Drinking Desalinated Water that Lack Calcium and Magnesium Has No Effect on Mineral Content of Enamel and Dentin in Primary Teeth

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Objective: *The present study compared the mineral contents of enamel and dentin of primary teeth from children exposed to desalinated water with those from children drinking ground water.* ***Study design:*** *The study comprised of two groups of teeth, seven primary teeth from children living in areas supplied exclusively with desalinated water and seven primary teeth from children that have been exposed solely to ground water from in-utero until the teeth were either extracted or naturally shed. Mineral content of three tooth regions was determined by scanning electron microscopy with an energy dispersive X-ray spectrometer (EDS). The main ion content of each region was calculated.* ***Results:*** *Children exposed to ground water presented higher levels of magnesium in pre- and post- natal enamel than children living in areas supplied exclusively with desalinated water but without significant differences. The same was found for calcium levels.*

Excluding post-natal enamel calcium level (of borderline statistical significance), no significant differences were found in magnesium and calcium levels of primary teeth enamel and dentin of children exposed to desalinated water in comparison to children exposed to ground water. ***Conclusion:*** *Mineral content of enamel and dentin in primary teeth is not affected by consuming desalinated water.*

Keywords: *Desalinated water, Enamel minerals, Dentin minerals, Primary teeth.*

INTRODUCTION

The World Health Organization (WHO) emphasizes the importance of drinking water as a source of daily dietary nutrition of calcium (Ca) and magnesium (Mg)[1]. Both are essential to human health and inadequate intake of either might impair health[2]. Global warming and climate changes together with population growth limit fresh drinking water supply. This shortage, in turn, catalyzed the construction of desalination water facilities that enable the exploitation of non-potable, salty water to produce high quality drinkable water[3, 4].

In 1999, the Israeli government initiated a long-term, large-scale Sea Water Reverse Osmosis (SWRO) desalination program. The program was designed to provide for the growing demands on the scarce water resources, and to mitigate the effects of drought conditions that have dominated most years since the mid-1990's[1]. Between the years 2005-2015 five large-scale reverse-osmosis desalination facilities were built. These, together with some smaller brackish water desalination facilities, currently supply the majority of Israel's potable water requirements to all sectors.

Currently, 75% of Israel's population consume desalinated water. In 2012, 42% of the drinking water was desalinated[3]. By 2025 desalinated water is expected to be 75% of the drinking water supplied through the national water system.

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The two-pass seawater reverse osmosis (RO) process used for desalination consists of driving high-pressure water forcefully through a semi-permeable membrane that removes all soluble minerals[1]. While desalinated water lack minerals, ground water contain calcium carbonate at varying concentrations: below 60 mg/l is generally considered as 'soft'; 60–120 mg/l- 'moderately hard'; 120–180 mg/l- 'hard'; and more than 180 mg/l- 'very hard'[5]. Magnesium is usually present in natural groundwater at lower concentrations (from negligible to about 50 mg/l). Both calcium carbonate and magnesium are practically lacking from desalinated water[6].

In an average 70 kg adult person, calcium constitutes 1100 grams of total body weight. It has a fundamental role in bone and teeth architecture and metabolism, neuronal conduction, coagulation, hemodynamics etc. Hard tissues contain ninety-nine percent of the calcium in the body. Decreased consumption has been associated with osteoporosis, osteomalacia and hypertension[6].

Magnesium also has a major role in body function. Total body magnesium weight is 20-25 gram in the average adult and stored mainly in the bone (50%)[1]. Magnesium is a co-factor for over 350 enzymes, essential to body metabolism, nucleic acids synthesis, cellular equilibrium, cardiovascular stability and hormonal function. Low consumption has been associated with osteoporosis, calcium imbalance, insulin-resistance and metabolic syndrome, as well as cardiac morbidity[1].

About 20% of magnesium intake comes from drinking water[7]. A retrospective 10-year study showed that low magnesium in desalinated drinking water was associated with increased cardiac morbidity and mortality[3]. Consumption of ground water with appropriate levels of calcium and magnesium was reported to have a protective effect on cardiac health. As presented by WHO in 2011, most populations lack adequate dietary intake of calcium and magnesium[7]. Remineralization of drinking water may mitigate such deficits[8]. Although calcium carbonate is added to the drinking water (as a dietary supplement and to prevent pipeline corrosion), calcium levels in desalinated water do not reach the levels found in ground water[9, 10].

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is the major inorganic component of all body hard tissues, including teeth. During amelogenesis, enamel proteins construct the scaffold for the growing lattice. As maturation of the enamel proceeds, the proteins are degraded and replaced mainly by calcium and phosphate resulting in the growth of the apatite lattice. The composition on the apatite lattice might change in the presence of magnesium and sodium (Na) ions (which replace the Ca) or by fluoride (F) or chloride (Cl) ions (which replace the Hydroxyl group)[11].

Although magnesium constitutes only 0.2-0.6wt% of enamel composition, its presence has been reported to restrict the growing of the enamel lattice and its lack is related to increased risk for caries[11, 12]. High levels of magnesium have been observed in areas of enamel with insufficient removal of enamel proteins[13].

