A study of water hardness and the prevalence of hypomagnesaemia and hypocalcaemia in healthy subjects of Surat district (Gujarat)

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Abstract. Background: Various sources of drinking water, with varying levels of total hardness, and calcium and magnesium concentrations, are used by populations in different regions. The use of water purifiers can compound the problem of maintaining the desired levels of hardness. An inverse relationship between various conditions, including cardiovascular disease, and hard water has been reported. Until this study, investigation of the hardness of drinking water from different sources, and serum magnesium and calcium in normal subjects from the Surat district, had not been undertaken. Objectives: This study was performed to assess the concentrations of calcium and magnesium, and total hardness in filtered and non-filtered water and the relationship with serum magnesium and calcium levels in normal subjects consuming such water. Methodology: Three water samples were collected, at 15-day intervals, from 12 urban and rural areas of Surat; and also 10 different brands of bottled water. Samples were analyzed for total hardness and calcium by complexometric and EDTA methods respectively. Magnesium concentrations were obtained by subtraction of the calcium concentration from total hardness. Serum samples from healthy individuals were analyzed for magnesium and calcium using calmagite and arsenazo methods respectively. The independent t-test was used to establish significance at a level of 95%. A p-value <0.05 was considered significant. Results: Mean total hardness, and calcium and magnesium concentrations in non-filtered, rural tube-well water were much higher than in filtered water from the same area, and the magnesium concentrations were significantly higher (p = 0.038). Filtered urban municipal had lower hardness and concentrations of calcium and magnesium (p = 0.01) compared to corresponding non-filtered water. Significantly lower levels were observed in bottled water compared to rural and urban sources of water. Serum magnesium was significantly lower in the population who were consuming filtered water compared to those drinking non-filtered water (p<0.05). No such difference was observed for serum calcium. Conclusion: Hypomagnesemia correlates with lower magnesium concentrations in drinking water (both rural tube-well and urban municipal waters), which can be attributed to the use of water purifiers. Assuming that a person consumes...
two liters of drinking water per day, it is estimated that there is an average loss of 160 mg (79%) of magnesium from total waterborne magnesium levels as a result of the filtration of both rural and urban water supplies. Bottled water is too hardness as in calcium and magnesium concentrations.

**Keywords:** water hardness, calcium, magnesium, water filters, RO water purifiers, cardiovascular diseases, hypomagnesemia

The biochemical roles of minerals, including magnesium and calcium, in health and disease are well established. Partial or complete lack of a variety of minerals leads to the development of a number of diseases. Subclinical deficiencies of iron, magnesium, zinc and calcium prevail in both the developed and developing world [1]. Water is one of the important sources of magnesium and calcium, along with diet. Magnesium and calcium content determines the level of water hardness. So the maintenance of appropriate levels of water hardness is beneficial to health. As in other parts of the world, drinking water standards also exist in India, but due to laxity in the implementation of these regulations, the desired levels of hardness are not maintained.

Asian drinking water generally contain 2-80 mg/L of calcium (Ca\(^{2+}\)) and less than 20 mg/L of magnesium (Mg\(^{2+}\)) [1]. Kumar et al. [2] analyzed water samples from 24 different locations in the Punjab and reported large variations in mineral contents in well water, with a range of 8-343 mg/L for calcium and 5-235.6 mg/L for magnesium. The popular concept of clean drinking water is that it is without pathogenic microbial or obvious anthropogenic or chemical contamination. Many consumers assume such “clean water” is harmless. Hence, in many Asian countries less priority is given to routine analysis for minerals such as calcium, magnesium and zinc in drinking water supplies, and is not included in regulatory monitoring [1].

