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Original article

The concentration of selected ions in bottled, commercial zamzam, and household water in Riyadh city and its effect on bone mineral content in growing rabbits

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Summary. *Objective*: Drinking safe water is an internationally accepted human right. This objective of this study was to evaluate some of the minerals responsible for bone health in various types of water and to assess the effect of bottled water, commercial zamzam water, and tap water on bone mineral content (Ca, Mg, and Zn) in growing rabbits, as well as to identify the association between the water mineral content and bone mineral content of rabbits that had consumed various water types. *Method*: Samples were collected locally. Water samples were analyzed directly by the inductively coupled plasma spectrometry (ICPS), while bone (femur) samples were digested in a microwave digestion system for 30 min and then aspirated into inductively coupled plasma mass spectrometer. *Result:* Highest concentration of calcium was observed in commercial zamzam water followed by bottled and tap water and the zinc concentration was highest in tap water followed by commercial zamzam and bottled water. Highest concentration of magnesium was found in one of the bottled water (B3) followed by commercial zamzam, than other bottled water and least in tap water. Statistically significant differences in mineral content were observed between the various water types ($p \leq 0.05$). All variety of water except zamzam provides magnesium and calcium in an amount of less than 10% of the amount recommended for children and in older people, these products supply even lower levels of the DRI of this component. Commercial zamzam and tap water provides high percentage of zinc. In bone (femur) concentration of calcium was highest in commercial zamzam water and least in bottled water. We detected significant differences when comparing the calcium content of commercial zamzam water and the other tested types ($p \leq 0.05$). But the differences in the mean concentration of magnesium and zinc were statistically insignificant ($p \geq 0.05$) between all varieties. A strong and highly positive correlation ($p \leq 0.05$) was observed between bone and water calcium ($R^2=0.9286$) and slight positive correlation was observed between bone and water magnesium ($R^2=0.2576$). *Conclusion*: This study inferred that the concentration of all analyzed elements was within the guidelines set by various agencies. A large variation in various mineral concentrations has been observed in bottled water. Zamzam water provides essential minerals required for bone health. This study will help in providing more focused design of further research.

Key words: mineral content; calcium, magnesium, zinc, bone, DRI

Introduction

Drinking safe water is an internationally accepted human right (1), and it is an elementary requirement for healthy living. The choice of water for consumption can vary, but it must be "clear, tasteless, odorless, colorless and absence of any harmful and pathogenic microorganisms and chemicals dangerous to humans" (2). Due to ostensible risks of tap water and the favored taste, and the convenience and the safety of bottled water (BW) (3), in the past decade there has been an enormous increase in the consumption of

BW (4). This surge in demand has produced one of the most vibrant sectors in the food and beverage industries (5). BW is defined as “water that is intended for human consumption, and that is sealed in bottles or other containers with no added ingredients except that it may potentially contain safe and suitable antimicrobial agents” (6). BW has turn out to be the main types of drinking water, second only to tap water; so, assessment of its safety and impact on health is now necessary (7,8).

In adults, approximately 60% of the total body weight is composed of water, which is distributed into intracellular and extracellular fluid compartments (9, 10). In the circulatory system, water functions as a carrier of nutrients and various other substances (2). Water is an essential source of mineral elements for human skeletal health (11). Although numerous chemicals might occur in drinking water, only a few of them are significant (12). Various metal ions, such as magnesium, calcium, and sodium, are important for the proper growth, development, and functioning of the human body as they comprise the material for bones, regulate the water-electrolyte balance and affect the course of metabolic processes (13). Certain trace elements (e.g., zinc) are also essential for optimal growth, development, and reproduction (12). Among the minerals found in BW, emphasis has been given to calcium and magnesium (14) because these mineral have a beneficial effect on bone metabolism. Various epidemiological studies have shown that the dietary intake of calcium is far below its recommended amount (12) and Ca-rich mineral waters are a substitute for dairy products (2). This study aims to evaluate some of the minerals responsible for bone health in various types of water and to assess the effect of BW, commercial zamzam water, and tap water on bone mineral content (Ca, Mg, and Zn) in growing rabbits, as well as to identify the association between the water mineral content and bone mineral content of rabbits that had consumed various water types.

Materials and methods

Sample Collection and Preparation

BW of different manufacturing dates and commercial zamzam water were purchased from the local shops in Riyadh. BW samples were given a code, and analytical grade chemicals and reagents were used. The bottles used for collecting samples were soaked in 0.5% nitric acid overnight followed by washing with household water and rinsing with deionized water.

