Comparative Analysis of Essential and Toxic Elements in Water and Blood Samples of Kidney Patients in Quetta, Pakistan

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Abstract. The current study based on the comparative analysis of essential elements such as sodium (Na), potassium (K), Calcium (Ca) and Magnesium (Mg) and toxic elements such as Al (Al) and lead (Pb), in drinking water, and biological sample of kidney disorder patients in Quetta capital city of Balochistan, Pakistan. In this study our main purpose was to compare the level of heavy metals (Pd & Al) and essential metals (Ca, Na, K & Mg) in blood samples of kidney disorder patients and in water samples of their respective areas. Level of these essential and toxic elements in blood samples as well as in water determined by atomic absorption spectrometer (AAS). It was evaluated that Ca and Mg in water might be related with patients of kidney disorders in Quetta Balochistan, Pakistan. The Ca and Mg contents in two types of drinking water, Ground Water (GW) and Munciple treated water (MTW) were found in the range of (510.7) mg/L and (319) mg/L respectively. In blood samples of kidney disorder patient’s concentration of essential elements were found in the range of Ca (118-140), Mg (20.1-43.2), Na (3156-4094) and K (211-299) mg/L while, the levels of Al and Pb were found in the range of (586-1380) and (278-645) µg/L, respectively. In the healthy referents concentration of electrolytes are lower than Kidney disorder patient (KDP), while the levels of toxic metals in blood samples of referents were three to six fold lower than kidney disorder patients. As water samples of Quetta area were not polluted with toxic elements (Pb and Al), but these elements were present in biological samples of KDPs due to the environmental exposure of Pb and Al to these individual. Eventually it is clear from present research work that only Ca is that element which is present in large amount in water samples of Quetta Area and contributed as one of the important factor for raising the Ca level in biological samples of KDPs and KDPs showing abnormal level of Ca element in their biological samples is due to the usage of unsafe water containing this element in excess amount.

1. Introduction

The kidneys are located in the middle of the back, below the rib cage. On average, they are the size of a closed fist. The main function of the kidneys is to remove toxic waste products and extra body fluids. Most people are born with two kidneys, but a single kidney can often function as well as two. They are
also important in regulating other body functions such as blood pressure, maintaining healthy bones and red blood cell production.

The prevalence of kidney disorder/disease (KD) is high in the general population and increases strongly all over the world (Cirillo et al., 2006; Coresh et al., 2007). When renal function decreases, a broad spectrum of metabolic complications like anemia and electrolyte disturbances develop. Early detection the symptoms of kidney disorder might be essential for preventing severe chronic diseases such as mineral and bone disease and cardiovascular diseases (Go et al., 2004; Fried et al., 2007). The toxic metals exposure is known to have adverse effects on the kidney and other vital organs of human body, but the kidney is accepted to be the critical organ.

An assessment of drinking water quality especially the presence of high and low levels of essential nutrients as well as various toxic materials including metals, often provides important clues about the sources of water pollution and guidelines for health protection. Therefore in present study we determined the levels of essential elements (Na, K, Ca, Mg) and toxic heavy metals (Al & Pb) in biological samples (blood) of kidney disorder patients (kidney failure and others) and compare it with presence of these metals in drinking water of Quetta areas of Balochistan, Pakistan.

Magnesium has a key role in many other crucial biological processes such as cellular energy metabolism, cell replication, and protein synthesis. Concentration of magnesium in drinking water and composition of food defines the magnesium intake. One of the important source of magnesium is drinking water especially the hard water which contains up to 30mg/L of magnesium. It is proclaimed that healthy volunteers and non-dialyzed patients with damaged renal function exhibit low average Mg concentration of erythrocytes than those patients who were on dialysis (Panhwar et al. 2015).

Potassium is a primary ion of body. Almost 98% of potassium is intracellular. Cellular membrane potential is estimated by ratio of potassium level in intracellular to extracellular. Function of cardiovascular and neuromuscular systems are greatly influenced by small change in potassium level in extracellular environment. Generally 50mEq/Kg of potassium is stored in body.