Households in both rural and urban areas use water purifiers, focusing on the removal of hardness. Water purifiers based on ion-exchange techniques or reverse osmosis or both, and distillation techniques are mainly used. Ion exchange resins remove each divalent ion (e.g. Ca\(^{2+}\) or Mg\(^{2+}\)) and replace them with two sodium ions [3]. Reverse osmosis and distillation devices remove virtually all of the minerals from the input water [4]. Populations all over the world use one sort or another of these water purifiers. There has also been a huge increase in the consumption of bottled water. Despite its high unit price compared to tap water, bottled water consumption has increased by 12% globally [1]. In India, a 50% annual growth has been observed, which is much higher than in the other parts of the world [5]. Drinking water is an important source of minerals. Rubenowitz et al. [6], using an oral loading test, observed the impact of drinking-water magnesium on body magnesium status. As filtration is known to change the composition of minerals, the shift in consumption from tap to bottled water may have important implications for health and disease. Extreme variations in the mineral content of packaged water are highly likely as a result of the use of various methods such as ion exchange, reverse osmosis and distillation, and large variations in the mineral content of commercially available bottled water have indeed been found [7, 8]. The magnesium and calcium content of bottled water available in North America ranged from 1 to 120 mg/L and 1 to 240 mg/L respectively, whereas in Europe, the concentrations range from 1 to 126 mg/L and 0 to 546 mg/L respectively. Calcium and magnesium play crucial roles in maintenance of bone architecture. Many epidemiological studies have linked hypertension and cardiac disease with “soft water” and water low in magnesium, and protection against cardiovascular diseases with “hard water” and water high in magnesium [9]. Rasic-Milutinovic et al. [10] reported an association between blood pressure and some components of metabolic syndrome, and the magnesium concentration in drinking water in some Serbian municipalities. Marx and Neutra [11] reviewed the strengths and weaknesses of the epidemiological evidence linking magnesium to the natural history of ischemic heart disease (IHD). Rubenowitz et al., [12] in a case-control study, reported a relationship between death from acute myocardial infarction and the content of magnesium in drinking water. The high incidence of deaths due to hypertension has been attributed to low magnesium concentrations compared to deaths due to other causes [13]. A relationship between the mineral content of drinking water and the incidence of cardiovascular mortality has
been observed by some workers [14, 15]. However, Leurs et al. [16] did not find an association between tap water hardness, magnesium or calcium concentrations, and IHD mortality or stroke mortality. Nevertheless, an important aspect is that they found an increased risk among males with an inadequate dietary magnesium intake. This illustrates the concept of risk groups in population studies of this kind and how an uneven distribution of the presence of risk groups may lead to erroneous conclusions.

Few studies are available regarding the incidence of hypomagnesemia in the general population. In Germany, a range of 2.5-15% was observed in blood donors, outpatients and children [17]. In another study, a prevalence of 2.5% of subjects with magnesium concentrations below 1.7 mg/dL, and 5% with concentrations below 1.83 mg/dL was reported [18]. Different workers have used different cut-off values to define hypomagnesemia. Some workers have used 1.7 mg/dL [19, 20] and 1.77 mg/dL [21] and others have used 1.6 mg/dL [22]. There is a lacuna in the literature regarding the status of drinking water with respect to the concentrations of calcium and magnesium and the status of serum magnesium in subjects who consume drinking water with different levels of hardness.

Based on present knowledge, our study was primarily aimed at the determination of total hardness, and calcium and magnesium concentrations of water samples from various sources, and serum magnesium and calcium concentrations in the local population who consume such water. We hypothesized that the water sources subjected to filtration processes, and bottled water would have low levels of total hardness, and low calcium and magnesium concentrations. The consumption of such water would have bearing on serum magnesium and calcium concentrations. A secondary objective was to determine the extent of hypomagnesemia and hypocalcemia in healthy subjects consuming filtered water compared to non-filtered water.

Drinking water samples were collected in clean, dry, plastic containers. Three samples of drinking water from 12 households from urban areas and five households from rural areas were collected at 15-day intervals. Similarly, 10 commercially bottled drinking water samples were also collected. Samples were analyzed for total hardness and calcium by complexometric and EDTA methods respectively. Magnesium concentrations were obtained by subtraction of the calcium concentration from total hardness. An average of three readings was taken for calculations. Simultaneously, blood samples taken from 185 healthy subjects from these areas were collected at their homes for analysis of serum magnesium and calcium levels. The study was approved by the Institutional Ethics Committee, and informed consent was obtained from all of the participants.

