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Evaluation of Calcium and Magnesium in Scalp Hair Samples of Population Consuming Different Drinking Water: Risk of Kidney Stone

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Abstract The objective of this study was to examine the relationship between calcium (Ca) and magnesium (Mg) in underground water (UGW), bottled mineral water (BMW), and domestic treated water (DTW) with related to risk of kidney stones. The water samples were collected from different areas of Sindh, Pakistan. The scalp hair samples of both genders, age ranged 30–60 years, consuming different types of water, have or have not kidney disorders, were selected. The Ca and Mg concentrations were determined in scalp hair of study subjects and water by flame atomic absorption spectroscopy. The Ca and Mg contents in different types of drinking water, UGW, DTW, and BMW, were found in the range of 79.1–466, 23.7–140, and 45–270 mg/L and 4.43–125, 5.23–39.6, and 7.16–51.3 mg/L, respectively. It was observed that Ca concentration in the scalp hair samples of kidney stone patients consuming different types of drinking water was found to be higher (2,895–4721 μg/g) while Mg level (84.3–101 μg/g) was lower as compare to referents subjects (2,490–2,730 μg/g for Ca, 107–128 μg/g for Mg) in both genders. The positive correlation was found between Ca and Mg levels in water with related to kidney stone formations in population, especially who consumed underground water. A relative risk and odd ratio were calculated; the relative risk had a strong positive association with incidence of kidney stone which depends on types of drinking water.

Keywords Calcium · Magnesium · Scalp hair · Kidney stone patients · Drinking water

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Introduction

The prevalence of kidney disorder is increasing all over the world [1, 2]. The perception that water sources may contribute to urinary stone formation is common. The mineral and electrolyte content of water varies dramatically throughout the world depending on the water source such as underground water (UGW), bottled mineral water (BMW), and domestic treated water (DTW) [3].

The role of magnesium (Mg) in the formation of calcium urolithiasis in human is unknown. The Mg appears to be a potent stone inhibitor in vitro and in animal studies [4–7]. The commonest type of stones contains calcium (Ca) in combination with either oxalate or phosphate with a small amount of protein [8]. The calcium oxalate and calcium phosphate make up at least 80 % of all kidney stones [7]. There is some evidence that increasing Ca and Mg in water may have a positive effect on stone formation. An increase in drinking water hardness results in enhancement of kidney stone incidence [9]. Another study suggests that drinking soft water increases ion activity and decreases crystal precipitation, which implies an inhibitory effect on stone formation [10]. By contrast, many studies have come out showing that water with increased Ca and Mg, i.e., mineral water, has beneficial effects on preventing stone events by different mechanisms, including excretion of citrate [11]. In developing countries, most of the people do not have access to safe drinking water. Intermittent water supply, insufficient chlorination, and sewage flooding seem to be associated with self-reported diseases [12]. Being a developing country, Pakistan needs to address a vast array of problems regarding water availability, quality, usage, and death caused by water-borne diseases. The low concentration of some trace elements in environmental and biological samples demands a technique with high sensitivity. Flame atomic absorption spectrometry is usually the method of choice, and numerous reports testify to this observation [12, 13].

The purpose of this study was to investigate the level of Ca and Mg in different types of drinking water (underground, domestically treated, and bottled mineral water) and its correlation with incidence of kidney stone in population of Sindh, Pakistan. Questionnaire survey was also conducted among the people residents of rural and urban areas to collect information about the drinking water sources and prevalence of kidney stone.

Study Population

This study involved 145 male as well as female patients who have kidney stone, age ranged of 30–60 years, admitted in the nephrology ward of Liaquat Medical University Hospital, Hyderabad, Pakistan, during 2011–2012. The prevalence of kidney stone was more in males of all three groups; among patients, 75 % (108) were males and 25 % (37) were females. The kidney stones in patients were diagnosed and detected by X-ray and ultrasound reports). The patients were divided according to consumption of drinking water such as underground, domestic treated, and mineral water. One hundred fifty age-matched healthy subjects who were receiving physical checkup were recruited as the referent group (relatives of patients) from rural and domestic areas of Sindh. The referent group had no history of kidney stone, and screening of these patients was done using ultrasound and X-ray reports. We obtained consent verbally and provided information on study objectives, procedures, and implications to participants. The information obtained were drinking water; demographic characteristics; environmental and occupational data; past or present history of smoking, diabetes, and hypertension; and duration and number of renal calculi formed. At the start of study, the participants' weight, height, and blood pressure were noted.