Animals and Care

Eighteen weanlings New Zealand white rabbits (6 weeks old, weighing 700–1300 g) were used in this study. Animals were obtained from the Experimental Animal Care and Experimental Surgery Center at the Faculty of Medicine, King Saud University, Saudi Arabia. This study is in accordance with the Animal Ethics Committee of the College of Science, King Saud University. The rabbits were randomly divided by weight into six groups (three in each group) and housed individually in stainless steel cages under controlled temperature (25 ± 2°C) and relative humidity (50 ± 5%), with a 12-h light/dark cycle.

Diets formulation and preparation

The Experimental Animal Care and Experimental Surgery Center at the Faculty of Medicine; King Saud University, Saudi Arabia provided the basal diet. Each rabbit was fed 500 g of basal diet and 500 ml of water (according to treatment group) every alternate day. Food cups were refilled every 2 days, and food provided, and the remaining food was weighed to calculate daily food consumption.

Collection of blood

At the end of the experiment, on the 100th day, animals were food deprived overnight and anesthetized under chloroform. Blood was collected from the retro-orbital plexus in the heparinized tube and centrifuged at 3500 rpm for 15 min for plasma separation and stored at 5-7ºC for further analysis.

Determination of Ca, Mg, and Zinc in various types of water and rabbit's bone by inductively coupled plasma spectrometry (ICPS)

A small portion of the samples was separated and acidified in 0.5% nitric acid for the analysis of Ca, Mg, and Zn in bottled, commercial zamzam, and tap water by ICPS. Samples were warmed at ambient temperature before analysis and then aspirated directly into ICPS.

Bones (femur) were obtained as previously described by Dekel *et al.* (15). Briefly, the femur was excised and carefully freed of soft tissue by gentle scraping with a scalpel, rinsed with normal saline (0.9% NaCl). Then, the femur was dried using a lint-free paper towel, and stored in a plastic container at −20°C until elicitation of bone marrow. All procedures were accepted by the Experimental Animal Care and Experimental Surgery Center at the Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia. The bones were thawed at room temperature; both bone epiphyses were removed using a fine saw, an incision was made along the bone using a sharp non-serrated knife, bone marrow was removed and weighed to the nearest 0.0001 g (AL204, Mettler-Toledo Inc., Shanghai). Finally, bone samples were again rinsed with normal saline, dried using a lint-free paper towel, and then stored in a plastic tube at −20°C until used for minerals analysis.

To determine calcium (Ca), magnesium (Mg), and zinc (Zn) contents, femur samples (300 mg) were digested in 6 ml of concentrated nitric acid (HNO3) and 2 ml hydrogen peroxide (H_2O_2) for 30 min in a microwave digestion system (START D, Milestone, Italy). Ca, Mg, and Zn concentrations were measured using an inductively coupled plasma mass spectrometer (ICP-MS 7500A, Agilent Technologies, Inc., Santa Clara, CA, USA).

Statistical Analysis

The data are presented as the average of replicates ± SD. The data of water and bone mineral content was subjected to statistical analysis by analyzing variance (ANOVA), using the SPSS software package (version 9.0). The significant differences identified using Turkey HSD tests, and p-values of < 0.05 were considered significant.

Results and discussion

Elemental analysis of water

Water consumption is related to most diseases that cause morbidity and mortality in humans, so water quality is an important aspect of human life (16). Water is a significant source of mineral intake because the gastrointestinal tract easily absorbs the minerals in water as they are available in an ionic form (17). Elemental analysis of water and bone was carried out using ICPS. Table 1 shows the mineral content of the water types given to the rabbits. The concentration of calcium was highest in commercial zamzam water, followed by bottled and tap waters, and the concentration of zinc was highest in tap water, followed by commercial zamzam and BW. The concentration of magnesium was highest in one of the BW (B3), followed by commercial zamzam, then other BWs, with the least in tap water. Statistically significant differences in mineral content were observed between the various water types ($p \leq 0.05$). All analyzed elements were in agreement with the maximum contaminants limits set by the regulatory agencies (18, 19, 20). In a previous study, compared to bottled and tap (municipal), relatively higher concentrations of anion and cations were reported in zamzam water (6). Doha *et al.* (21) reported previously that commercial zamzam water has a rich mineral profile, which is in accordance with the results obtained in this study. However, unlike this study, the previous study reported a higher concentration of major cations in tap water (21). This difference might be due to the regional differences; wide variations in mineral content have been noted when comparing regions (21). The concentration of calcium in BW was previously reported as between 0.3 and 40.0 mg/L, with an average value of 14.4 mg/L, while the concentration of magnesium ranged between 0.01 to 25.0 mg/L, with an average value of 4.7 mg/L (22). The mean mineral concentrations in BW reported in this study are higher (18.37 mg/L Ca and 5.83 mg/L Mg) than those reported previously (22). Saudi Arabia uses conventional (surface water and groundwater) and unconventional (desalination of seawater and treated wastewater) water resources to fulfill its continuous increasing water demand (23) and is the world's largest producer of desalinated water, which accounts for 70% of the total water demand (24).