**Inclusion and exclusion criteria**

Households using only one source of water were included: subjects suffering from any type of disease or who were on medication, and subjects moving from rural to urban areas, or vice versa, on a day-to-day basis, were excluded.

**Laboratory analysis**

A complexometric method was used to determine total hardness involving erichrome black T as indicator. The calcium concentration was determined using an EDTA titration method with Patton’s and Reeder’s indicators [23, 24]. Total hardness was expressed as the amount of CaCO₃ in mg/L, and also in terms of German degrees. The magnesium concentration was obtained by subtracting the calcium concentration from total hardness. Three readings were taken for each sample and an average value was derived. Serum magnesium levels were determined using the calmagite method [25], and serum calcium levels using the arsenazo method [26], with analysis being performed in an ERBA-XL auto analyzer (Transasia Biomedical Ltd. Mumbai, India, a subsidiary of ERBA Manheim, Germany). In the present study, the reference range for serum magnesium and calcium were 1.60-2.60 mg/dL and 8.6 to 10.2 mg/dL respectively [22]. Both internal and external quality control samples were run and found to be within the permissible limits.

**Methods and materials**

The present study was conducted in the Biochemistry Department of Surat Municipal Institute of Medical Education and Research (SMIMER), Surat, India, between June 2011 and June 2012.
Table 1. Comparison of mean total hardness, calcium and magnesium concentrations: non-filtered versus filtered rural tube-well water samples.

<table>
<thead>
<tr>
<th>Water hardness</th>
<th>Rural tube-well water (n = 5)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without filter</td>
<td>With filter</td>
</tr>
<tr>
<td>Total hardness</td>
<td>330.0 ± 121.0</td>
<td>154.0 ± 131.0</td>
</tr>
<tr>
<td>German degrees</td>
<td>18.5 ± 6.8</td>
<td>8.6 ± 7.3</td>
</tr>
<tr>
<td>Calcium (mg/L)</td>
<td>182.0 ± 61.0</td>
<td>104.0 ± 83.0</td>
</tr>
<tr>
<td>Magnesium (mg/L)</td>
<td>148.0 ± 71.0</td>
<td>62.0 ± 45.0</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SD. *Significance: p value (<0.05) calculated using a paired t-test between non-filtered versus filtered, rural, tube-well water.

Table 2. Comparison of mean total hardness, calcium and magnesium concentrations: non-filtered versus filtered urban, municipal water samples.

<table>
<thead>
<tr>
<th>Water hardness</th>
<th>Urban municipal water (n = 12)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without filter</td>
<td>With filter</td>
</tr>
<tr>
<td>Total hardness</td>
<td>226.7 ± 16.1</td>
<td>65.0 ± 39.2</td>
</tr>
<tr>
<td>German degrees</td>
<td>12.7 ± 0.9</td>
<td>3.6 ± 2.2</td>
</tr>
<tr>
<td>Calcium (mg/L)</td>
<td>133.3 ± 19.2</td>
<td>50.0 ± 34.9</td>
</tr>
<tr>
<td>Magnesium (mg/L)</td>
<td>90.8 ± 17.8</td>
<td>15.0 ± 6.7</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SD. *Significance: p value <0.05 calculated using the independent t-test between non-filtered versus filtered urban water.

Statistical methods

Data analysis was performed using SPSS (Statistical Package for the Social Sciences) version 12.0. Results were expressed as mean ± SD. The independent t-test was used for significance at a 95% confidence interval. A p-value <0.05 was considered as significant.

Results

Table 1 shows comparative total hardness and calcium and magnesium concentrations between non-filtered and filtered, rural tube-well water samples. Total hardness and calcium concentration were much higher in non-filtered water than filtered water, and magnesium concentrations were significantly higher (p = 0.038) in non-filtered water. The minimum concentrations found in the rural, non-filtered water samples were 110 mg/L for calcium and 70.0 mg/L for magnesium; in filtered water samples the concentrations were 20.0 and 0 mg/L respectively.