Apparatus

The analysis of elements was carried out by means of a double beam Perkin-Elmer atomic absorption spectrometer model 700 (Perkin Elmer, Norwalk, CT, USA) equipped with a flame burner HGA-400 (Perkin Elmer). The instrumental parameters were set according to manufacturer guideline. The Ca and Mg were measured under optimized operating conditions using FAAS with an air–acetylene flame. Signals were measured as absorbance peaks in the flame absorption mode. A Pel (PMO23, Osaka, Japan) domestic microwave oven (maximum heating power of 900 W) was used for digestion of the biological samples. Acid-washed polytetrafluoroethylene (PTFE) vessels (Kartell, Milan, Italy) and flasks were used for preparing and storing solutions.

Reagents and Glasswares

Ultrapure water obtained from an ELGA LabWater system (ELGA, Bucks, UK) was used throughout the work. Concentrated nitric acid (65 %) and hydrogen peroxide (30 %) were obtained from Merck (Darmstadt, Germany) and checked for possible trace metal contamination. Working standard solutions of Ca and Mg were prepared immediately before their use by stepwise dilution of certified standard solution (1,000 ppm), Fluka Kamica (Buchs, Switzerland), with 0.2 M HNO_3.

Water Sampling

A total of 150 tap water samples were sampled from private houses or public places all over the Hyderabad City between 2011 and 2012. The tap water was allowed to run for 10 min, and about 1,000 ml of water was collected in a beaker. Groundwater samples were collected on monthly basis, from four towns of two sub-districts of Naushahro Feroze with the help of Global Positioning System during 2012. All groundwater samples were collected from >40 ft depth. The groundwater samples were collected from hand pumps and motor pumps directly with polyethylene plastic bags. The collected water samples were kept in well stopper polyethylene plastic bottles previously soaked in 10 % nitric acid for 24 h and rinsed with ultrapure water.

The containers were rinsed several times with water flushed from the distributing system for at least 2 min, preferably at the end of the day. This procedure should minimize the effect of interaction between the local casing system and water, which is strong if limited flushing occurs. No filtration was performed during sampling. Twenty BMW samples of different brands packed on four different dates (five samples of each brand) were collected from local market of Pakistan. On arrival to laboratory, the BMW was stored at −4 °C until further analysis. All water samples were filtered through a 0.45-mm pore size membrane filter (Millipore Corporation, Bedford, MA, USA).

The pH of all water samples was checked with a pH meter, and then the pH was adjusted to 2 with 0.1 M HNO_3 and subjected to analysis as described in our previous work [13].

Hair Sampling

The hair samples were obtained using stainless steel scissors from the nape of neck. The hair samples were cut into approximately 0.5 cm pieces in length and mixed to make a representative hair sample. Collected hair samples were sequentially washed with tap water, distilled water, 1 % neutral detergents (Triton X-100; Sigma, USA), deionized water, and acetone to remove surface contamination. The wet hair samples were placed in a cleaned beaker and then dried in an oven at 40–50 °C for 2 h. Each hair sample was stored in labeled plastic bottles. A file of complete information and all of the demographical data on each contributor was made.

Microwave-Assisted Acid Digestion Method

Duplicate samples of dried scalp hair samples of each patients and referents subjects were directly taken into Teflon PTFE flasks. Two milliliters of a freshly prepared mixture of concentrated HNO_3–H_2O_2 (2:1, v/v) was added to each flask and kept for 10 min at room temperature and then placed the flasks in covered PTFE container. This was then heated following a one-stage digestion program at 80 % of total power (900 W), and 5–8 min was required for complete oxidation of hair matrix. After cooling, the digestion flasks were cooled and resulting solution was evaporated to semidried mass to remove excess acid and then diluted to 10.0 ml in volumetric flasks with 0.1 M nitric acid. Duplicate blanks (without sample) were carried through the complete procedure. The concentrations were obtained directly from calibration graphs after correction of the absorbance for the signal from an appropriate reagent blank. The validity and efficiency of the microwave-assisted digestion method were checked with certified sample of hair (BCR397) and with those obtained from conventional wet acid digestion method mention elsewhere(Table 1) [14]. Microwave digestion method required less time as well as low amount of acid mixture than conventional digestion method.