Several factors (e.g., the geological settings, rock mineralogy, the source of climate, treatments, length of storage, and type of material used for storage) might be responsible for the difference in the chemical composition between the samples (25,26). BW goes through a purification process that incorporates reverse osmosis; ultraviolet and ozone sterilization. Municipal tap water goes through a purification process that incorporates desalination, followed by filtration (reverse osmosis), and zamzam water is filtered through a series of sand filters and cartridge filters and then sterilized by UV irradiation at these treatment plants, before distribution to consumers. Filtration is less effective in removing solids or dissolved particles that are smaller than the filters pore size (27). In this study, the pH of all water (bottled, tap and commercial zamzam) was ≥ 7.00 (data not shown), which is within the values recommended by the WHO (6.5-8.0) (28). Previously, it has been reported that drinking water with a pH of ≥ 7.0 reduced the risk of forearm fractures relative to drinking water with a pH < 7.0 (29). The ratio of calcium and magnesium is important for optimal utilization of minerals in the body; the optimum value of calcium and magnesium ratio is 2:1(Ca:Mg) (30). In this study, none of the water samples had an optimum calcium-magnesium proportion (Table 1). Gątarska *et al*. (14) reported that only a few of the many tested samples featured the optimum calcium-magnesium proportion.

Calcium, magnesium, and zinc intake from water relative to DRIs

The issue of the adequate amount of water intake is not clear (31). Proper hydration is necessary to sustain the body water equilibrium, and its demand varies from individual to individual and largely depends on various factors, such as age, physical activity, personal circumstances, weather conditions, and other sources of fluids (2). Gątarska *et al*. (14) reported that for determination of various minerals in water, the assumption of daily consumption of the 1-liter volume of the product allows for the estimation of a given water percentage in the RDA of a given mineral. Dietary Reference Intake (DRIs) includes the perception of preventing nutrient deficiencies as well as risk reduction for chronic conditions (32). The results reported here can be used to estimate the amounts of ingested elements (22). To determine the clinical importance of mineral intake from drinking water, mineral level in bottled, commercial zamzam water, and tap water has been compared to DRIs (33). Comparison between DRI and mineral intake from drinking water is made using a reference volume of 1 L. It has been assumed that adults typically consume about 1 L of water per day (about 500 ml for children), and the remaining drinking water requirement is fulfilled by other sources, such as tea and juices. From Table 2, it can be concluded that all variety of water, except commercial zamzam, provide cations (Ca and Mg) in an amount of less than 10% of the amount recommended for children, and the contribution of tap water was found to be very low. In older people (relative to children and other population age groups), these products supply levels lower than the DRI. Variation has been observed between the mineral contents of the samples. Commercial zamzam and tap waters provide 50% and 70% of the recommended amount of zinc for children up to 3 years and more than 32% and 42% of the amount recommended for children under 8 years, respectively. Zinc was not detected in two BWs, and the rest of the BWs provide less than 3% of the amount recommended for children under 8 years old, and even lower levels of the DRI of this component were found in adolescents and adults. Gatarska *et al.* (14) also analyzed three mineral waters with different mineral levels (low, medium and high) and reported that daily consumption of 1 L of these waters provides a good percentage of the RDA in younger children, but in older and special need persons these products supply lower level of RDAs of various minerals, which is quite similar to the results reported in this study.

Table 1: Concentration of Ca, Mg and Zn in bottles, tap and commercial zamzam water.