Table 2 describes the total hardness and calcium and magnesium concentrations of urban, municipal water supplies from 12 areas of Surat city. As in rural, tube-well water, a similar pattern was found in these urban areas before and after filtration.

Table 3. Total hardness, calcium and magnesium concentrations in different brands of commercially available bottled water.

<table>
<thead>
<tr>
<th>Water hardness</th>
<th>Mean ± SD (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total hardness</td>
<td>40.0 ± 8.0</td>
</tr>
<tr>
<td>German degrees</td>
<td>2.2 ± 0.5</td>
</tr>
<tr>
<td>Calcium (mg/L)</td>
<td>27.0 ± 9.0</td>
</tr>
<tr>
<td>Magnesium (mg/L)</td>
<td>20.0 ± 13.0</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SD.
Results expressed as mean ± SD. Significant p values among different groups

<table>
<thead>
<tr>
<th></th>
<th>Gr. 1 without filter</th>
<th>Gr. 1-without filter</th>
<th>Gr. 2 without filter</th>
<th>Gr. 2-with filter</th>
<th>Gr. 3 without filter</th>
<th>Gr. 3-without filter</th>
<th>Gr. 4 without filter</th>
<th>Gr. 4-with filter</th>
<th>Gr. 5 Total hardness (mg/L)</th>
<th>Gr. 5-Calcium concentration (mg/L)</th>
<th>Gr. 5-Magnesium concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total hardness</td>
<td>330</td>
<td>154</td>
<td>182</td>
<td>104</td>
<td>148</td>
<td>62</td>
<td>65</td>
<td>133</td>
<td>40</td>
<td>27</td>
<td>20</td>
</tr>
<tr>
<td>Calcium concentration</td>
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<td>Magnesium concentration</td>
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</table>

**Rural tube well water (n = 5)**  
**Urban municipal water (n = 12)**  
**Commercial bottled water (n = 10)**

**Figure 1. A)** Mean total hardness, calcium and magnesium concentration(s) (expressed as mean) for non-filtered and filtered water samples from various sources.

Mean total hardness, and calcium and magnesium concentrations in different types of water samples are presented in figure IA. Total hardness of non-filtered, rural water was significantly higher compared to urban municipal supplies (p = 0.03). Total hardness of tube-well water was significantly higher (p = 0.02), even after filtration, when compared to commercially bottled water. In the filtered water category, the calcium concentration was found to be significantly higher in tube-well water when compared to bottled water. Magnesium concentrations were significantly higher in tube-well water than urban municipal water supplies, with or without filtration (p<0.01).

**Figure 1B** shows mean total hardness, and calcium and magnesium concentrations in non-filtered and filtered water, from both rural and
urban areas. Total hardness, and calcium and magnesium concentrations of filtered water were significantly lower (p < 0.05) than seen in non-filtered water.

Serum concentrations of calcium and magnesium are given in table 4. In the filtered-water group, the serum magnesium concentration was significantly lower (p < 0.01) than that of non-filtered water group.

Figure 2 shows the prevalence of hypomagnesemia in subjects consuming non-filtered and filtered water. A significantly higher prevalence of hypomagnesemia (p < 0.05) was observed in subjects consuming filtered water.

Table 5 shows the estimate of magnesium intake per day through various types of drinking water, assuming that a person consumes two liters of drinking water per day.

Discussion

Ground-water collected through tube-wells was the main source of drinking water in the rural areas of Surat included in this study. In households using non-filtered groundwater, total hardness varied from 230.0-500.0 mg/L, with an average of 330.0 mg/L (SD 121.0 mg/L), which was much higher than the levels reported from other countries, such as Canada (<5 to 165 mg/L) [1].

In Asian countries, drinking water supplies have a range for calcium from 2-80 mg/L, and less than 20 mg/L of magnesium [27]. In this study, calcium and magnesium concentrations varied between 110-250 mg/L (182 ± 61 mg/L) and 70-250 mg/L (148 ± 71 mg/L) respectively in the non-filtered, rural, groundwater, which were comparable to those found in well-water samples from various places in the Punjab [2].