Humans are commonly exposed to Al which is considered to be extraneous and poisonous metal. Al is common in nature in water, in air and plants and therefore in food. Many diseases like Alzheimer’s disease, Parkinson’s disease and renal osteodystrophy are considered to be consequence of this metal ion. Al is one of the interesting ion which due to the diversified metabolism of aluminium in KFP causes intoxication in them. Those KFP who were on hemodialysis for long period of time can be affected by Al contamination and this poisoning is perceived to be a contributing factor to encephalopathy syndrome, anemia and osteomalacia. It is acknowledged that long term utilization of Al containing factors can be probably pernicious to human. Therefore there is utmost requirement for Al control in natural water resources (Panhwar et al., 2015).

2. Methodology

The practical operation segment of this research work was comprised of:

- Blood samples collection of KDPs and referents who were residents of Quetta city.
- Blood samples collection of KDPs were carried out in Civil Hospital of Quetta city.
- Assessment of electrolyte (Na, K, Mg, Ca) concentration and their contribution in KDPs and referent’s body system, who were resident of Quetta city.
- Investigation of toxic elements (Pb & Al) exposure and analysis of their level in blood samples of KDPs and comparison with HRs.

2.1 Study Population

1. Dialysis patients and other kidney patients admitted were selected for collecting blood samples.
2. N = 30 KDP (male and female) with an age range of 25-60 years, admitted in nephrology department and Urology department of Civil Hospital Quetta, Pakistan were chosen as study patients.
3. While N = 30 healthy persons were selected as healthy referents who were relatives of study patients. All patients were permanent resident of Quetta Area especially residents of Quetta city for 30 years. Agreement of participants was retrieved orally and information regarding study objectives provided. The information obtained was given in questionnaire.
4. Good water treatment devices were installed in dialysis unit.
5. Age, geographical area (province Balochistan, Quetta area), socioeconomic status and occupation of healthy referents or control group were frequency-matched to cases.
6. Connection of hemodialysis patients & kidney stone patients with medication was not estimated in the present research work. As all patients were treated with same dialysate solution and on drug.

Table 1. Measurement conditions for atomic absorption spectrometer Perkin Elmer Model 700.

| Elements | Wave length (nm) | Slit width (nm) | n current (mA) | Oxidant (Air L min⁻¹) | Fuel (acetylene L min⁻¹) |
|----------|-----------------|-----------------|----------------|------------------------|-------------------------|
| Al       | 309.3           | 1.30            | 10.0           | 16.0                   | 7.80                    |
| Ca       | 422.7           | 0.70            | 30.0           | 17.0                   | 2.20                    |
| K        | 766.5           | 0.7             | 7.50           | 17.0                   | 2.00                    |
| Mg       | 285.2           | 0.7             | 7.50           | 17.0                   | 2.00                    |
| Na       | 589.0           | 0.2             | 10.0           | 17.0                   | 2.00                    |

- D₂ lamp used for background correction. Nitrous oxide as an oxidant.

2.2. Analytical procedures

Different water quality parameters, their units, abbreviations and methods of analysis applied in triplicate manner on each composite samples of water. In the field, we measured water pH, electrical conductivity (EC) and total dissolved solids (TDS) using thermometer, pH meter and conductivity meter, respectively (Lenore et al., 1998). Where, according to USEPA database the oral toxicity reference dose values (RfD) are 5.0E⁻⁰⁴ and 3.6E⁻⁰² mg/kg-day for Al, and Pb respectively (Forum, 2005). The exposed population is assumed to be safe when Hydro Quality HQ<1. Dilutions were prepared by using above standard solutions of elements Na, K, Ca, Mg, Pb and Al. After calibrating FAAS by these dilutions, all blood and water samples were analyzed for essential and toxic elements in these samples.

3. Results and Discussions

Figure 1: Graphical representation of important parameters in GW & MTW samples.

In the present study, we evaluated the effect of Ca and Mg intake via different types of drinking water (GW and MTW) in Quetta, Balochistan Pakistan, in adults (male and female). The MTW and GW were mostly used as the sole source of drinking water, cooking and personal hygiene in domestic and
rural areas, respectively. The GW samples collected from different locations of Quetta contained high concentrations of Ca exceeding the guideline level for drinking water (100 mg/L for Ca) (WHO, 2004).

