney diseases. The higher level of the heavy metals in male patients as compared to female patients revealed that males face more exposure to heavy metals as compared to the females. Females in central Punjab most commonly act as house wife and face least heavy metal exposure. Perhaps this was the reason that the risk of kidney disease is more frequent in male.

Overall results show that intake of heavy metals may play role in creating disorder in certain functions of nephrones and also take the place of certain co-enzymes such as Zn and Cu that may prove remedy for normal functioning of the kidneys.

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