In these rural areas, there was also a general trend of using water purifiers for cooking as well as drinking purposes. These purifiers also remove hardness (e.g. calcium and magnesium) and iron from water. The impact of water filters on the mineral composition and hardness of groundwater supplies in these rural areas was clearly demonstrated by an average decrease of 52.7% total hardness after filtration. A similar trend was observed regarding calcium and magnesium concentrations (decreases of 42.8% and 57.8% respectively).

In Surat city, drinking water is supplied by the Municipal Corporation through a piped system following passage though water treatment plants involving treatment that uses a filtration
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Table 4. Concentrations of serum calcium and magnesium in healthy subjects drinking non-filtered or filtered drinking water.

<table>
<thead>
<tr>
<th>Serum concentration(s)</th>
<th>Non-filtered water group (n = 125)</th>
<th>Filtered water group (n = 60)</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.4 ± 1.1</td>
<td>9.4 ± 1.2</td>
<td>1.00</td>
</tr>
<tr>
<td>Magnesium (mg/dL)</td>
<td>2.0 ± 0.5</td>
<td>1.8 ± 0.4</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SD. *p values were calculated using t-test between non-filtered water group versus filtered water group.

Table 5. Estimate of magnesium intake per day through various types of drinking water.

<table>
<thead>
<tr>
<th></th>
<th>Non-filtered</th>
<th>Filtered</th>
<th>Commercially bottled water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of magnesium detected (mg/L)</td>
<td>60-250</td>
<td>0-100</td>
<td>0-20</td>
</tr>
<tr>
<td>Amount (mg) of magnesium available if water consumption is 2 L/day</td>
<td>120-500</td>
<td>0-200</td>
<td>0-40</td>
</tr>
</tbody>
</table>

process called a Lamella Sludge Blanket clarifier. This involves a rapid gravity sand filter, which allows the mineral content to be maintained as per the guidelines [28]. The total hardness of urban municipal water ranges between 210-250 mg/L, which is comparable to the hardness found in developed countries such as the USA [7] and Great Briton [7, 29]. Urban municipal water may be categorized as very hard water, as total hardness is well above the figure of 181 mg/L.

Correspondingly, calcium and magnesium concentrations in urban municipal water (133.3 ± 19.2 and 90.8 ± 17.8 mg/L respectively) were as per Indian standards [28]. However, these were very high compared to other countries such as Nigeria (calcium: 2 to 60 mg/L) and Los Angeles, California (magnesium: 5-29 mg/L), as well as most Asian countries (2-80 mg/L for calcium and below 20 mg/L for magnesium) [1].

As in to rural areas, many urban households use reverse osmosis water purifiers, in spite of receiving treated, piped water, and this reduces total hardness drastically by 71.3%. Similar filtration effects were observed in the case of calcium and magnesium, where reductions of 62.5 % and 75.8 % respectively were seen. Results obtained from this study reveal that the municipal water supplies adhered strictly to Indian standards in maintaining total hardness, calcium and magnesium concentrations, but the overcautious approach by the consumers in the concomitant use of water purifiers, without monitoring, led to the undesirable effect of converting hard water into soft water with a low mineral content.

In recent years, there has been a dramatic rise in use of bottled water in India, as elsewhere [1]. Labels on bottled water provide only no or only partial details of chemical analysis making it difficult for consumers to interpret. For instance, none of the samples treated using reverse osmosis displayed information on total dissolved solid levels [3]. In the present study, total hardness ranged from 30-50 mg/L, which falls into the category of soft water as these levels were much lower than the standard of 60 mg/L [30].

The present study found variations in the mineral content of bottled water used in Surat city, but these variations were not as great as those reported for North America or Europe [7, 8, 31]. Calcium and magnesium concentrations were much lower than those found in North America and Europe, but slightly higher than the levels reported from Sweden.