The drinking water tested included a range of pH, electro-conductivity, TDS, Ca, Mg, Na, K, Carbonate and Bicarbonate from both types of water. The both types of drinking water used had statistically significant differences in levels of pH, electro conductivity, TDS, Ca, Mg, Na, K, Carbonate and Bicarbonate; however, all the studies water samples were consisted in terms of pH (Table 2).

pH of GW and MTW was 7.59 ± 0.61 and 7.21 ± 0.19 respectively which were according to the World Health Organization (WHO) values (6.5-8.5) and also according to the United States Environmental Protection Agency (USEPA) as well as according to the EU (1998). Same results were reported by (Mohod & Dhote., 2013 & Dkhar et al., 2014). The strength of acidity and alkalinity of water is estimated by the pH of water and has a powerful impact on the coagulants reaction with raw water. Concentrations of certain metals (NO₃ and PO₄) in treated water is controlled by most crucial water quality parameter known as pH (Dkhar et al., 2014).

TDS of MTW was according to WHO recommended value 1000mg/L (given by Ghoraba & Khan, 2013) but it was exceeded for GW having the value of (1530.2 ± 306.81). Similarly it was also observed that conductivity value was higher for GW as compare to MTW. Ions which are considered as charge carriers, if absent in distilled water then this water sample is considered theoretically insulator. Thus TDS value can be comparable with EC of water samples (Ghoraba & Khan, 2013). GW and MTW samples exhibited the greater value of SO₄ than the WHO recommended value. Despite the fact that SO₄ is not thought out to be a dominant pollutant, high concentration of SO₄ in drinking water can produce gastrointestinal effect. The existence of gypsum in the neighboring rocks of Quetta valley could be partly responsible to the SO₄ in GW of Quetta valley. According to some researchers, SO₄ concentration from 500-700mg/L in drinking water can be a reason of diarrhea (Khan et al., 2010).

3.1. Biochemical Parameters

Biochemical data concerning the KDP and referent groups obtained from the pathological laboratories of hospital are shown in Table 4.3. It can be seen from the table that lower level of hemoglobin in KDPs (9.36 ± 1.73) than in healthy referents (14.27 ± 2.08). Chronic kidney disease (CKD) exhibit common complexity of anemia because of the production of erythropoietin by kidney. In chronic renal failure (CRF), when kidney function is damaged or loss, RBC count decreases. Similarly it was observed that KDPs showed higher level of creatinine and BUN i.e. 4.25 ± 1.8 & 33.73 ± 7.13 respectively. It is happened because in CRF, Glomerular Filtration rate (GFR) reduces continuously and therefore Urea, creatinine and other chemicals in blood are gathered. It was also observed that creatinine clearance is lower in KDPs than referents and normal range.

**Table 2. Biochemical parameters of referents and Kidney disorder patients**

| Parameters               | Normal Range | Referents   | KDPs       |
|--------------------------|--------------|-------------|------------|
| Hemoglobin g/dL          | 13-19        | 14.27 ± 2.08| 9.36 ± 1.73|
| Creatinine mg/dL         | 0.5-1.4      | 0.99 ± 0.22 | 4.25 ± 1.8 |
| Blood Urea nitrogen mg/dL| 9-19         | 13.47 ± 2   | 33.73 ± 7.13|
| HDLP mg/dL               | 28-88        | 61.9 ± 6.4  | 85.63 ± 10.89|
| Creatinine clearance mL/min| 88-137       | 115.14 ± 10.15 | 62.47 ± 14.99|


3.2. **Comparative evaluation of essential and toxic elements in the blood of kidney disorder patients and healthy referents with water samples**

The average electrolytes and toxic elements concentrations in blood samples of kidney failure patients and healthy referents are shown in Table 2 & 3, respectively.