Water quality parameters, their units, and methods of analysis are summarized in Table 2. The pH, electrical conductivity (EC), total dissolved solids (TDS), and salinity of each water sample were measured at the sampling points by digital pH and EC meters, respectively. Total hardness and Ca hardness were measured by EDTA complexometric titration, with the indicators being Eriochrome Black T and Murexide at pH 10 and 12, respectively [15].

Statistical Evaluation

Statistical analyses were performed using computer program Excel XL State (Microsoft Corp., Redmond, WA) and Minitab 15.2 (Minitab Inc., State College, PA). The differences between concentrations of metals recorded in the biological samples of kidney stones and normal subjects of both genders were calculated by unpaired (two samples) t test. Association between Ca and Mg levels with kidney stone patients was assessed using univariate logistic regression analysis.

Analytical Figure of Merit

The linear range of calibration curve reached from the detection limit up to 2 mg/L for Ca and Mg. The limit of detection (LOD) was defined as $3s/m$, where s is the standard deviation corresponding to 10 blank injections and m is the slope of the calibration graph. The LOD of 164 and 2.46 μg/L were calculated for Ca and Mg, respectively. The validity and efficiency of the microwave-assisted digestion method were also checked in CRM BCR 397 (Table1). The precision of the microwave-assisted acid digestion was expressed as the percent of coefficient of variation (%CV) calculated as <5 %.

Results

The goal of this study was to examine the effects of Ca and Mg in different types of drinking water such as ground, domestic treated, and mineral water on kidney stone risk assessment. The pH of water samples was found within the recommended value of WHO [16].

The Ca and Mg concentrations in domestic treated water, underground water, bottled mineral waters, and scalp hair samples are shown in Tables 3and 5, respectively. The Ca concentration in the UGW, DTW, and BMW were observed in the range of 79.1–466, 23.7–140, and 45–270 mg/L, while Mg concentrations were ranged as 4.43–39.6, 4.43–125, and 7.16–51.3 mg/L (Table 3).

Biochemical data concerning the kidney stone patients and referent groups obtained from the pathological laboratories of

Table 1 Trace elements concentration in certified sample of human hair (CRM) by conventional (CDM) and microwave digestion method (MDM) ($n = 10$)

Element	Conventional digestion method (CDM)	Microwave digestion method (MWD)	T value[a]	% recovery[b]	Certified values
Ca	1,550.6±55.3 (3.56)	1,546.0±45.6 (2.95)	0.889	99.7	1,560.0±40[c]
Mg	199.0±13.5 (6.79)	198.2±12.3 (6.2)	0.838	99.6	200±5[c]

[a] Paired t test between CDM and MWD, DF=9, T (critical) at 95 % CI=2.262, p=0.05, means in percentage, values in parentheses are RSD

[b] % recovery was calculated according to [MDM]×100/[CDM]

[c] Informative value

hospital are shown in Table 4. In kidney stone patients, the levels of hemoglobin, creatinine, creatinine clearance, and blood urea nitrogen were higher than control group ($p<0.05$), while the glucose was much lower than referent group. The mean creatinine clearance in urine samples of the referent group was observed as 29.7±1.56 mL/min; however, the creatinine clearance was significantly increased in kidney stone patients (33.2±3.21 mL/min). Blood urea nitrogen in kidney stone patients (22.4±2.4) was higher than referent subjects at $p> 0.05$. The blood creatinine level in kidney stone patients (1.8±0.42 mg/dL) was higher than referent groups. Glucose random (94.2±6.21 mg/dL) in blood samples of kidney stone patients was significantly lower than referents at $p< 0.05$.