	Ca (mg/L)	Mg (mg/L)	Zn (mg/L)	Ca: Mg
B1	34.96 ± 0.157^{e}	4.74±0.010^{c}	0±0^{a}	7.37:1
B2	12.597±0.169^{c}	3.39±0.001^{b}	0±0^{a}	3.71:1
B3	11.007± 0.074^{b}	12.99 ±0.047^{e}	0.066±0.0003^{c}	0.84:1
B4	14.92±0.039^{d}	2.21±0.036^{a}	0.012±0.0003^{b}	6.75:1
Zamzam	51.03±0.346^{f}	11.06±0.049^{d}	1.65±0.00089^{d}	4.61:1
Tap	10.082 ±0.0977^{a}	10.08±0. 053^{d}	2.118± 0.0007^{e}	1:1

Data are expressed as the mean ± standard deviation; Model ANOVA, p values < 0.05 are significant. Superscript ^{abc} indicate significant differences among various groups as indicated by ANOVA followed by Turkey HSD test.

Table 2: The percentage of daily recommended index (DRI) for calcium, magnesium and zinc at the assumed consumption of 1 liter of water in different age group of consumer

Life stage	Mineral* (mg/d)	DRI	B1	B2	B3	B4	Zamzam	Tap
Children								
1-3 years	Ca	500	6.99	2.51	2.20	2.98	10.21	2.02
1-3 years	Mg	80	5.93	4.23	16.24	2.76	13.82	12.6
1-3 years	Zn	3	0	0	2.3	0.4	54.87	70.6
4-8 years	Ca	800	4.37	1.57	1.38	1.87	6.38	1.26
4-8 years	Mg	130	3.65	2.61	9.99	1.7	8.51	7.75
4-8 years	Zn	5	0	0	1.32	0.24	32.92	42.36
Males								
9-13 years	Ca	1300	2.69	0.97	0.85	1.15	3.93	0.78
9-13 years	Mg	240	1.98	1.41	5.41	0.92	4.60	4.2
9-13 years	Zn	8	0	0	0.83	0.15	20.58	26.475
14-18 years	Ca	1300	2.69	0.967	0.85	1.15	3.93	0.78
14-18 years	Mg	410	1.16	0.83	3.17	0.53	2.69	2.46
14-18 years	Zn	11	0	0	0.6	0.11	14.96	19.25
19-30 years	Ca	1000	3.49	1.26	1.10	1.49	5.10	1.101
19-30 years	Mg	400	1.19	0.85	3.25	0.55	2.76	2.52
19-30 years	Zn	11	0	0	0.6	0.11	14.96	19.25
31-50 years	Ca	1000	3.49	1.26	1.1	1.49	5.10	1.101
31-50 years	Mg	420	1.13	0.81	3.09	0.53	2.63	2.4
31-50 years	Zn	11	0	0	0.6	0.11	14.96	19.25
51-70 years	Ca	1200	2.92	1.05	0.92	1.24	4.25	0.840
51-70 years	Mg	420	1.13	0.81	3.09	0.53	2.63	2.4
51-70 years	Zn	11	0	0	0.6	0.11	14.96	19.25
>70 years	Ca	1200	2.92	1.05	0.92	1.24	4.25	0.840
>70 years	Mg	420	1.13	0.81	3.09	0.53	2.63	2.4
>70 years	Zn	11	0	0	0.6	0.11	14.96	19.25
Females								
9-13 years	Ca	1300	2.69	0.97	0.85	1.15	3.93	0.78
9-13 years	Mg	240	1.98	1.41	5.41	0.92	4.61	4.2
9-13 years	Zn	8	0	0	0.83	0.15	20.58	26.475
14-18 years	Ca	1300	2.69	0.97	0.85	1.15	3.93	0.78
14-18 years	Mg	360	1.32	0.94	3.61	0.61	3.07	2.8
14-18 years	Zn	9	0	0	0.73	0.13	18.29	23.53
19-30 years	Ca	1000	3.49	1.26	1.10	1.49	5.10	1.101
19-30 years	Mg	310	1.52	1.09	4.19	0.71	3.57	3.25
19-30 years	Zn	8	0	0	0.83	0.15	20.56	26.475
31-50 years	Ca	1000	3.49	1.26	1.10	1.49	5.10	1.101
31-50 years	Mg	320	1.48	1.06	4.06	0.69	3.46	3.15
31-50 years	Zn	8	0	0	0.83	0.15	20.56	26.475

Table 2: (Continued) The percentage of daily recommended index (DRI) for calcium, magnesium and zinc at the assumed consumption of 1 liter of water in different age group of consumer