**Table 3:** Essential & toxic element concentration in blood samples of GW consumers (HR and KDPs)

| S.No. | Elements | Healthy Referents | Kidney disorder patients |
|-------|----------|-------------------|-------------------------|
|       |          | mg/L              | mg/L                    |
| 1     | Na       | 3479.33 ± 297.3   | 3537.2 ± 276.79         |
|       |          | (3018-3898)       | (3156-4094)             |
| 2     | K        | 215.89 ± 18.31    | 256.4 ± 30.63           |
|       |          | (189-235)         | (211-299)               |
| 3     | Ca       | 114.67 ± 4.6      | 131.3 ± 7.6             |
|       |          | (107-121)         | (118-140)               |
| 4     | Mg       | 23.97 ± 3.67      | 35.48 ± 9.69            |
|       |          | (17.6-28.1)       | (20.1-43.2)             |
| 5     | Al       | 241 ± 35.77       | 879.8 ±246.21           |
|       |          | (189-235)         | (586-1380)              |
| 6     | Pb       | 269 ± 39.27       | 421.1 ± 127.13          |
|       |          | (211-321)         | (278-645)               |
Figure 3: Bars showing Essential & toxic element concentration in blood samples of GW consumers (HR and KDPs)

Table 4: Essential & toxic element concentration in blood samples of MTW consumers (HR and KDPs)

| S.No. | Elements | Healthy Referents | Kidney disorder patients |
|-------|----------|-------------------|--------------------------|
| 1     | Na       | 3394.83 ± 377.65  | 3444.8 ± 512.52          |
|       |          | (2944-3810)       | (3000-4018)              |
| 2     | K        | 216 ± 18.47       | 262.2 ± 37.44            |
|       |          | (184-238)         | (205-300)                |
| 3     | Ca       | 94.33 ± 4.76      | 107 ± 4.47               |
|       |          | (88-100)          | (102-113)                |
| 4     | Mg       | 20.3 ± 2.37       | 30.28 ± 6.9              |
|       |          | (16.3-23.4)       | (21.5-40.9)              |
| 5     | Al       | 236.33 ± 64.03    | 834.8 ± 206.97           |
|       |          | (139-297)         | (496-1011)               |
| 6     | Pb       | 275 ± 57.94       | 407.6 ± 135.53           |
|       |          | (199-328)         | (261-621)                |
Figure 4: Essential & toxic element concentration in blood samples of MTW consumers (HR and KDPs)

3.3. Na Level in Blood Samples of KDPs and in Water samples:
As it was noticed that GW and MTW exhibited higher level of Na, even go beyond the level of WHO recommended value i.e. 200mg/L. Therefore those referents and KDPs who were ingesting GW exhibited higher average level of Na in blood samples. GW and MTW consumers (healthy referents and KDPs) exhibited mild hypernatremia (showed the Na level greater than 3082-3335mg/L).

3.4. K Level in Blood Samples of KDPs and in Water samples:
It was observed that the average concentration of potassium was higher in blood samples of GW consumers (referents and KDPs) and MTW consumers due to exhibiting the higher level of K than the permissible limit of K i.e. 126.5mg/L in blood samples (value given by Chakraborty et al., 2016) and show hyperkalemia. It was observed that MTW exhibit low K level than GW but level of K in KDPs consuming GW and KDPs consuming MTW has no significant difference. Same condition was observed in referent consumers of GW and MTW, they didn’t have significant difference in K level.

3.5. Ca level in Blood Samples of KDPs and in Water Samples:
In this research work, two types of water samples (GW and MTW) were examined for concentration of Ca. According to results obtained, level of Ca in GW and MTW higher than the permissible limit i.e. 100mg/L and 100mg/L (value given by Panhwar et al., 2015). And it was also observed that GW sample (510 ± 129.19) showed higher value of Ca than MTW samples (319 ± 109.09) mg/L. Ca in water exist as hydrated ions and consequently are more effortlessly captivated from water than from food. Thus main source of Ca ion is considered to be drinking water as compare to food. Various research work showed the formation of kidney stone can be influenced by increased Ca ingestion. Thus studies have exhibited that absorption of Ca in intestine increases than Ca is taken in lesser amount, there lesser amount of Ca is present in intestine to produce insoluble oxalate complexes.

3.6. Mg Level in Blood Samples of KDPs and in Water Samples:
In this research work, the level of Mg in GW samples and MTW samples were 50.9 ± 25.48 and 18.2 ± 4.52, which is within the permissible limit given by WHO i.e. 50mg/L. but it was observed that GW Mg concentration is higher than MTW Mg level. Likewise, level of Mg also determined in HR and KDPs who were utilizing GW and MTW for drinking purposes. Results showed that level of Mg in blood samples of GW consumers (HR) and MTW consumers (both HR and KDPs) were within the normal range of Mg level i.e. 18-30 mg/L (value given by Kanbay et al., 2010). Due to hypermagnesemia, secretion of PTH is prevented which is contemplated as important agent for vascular calcification and left ventricular hypertrophy and mortality in ESRD patients. From above results and discussion it was observed that there is no notable association between concentration of Mg in water and level of Mg in blood samples of HR and KDPs.