The concentrations of Ca and Mg in the scalp hair samples of referents and kidney stone patients are shown in Table 5. The concentration of Ca in scalp hair samples of males having kidney stone was correlated with the levels of both elements in drinking water. The levels of Ca in kidney patients consuming underground water had significantly higher levels at 95 % confidence interval [CI 4,554, 4,642], as compared to those patient drinking DTW and BMW [CI 3,660, 3,721] and [CI 3, 182, 3,270] μg/L, respectively (p=0.008–0.001).The levels of Ca in scalp hair of referents consuming UGW, DTW, and BMW were found as [CI 2,615, 2,741], [CI 2,462, 2,546], and [CI 2,496, 2,578] μg/L, respectively. It was observed that the level of Ca was significantly higher in those referents who consumed UGW as compared to other two groups ($p<0.05$). The same trend was observed for patients and referent females.

The referent males and females who consumed UGW, DTW, and BMW had higher Mg concentration in the scalp hair samples at 95 % {[CI 123.0, 131.7], [CI 118.2, 124.6]}, {[CI 104.1, 123.8], [CI 117.5, 145.1}, and {[CI 111.8, 125.0], [131.8, 152.2]} μg/g, respectively. While, the level of Mg in scalp hair samples of male and female kidney stone patients who consumed UGW, DTW, and BMW was significantly lower at 95 % confidence interval{[CI 83.30, 95.13], [CI 85.8, 96.28]}, {[CI 87.2, 99.2], [CI 96.21, 103.5]}, and {[CI 86.35, 96.01], [CI 83.51, 91.98]} μg/g, respectively.

The logistic regression analysis indicates the higher relative risk for incidence of kidney stone formation in those people who drink underground water as compared to those who drink domestic treated water and bottle mineral water. The results show that the prevalence of kidney stone formation in studied population who consumed UGW, DTW, and BMW water were 50, 20, and 25 %, respectively (Table 5). The odds ratio showed a 4.0-fold (95 % CI, 2.13 to 7.49) higher incidence of kidney stone formation in those who consumed UGW as compared to those who drink DTW. The odds ratio of kidney stone patient drinking UGW with respect to BMW was found to be 3.0-fold higher (95 % CI, 1.64 to 5.46), after adjusting for age and gender (Table 6). The relative risk of prevalence of kidney stone is 1.86 (CI, 1.43 to 2.42) in those who consumed UGW water with respect to those who consumed DTW and BMW which is 1.67 (CI, 1.42 to 2.22) (Table 6). Regression

Table 2 The statistical data of the various parameters of the ground water (GW), mineral water (MW), and domestic treated water (DTW)

Parameter	WHO limits	GW	MW	DTW
pH	6.5–8.5	7.12±0.14 (6.9–7.5)	6.79±0.41 (6.3–7.3)	6.9±0.32 (6.6–7.8)
EC (μS/cm)	2,500	1,895±1,002 (597–3,616)	363±98 (151–538)	550±110 (250–1,050)
Salinity (mg/L)	–	0.81±0.3 (0.5–1.1)	0	0.5±0.2 (0.2–0.7)
TDS (mg/L)	1,000	1,300±75.2 (1,072–1,628)	178±81 (69–450)	350±35 (250–550)
Total hardness (mg/L)	500	493±32.4 (410–610)	175±33 (69–450)	250±25 (65–300)
Ca-hardness (mg/L)	100	235±15 (195–420)	56±5.2 (10.0–95)	180±13 (65–300)
Mg-hardness (mg/L)	50	158±36 (80–300)	25±19 (5.0–75)	110±15 (60–150)

Table 3 Calcium and magnesium concentrations in the domestic treated water, underground water, and bottled mineral waters from study areas

Types of water	Calcium	Magnesium
Underground water (n=20)	281±126 (79.1–466)	56.7±33.5 (4.43–125)
Domestic treated water (n=20)	84.5±37.7 (23.7–140)	23.0±10.1 (5.23–39.6)
Bottled mineral water (n=20)	157±58.7 (45–270)	22.5±10.4 (7.16–51.3)

analyses have been carried out between the Ca and Mg concentrations in water and in scalp hair samples of the studied subjects. The linear regressions showed Ca and Mg concentrations in water versus scalp hair (R^2=0.862, 0.629, p<0.001).