Life stage	Mineral* (mg/d)	DRI	B1	B2	B3	B4	Zamzam	Tap
51-70 years	Ca	1200	2.92	1.05	0.92	1.24	4.25	0.840
51-70 years	Mg	320	1.48	1.06	4.06	0.69	3.46	3.15
51-70 years	Zn	8	0	0	0.83	0.15	20.56	26.475
>70 years	Ca	1200	2.91	1.04	0.92	1.24	4.25	0.840
>70 years	Mg	320	1.48	1.06	4.06	0.69	3.46	3.15
>70 years	Zn	8	0	0	0.83	0.15	20.56	26.475
Pregnancy								
14-18 years	Ca	1300	2.69	0.97	0.85	1.15	3.93	0.78
14-18 years	Mg	400	1.19	0.85	3.25	0.55	2.76	2.52
14-18 years	Zn	12	0	0	0.55	0.1	13.72	17.65
19-30 years	Ca	1000	3.49	1.26	1.1	1.49	5.10	1.101
19-30 years	Mg	350	1.35	0.97	3.71	0.63	3.16	2.88
19-30 years	Zn	11	0	0	0.6	0.11	14.96	19.254
31-50 years	Ca	1000	3.49	1.26	1.1	1.49	5.10	1.101
31-50 years	Mg	360	1.32	0.94	3.61	0.61	3.07	2.8
31-50 years	Zn	11	0	0	0.6	0.11	14.96	19.254
Lactation								
14-18 years	Ca	1300	2.69	0.97	0.85	1.15	3.93	0.78
14-18 years	Mg	360	1.32	0.94	3.61	0.61	3.07	2.8
14-18 years	Zn	13	0	0	0.51	0.09	12.66	16.292
19-30 years	Ca	1000	3.49	1.26	1.1	1.49	5.10	1.101
19-30 years	Mg	310	1.53	1.09	4.19	0.71	3.57	3.25
19-30 years	Zn	12	0	0	0.55	0.1	13.72	17.65
31-50 years	Ca	1000	3.49	1.26	1.1	1.49	5.10	1.101
31-50 years	Mg	320	1.48	1.06	4.05	0.69	3.46	3.15
31-50 years	Zn	12	0	0	0.55	0.1	13.72	17.65

Elemental analysis of rabbit bones (femur)

Table 3 reports the mineral content of the bone (femur) of rabbits that consumed bottled, commercial zamzam, and tap waters. The concentration of calcium was highest in commercial zamzam water and least in BW. We detected significant differences when comparing the calcium content of commercial zamzam water and the other tested types ($p \le 0.05$). Even though the concentrations of magnesium and zinc were highest in BW, these failed to reach statistical significance ($p \ge 0.05$). Even though many different chemicals are found in drinking water, only a few are significant in any given

Table 3. Concentration of Ca, Mg and Zn in bone (femur) of male rabbits consuming bottled, zamzam and tap water

	Ca (mg/L)	Mg (mg/L)	Zn (mg/L)
B1	46±22.596^{ab}	7.57±0.541^{a}	4.01±0.671^{a}
B2	22.67± 0.963^{a}	7.29±0.4413^{a}	3.25± 0.011^{a}
B3	20.59±1.944^{a}	7.89±0.314^{a}	4.21±0.450^{a}
B4	36.71±8.701^{a}	7.49±0.589^{a}	4.30±0.576^{a}
Zamzam	78.37±4.586^{b}	7.37±0.076^{a}	3.87±0.364^{a}
Tap	25.22± 15.492^{a}	7.49± 1.007^{a}	4.14± 0.57^{a}

Data are expressed as the mean ± standard deviation; Model ANOVA, p values < 0.05 are significant. Superscript ^{abc} indicate significant differences among various groups as indicated by ANOVA followed by Turkey HSD test.