3.7. Pb Level in Blood Samples of Referents and KDPs and Water Samples:
In research study, it was observed that water samples lack the Pb toxic element in both GW and MTW samples. But it was noticed that HR and KDPs (consumer of both type of water samples) exhibited certain level of Pb in their blood samples. It was observed that there was no significant difference in level of Pb in their blood samples of HRs (consumer of MTW and GW) and same condition was observed for KDPs (consumer of both MTW and GW). According to World Health Organization’s Regional Office of Europe, recently suggested that blood lead should be below 200 ug/L in 98% of adult population. According to another study, in whole blood >100 ug/L for Pb is considered to be a normal range. While when Pb level reach between 100-140 ug/L, concerned and consideration is needed (Kazi et al., 2008). It was observed that all four groups showed Pb level higher than the permissible limit. But is also noticed that KDPs showed two times higher Pb level in their blood sample than HRs. Our results were consistent with Kazi et al., 2008.
3.8. **Al in Blood Samples of KDPs and Water Samples:**

Al was not found in any GW sample and MTW samples. But consumers (HRs and KDPs) showed higher level of Al in their blood samples. It was examined that blood samples of HR and KDPs showed the level of Al greater than permissible limit i.e. 10ug/L (value given by Długaszek et al., 2009). And it was also observed that CKD patients exhibited large concentration of Al in their blood samples as compare to the HRs but HRs group (consumer of GW and MTW) and KDPs (consumer of GW and MTW) exhibited almost equal level of Al in their blood samples.

The main reason for the high level of Al was that most of the food products (juice, soft drinks, bakery products, bread, and rice) have Al in large amount. Al absorption in gastrointestinal is usually hindered by the digestive tract and large amount of Al which is consumed is eliminated in the feces is in unabsorbed condition and little amount which is absorbed, readily eliminated in the urine. However little bit is retained in the skeleton. Although protective operation which is performed by intestinal barrier, is less compelling in ESRD patients as compare to healthy referent.

3.9. **Questionnaire Analysis:**

From questionnaire, following information were gathered:

- 60% respondents were male and 40% were female. Same percentage was considered for sampling of healthy referents.
- 50% respondents were GW consumers and 50% were MTW consumer
- 100% KDPs were non-smokers. Same condition was observed for healthy referents
- 25% respondents were educated, while 75% uneducated in fact unable to speak Urdu.
- Most of the respondents were belong to poor or lower middle class family.
- All KDPs were restricted to take less salt in their dietary food.
- About 75% KDPs were also the patients of high blood pressure and 25% were only KDPs.
- All those patients who were diagnosed other diseases like Hepatitis, Diabetes, Cardiovascular diseases were excluded from the analysis.
- All KDPs were prohibited to take proteinaceous diet daily.

4. **Conclusion**

The present study reveals that the prevalence of kidney disorder was high among population who consumed underground hard water as compared to those who drink domestically treated l water. The conclusion is based on the correlation of kidney disorders and electrolyte and toxic element levels in two types of drinking water and biological samples of kidney disorder patients and healthy referent subjects. It is clear from present research work that only Ca is that element which is present in large amount in water samples of Quetta Area and contributed as one of the important factor for raising the Ca level in biological samples of KDPs and KDPs showing abnormal level of Ca element in their biological samples is due to the usage of unsafe water containing this element in excess amount.

Concentration of toxic metals in blood of kidney disorder patients was significantly higher than in the referents. The adverse impact of both toxic elements produces nephrotoxicity in humans, as indicated by increased N-acetyl beta glucosaminidase (NAG) values and decreased clearance of urea creatinine in patients live in different areas of Quetta and consumed ground water as compared to those kidney patients consumed domestically treated water. The correlation study indicated the balance of essential elements, such as Ca, Mg, and Na, had been disturbed by toxic elements in the KDP. Further studies are needed, possibly including additional new biomarkers and improved exposure assessment technology, to firmly establish this interaction.

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