The total hardness, as well as calcium and magnesium concentrations, of all the supplies of water, apart from the filtered, urban municipal water used by the urban households in our study, were significantly higher than those found in commercially bottled water.

This pattern can be attributed to the change from traditional methods to more modern lifestyles, which have involved changes in dietary and drinking water habits. The main disadvantage of the modern methods used in the
purification of drinking water, is the transformation of naturally available hard water into soft water with a deficient mineral content. The use of soft water may increase the longevity of piping and other articles; however, this is detrimental to the health of the consumer. In both developed and developing countries there is a prevalence of subclinical deficiencies in iron, magnesium and calcium. An inverse relationship between water hardness and various diseases, including coronary morbidity, cerebrovascular diseases and gastrointestinal cancers has been observed [32-35].

The prevalence of hypomagnesemia (cut-off value <1.6 mg/dL), found to be 17.83% in the present study, was higher than the 14.5% reported by Schimatschek et al. [17] in Germany, but lower than the 20% prevalence reported by Fox et al. [21] (cut-off value <1.77 mg/dL) in an African-American population examined during routine medical care.

In the healthy subjects of the present study, serum magnesium concentrations (1.93 ± 0.46 mg/dL) were within the normal range, but these results appear to be misleading when the serum magnesium concentrations found in non-filtered- and filtered water-consumers are compared. Serum magnesium concentrations in the non-filtered water consumer category were significantly higher than those seen in the filtered water category. More hypomagnesemic and borderline cases were found in the filtered water category compared to the non-filtered category.

Most of the body’s magnesium requirements are fulfilled through diet, only 10% being supplied by drinking water; however, it is actually a very important contributor to body magnesium levels because of its 30% higher bioavailability than that of dietary magnesium [11]. Water-borne magnesium intake may in fact quantitatively represent a critical contribution compensating for a marginal dietary intake [36-39], thus fulfilling recommended daily allowances [40]. The subjects involved in this study had similar dietary habits, but used different sources of drinking water. It has been suggested that individuals with insufficient dietary magnesium intake would benefit particularly from magnesium in drinking water [41]. Leurs et al. [16] reported contrasting results in men and women, and were cautious about drawing conclusions from results obtained in subjects with a low dietary magnesium intake. This illustrates the need to consider the presence of risk groups when researching relationships between health and magnesium, as well as the effect of interventions.

When considering the available magnesium in the drinking water supplies from different sources, and assuming that a person consumes two liters of water per day, the contribution to intake of magnesium from filtered water sources ranges from 0-200 mg/day, much lower than the non-filtered sources which ranges from 120 to 500 mg/day, a reduction of 79% in waterborne magnesium (table 5). The higher number of hypomagnesemic subjects in the filtered water category reiterates the significant nutritional impact of drinking water in the maintenance of body magnesium levels.

We did not find any significant incidence of hypocalcemia in the normal, healthy controls, but a lower concentration of serum calcium was observed although this was still within the normal range in subjects with hypomagnesemia compared to normomagnesemic subjects.

### Conclusion

In the present study, hypomagnesemia correlated with lower magnesium concentrations in the drinking water (both rural tube-well and urban municipal waters), which is attributed to the use of
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water purifiers. Bottled commercial water was too soft, with inadequate mineral content and hardness. One important reason for the presence of hypomagnesaemia in the local population may be due to the use of filtered water for both drinking as well as cooking purposes; it is well known that the loss of magnesium from food is less when it is cooked in magnesium-rich water. No correlation was observed in the case of serum calcium levels, but lower concentrations of serum calcium were found in the hypomagnesemic subjects compared to those who were normomagnesemic. Further studies are required, involving larger sample sizes from both rural as well as urban areas, to confirm these findings.

Limitations

Water samples were analyzed using a titrimetric method, and serum samples were analyzed using a spectrophotometric method. Atomic absorption spectrophotometry is the recommended method, but due to the lack of this facility, this study was carried out using other methods. Although dietary analyses were not carried out, all of the subjects had similar socio-economic, cultural backgrounds and had similar dietary habits.

Disclosure


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