circumstances (12). Bone plays a significant role in mineral homeostasis regulation, and it is the core reservoir for minerals (34). The repetitive cycle of destruction and rebuilding maintains the bone mass, as well as the balance between bone formation and bone resorption, which are controlled by osteoblasts and osteoclasts respectively (35). Magnesium and calcium play significant roles in human dietary needs (36, 37). The skeleton stores calcium and thus regulates blood calcium levels, but a dearth of calcium leads to depletion of the bone reservoirs, causing brittleness in the bones and greater risk of fractures (osteoporosis) (38). Various epidemiological, clinical, and animal studies have reported an inverse relationship between calcium intake and incidence of osteoporosis (39, 40). Deficiency in calcium intake is generally combined with a vitamin D insufficiency (41), which leads to decreased calcium absorption. This causes a reduction in ionized extracellular fluid calcium, which in turn stimulates parathyroid gland secretion. Augmented parathyroid secretion upsurges bone turnover, which is the main determining factor for bone loss and fracture risk (42). Various authors have studied the bioavailability of calcium from mineral waters, concluding that it is very high (43, 44). Previous studies have reported an increase in bone mineralization, considering both femoral and spinal bone mineral density with consumption of calcium-rich mineral water (45, 46). Magnesium (Mg) is the second most abundant in the intracellular fluid and the fourth most abundant cation in the human body (47). Absorption of waterborne Mg is almost 30% faster and better than Mg from food (48) and its bioavailability increases with a light meal (49). An appropriate magnesium intake maintains adequate bone mineral density (50, 51). Relative to dairy products and other sources, waterborne calcium and magnesium are advantageous because unlike them neither it has any side effects nor it brings any, calorie, or lipid supplements (52).

Zinc is an essential trace metal, and its deficiency leads to a decrease in bone weight, retardation of bone growth and maintenance, and delays growth in bone metabolism (53). Zinc stimulates cellular protein synthesis by activating aminoacyl tRNA synthetase in osteoblastic cells and inhibits osteoclastic bone resorption by inhibiting osteoclast-like cell formation from marrow cells (54). Drinking water contains this trace metal in minute quantities, which might decrease the likelihood of its deficiency in the diet. However, its deposition in the human body causes harmful effects, such as acceleration of anemic condition (55). As compared to BW, higher levels of zinc have been reported in tap water, which is similar to the result obtained in this study (12).

Correlation between water and bone mineral contents in rabbits

A strong and highly positive correlation ($p \leq 0.05$) was observed between bone and water calcium levels ($R^2 = 0.9286$), and a slight positive correlation was observed between bone and water magnesium levels ($R^2 = 0.2576$). The correlation was very weak between bone and water zinc levels ($R^2 = 0.0169$) (Figure 1 A-C). The associations observed in this study provide further evidence of the links between nutrients and bone metabolism (56). These results might reflect the positive influence of the consumption of water on bone health.

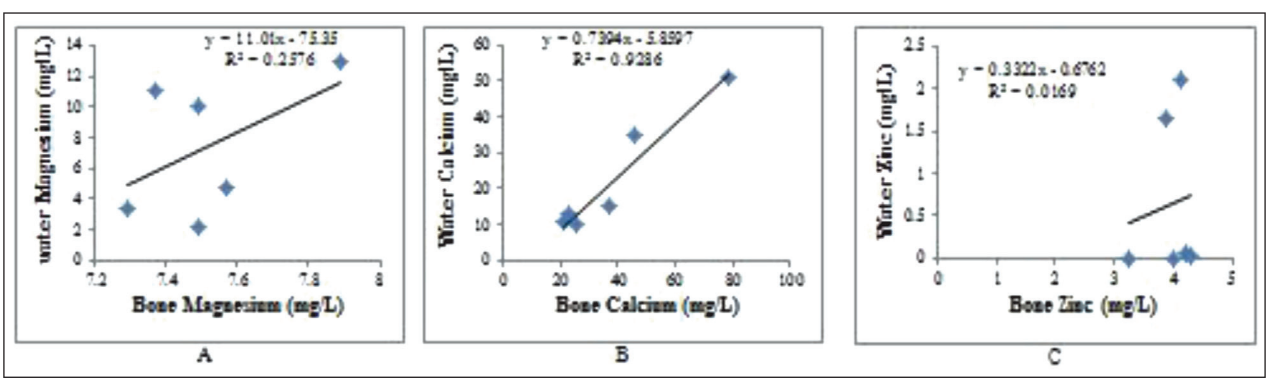

Figure1. Pearson's correlation coefficient between ions in water and bone (femur) of male rabbits.

Conclusion

In conclusion, this study shows that the concentration of all analyzed elements was within the guidelines set by various agencies. A large variation in various mineral concentrations was detected among BWs. We found that commercial zamzam water provides the essential minerals required for bone health. Further studies are required to understand the biochemical pathways involved in health. This study will help in supporting a more focused design of further research and can be considered as a baseline for future reference.

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