Discussion

In the present study, we evaluated the effect of Ca and Mg intake via different types of drinking water (UGW, DTW, and BMW) in rural and urban areas of Sindh, Pakistan, in adults (male and female). The DTW and UGW were mostly used as the sole source of drinking water, cooking, and personal hygiene in domestic and rural areas, respectively. The UGW samples collected from rural area contained high concentrations of Ca and Mg, exceeding the guideline level for drinking water (100 mg/L for Ca and 50 mg/L for Mg) [16]. BMW production is distributed all over the main cities of Pakistan, and its sampling has covered important productive areas.

The drinking water tested included a range of total hardness, electric conductivity, TDS, salinity, Ca-hardness, and Mg-hardness from all types of water. The different types of drinking water used had statistically significant differences in levels of total hardness, electric conductivity, TDS, salinity, Ca-hardness, and Mg-hardness; however, all the studies water samples were consisted in terms of pH (Table 2).

Table 4 Characteristics of kidney stone patients and referent subjects

Parameters	Normal range	Healthy controls	Kidney stones patients
Hemoglobin (g/dL)	13–19	11.57±0.63	10.9±0.52
Creatinine (mg/dL)	0.5–1.4	0.93±0.054	1.8±0.42
Blood urea nitrogen (mg/dL)	9–19	12.34±1.23	22.4±2.4
High-density lipoprotein (mg/dL)	28–88	50.5±4.12	95.21±6.4
Creatinine clearance (mL/min)	27–32.5	29.7±1.56	33.2±3.21
Glucose (random)	160–180	170±8.5	94.2±6.21
Sodium (M eq/L)	135–145	136±6.86	155±8.23
Potassium (M eq/L)	3.7–5.2	4.7±0.69	6.24±0.24
Uric acid (mg/dL)	3.5–7.2	3.7±0.42	10.2±0.82
Phosphorus (mg/dL)	2.4–4.1	3.2±0.39	5.21±0.32

It was reported in literature that BMW may have sometimes high levels of hardness, used as single source for drinking purposes [17]. In the present study, the villagers consumed UGW, as well as consumed the same types of diet and drinking water. So, the kidney stone patients and referents in these areas were exposed to identical environmental conditions, while the population who consumed DTW and BMW with related to their eating and socioeconomic data were not studied. So here, we only considered the incidence of kidney stone in different patient with related to referent (relatives of patients). The frequency of stone formation was found to be significantly higher (two to three times) in the patients belonging to rural area who consumed UGW as compared to the patients who drink DTW and BMW.

Among the study population, the rate of kidney stone formation was more in males as compared to females of three groups who consumed different type of drinking water, and this is consistent with reported results [18]. The Ca and Mg in water appear as hydrated ions and therefore are more easily absorbed from water than food [19, 20]. Thus, drinking water would serve as a more readily available source for Ca and Mg ions as compared to food. Our study indicates a possible link between Ca and Mg intake via drinking water for kidney stone formation. The results show that there is a significant positive association between Ca and Mg levels in drinking water and mortality risk attributed to kidney stone formation in patients.

A majority of understudy people do not obtain the recommended amount of Ca and Mg from their diets. The relative contribution of water Ca would be even more important for renal stone formation patients as these individuals tend to have lower dietary Ca intake compared to referents [21]. Despite decreased Ca intake, most renal stone patients have positive Ca balances due to decreased renal excretion and the resultant whole-body Ca retention [21–24]. Several other studies have debated the potential effects of increased Ca intake on kidney stone formation. These studies have shown that low Ca intake increases the intestinal absorption of Ca, thus decreasing the amount of Ca available in the intestinal tract to form insoluble complexes with oxalate [25]. Consequently, a higher amount of oxalate is available for intestinal absorption, and as a result, urinary oxalate excretion increases [26–29]. This would mean that little or no oxalate was available in the intestinal tract to bind to the Ca from the drinking water as shown in prior studies [30, 31]. However, hyperoxaluria has been shown to be one of the most important risk factors for Ca stone formation [32]. The relation between Mg content of drinking water and urolithiasis incidence is not well clear in epidemiological

Table 5 Calcium and magnesium concentrations in scalp hair of kidney stone patients, age group 30–60 years

Underground water		Domestic treated water		Mineral water	
50 %		25 %		20 %	
Male					
Referents	Kidney stones patient	Referents	Kidney stones patients	Referents	Kidney stones patients
2,690±256	4,590±160	2,670±110	3,690±112	2,560±120	3,240±130
110±13	87.5±7.2	120±15.3	95±6.2	115±12	92±5.35
Female					
2,580±170	4,460±275	2,560±128	3,525±108	2,530±115	3,120±225
125±15	92.5±7.2	130±14.7	98±4.56	112±5.28	88.5±4.2

studies. Many epidemiological studies focused only on the total hardness yet not the individual water Mg impact [33–36]. Others did not observe any significant role for Mg in the urinary calculus incidence [31, 37–39].

It was also reported that to prevent the incidence of Ca nephrolithiasis, the intake of soft water is may be preferable to hard water since it was associated with a lower risk for recurrence of Ca stones [31]. Our study has shown an inverse correlation between the Mg levels of the studied drinking water, UGW, DTW, and BMW, and the rate of kidney stone formation in different population, which is also consistent with other study which indicated that low levels of Mg are encountered in stone formation [40]. Mg/creatinine ratio serves as an indicator for insufficient Mg intake. Intestinal malabsorption (including low dietary Mg) or renal losses cause hypomagnesaemia.

The interplay between Mg and Ca is complex and crucially influences Ca homeostasis. Hypomagnesaemia is a relatively common and often overlooked cause of ion disturbances, such as hypocalcaemia and hypokalemia. Although its causes are diverse, if chronic, it can induce changes in the parathyroid hormone functions and Ca regulatory axis [41]. The absorption and metabolism of Ca and Mg are of mutual dependence, and therefore, the balance between these two minerals is especially important. If Ca consumption is high, Mg intake needs to be high also [42]. It was reported in study that the water Ca or Mg levels alone had an effect on the incidence of urolithiasis, while the Mg-to-Ca ratio had also importance in kidney disorders. It was also investigated that the lower Mg-to-Ca ratio to be associated with a higher risk for urolithiasis regardless of type and its incidence to correlate with the type of geological subsoil [43], and another study found correlation between the higher Mg-to-Ca ratio and higher incidence of infectious phosphate urolithiasis [44]. It has been demonstrated by some authors that imbalance between Ca and Mg in hair samples may results in kidney disorders [45]. It was also reported that Mg iron may act as inhibitors of calcium oxalate growth [46]. The body uses Mg and vitamin B6 to convert calcium oxalate into magnesium oxalate—a soluble compound.

Table 6 Odd ratios, relative risk, and confidence interval of kidney stone occurrence in consuming different drinking water

Water samples	Odd ratio	Relative risk	95 % confidence interval	
GW to MW	3.00	1.67	1.64 to 5.46	1.42 to 2.22
	<0.001	$P<0.001$		
	4.00	1.86	2.13 to 7.49	1.43 to 2.42
GW to DTW	<0.001	<0.001		

There is a need of the existence and implementation of strict laws with no compromise on quality of public drinking water for rural and urban populations. Public awareness campaigns should be launched to educate the population about the importance of safe drinking water. The public should receive guidance to adapt safety measures for stored water inside the houses.

Conclusion

The present study reveals that the prevalence of kidney stone formation was high among population who consumed underground hard water as compared to those who drink domestically treated and bottled mineral water. Our results support the previous reported data that disturbed ratio of Ca and Mg (lower value of Mg) may increase the kidney stone formation as compared to those subjects who used DTW and BMW, which have proper ratio of Ca to Mg (2:1). Underground water source in Pakistan is not safe for human consumption as most of the pollutants exceed the quality standards for drinking water. There is a lack of proper monitoring of water quality particularly in rural areas. However, comparatively little data are available regarding water-related physiological disorders due to the lack of diagnostic facilities and maintenance of records in Pakistan.

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