The present study compared the mineral contents of enamel and dentin of primary teeth from children exposed to desalinated water with the mineral contents of enamel and dentin of primary teeth of children drinking ground water.

MATERIALS AND METHOD

The study comprised of two groups of teeth:

1. Study group included seven primary molars (two mandibular second primary molars, four maxillary second primary molars and one mandibular first primary molar) from children living in areas supplied exclusively with desalinated water (city of Eilat and kibutz Ma'agn-Michael). Those children have been exposed to desalinated water both in-utero and until the teeth were either extracted or naturally shed.

2. Control group included seven primary teeth (two mandibular first primary molars, three maxillary first primary molars, one mandibular primary incisor and one maxillary primary canine) from children residing in the northern parts of Israel that have been exposed solely to ground water in-utero and until the teeth were either extracted or naturally shed.

The teeth were extracted during routine dental procedures or collected after normal exfoliation with the appropriate parental and child consent.

Information about water composition was obtained through the Israeli National Water Authority.

Ethical Considerations

Study protocol was approved by the Institutional Human Subjects Ethics Committee of Hadassah Medical Organization IRB, Jerusalem, Israel. Parental consent was given.

Scanning electron microscopy:

In accordance with the protocol of Caropreso et al. the teeth were embedded in epoxy (Epofix kit, Struers GmbH, Willich, Germany) and sliced bucco-lingually parallel to their sagittal axes, using a wafer blade (Isomet 1000, Buehler Ltd. Esslingen, Germany)[13]. Since microanalysis requires a smooth surface of the tested area, a slice of 80-100 μm was gradually polished using Isomet 1000 (IsoMet™ 1000 Precision Sectioning Saw, Buehler Ltd. Esslingen, Germany) and Minimet Polisher (MiniMet™ 1000 Grinder Polisher, Buehler Ltd. Esslingen, Germany). Under a microscope (BestScope T3040, BestScope International Limited, Beijing, China) at ×10 and ×20 enlargements the neonatal line (incremental growth line seen in histologic sections of enamel of primary teeth formed at birth) was detected[12]. By using scanning electron microscopy (SEM; Quanta 200, Oregon, USA) under high vacuum mode in conjugation with an energy dispersive X-ray spectrometer (EDS) the mineral content (e.g., calcium, phosphate, magnesium) of three regions, squares of 0.2 × 0.2 mm, was determined in the two groups (Fig. 1). Two regions in the enamel and one region in the dentin were analyzed: 1) Enamel in the upper section of the crown close to the dentin-enamel junction (DEJ) representing pre-natal enamel formation, 2) Enamel in the cervical region of the crown close to the outer surface of enamel representing the post-natal enamel 3) Dentin in the coronal third closer to the DEJ and distant from the pulp. More than 5000 readings were performed by the EDS on each square and the results were given as mean and standard deviation (SD). The main ion content of each region was calculated, and the results were recorded in mol. wt% (molecular weight) units.

Statistical analysis

All analyses were performed using IBM-SPSS software (version 22.0; SPSS, Inc., Chicago, Illinois, USA), statistical analyses (two-tailed student's t-test) were performed to compare the ion content between the two groups and p-value < 0.05 was regarded as significant.

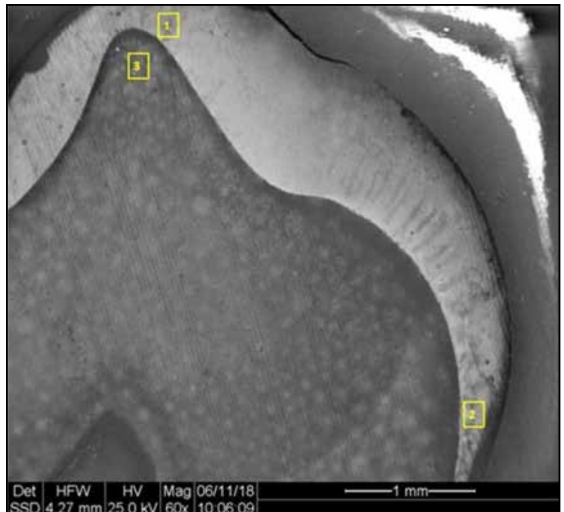
RESULTS

Enamel analysis presented higher levels of magnesium in pre and post-natal enamel in the control group compared with the study group but with no statistically significant differences (Table 1). The same was found for calcium levels in the post-natal enamel that were higher in the control group but with only borderline significance (p-value = 0.05). Children exposed to desalinated water showed higher levels of carbon and lower levels of phosphate in comparison with the control group but without statistically significant differences.

Dentin analysis (Table 2) revealed statistically non-significant differences, although there were higher levels of calcium and magnesium in the control group.

DISCUSSION

Excluding the post-natal enamel calcium level (of borderline statistical significance), no significant differences were found in the magnesium and calcium levels of primary enamel and dentin of children exposed to desalinated water in comparison to children exposed to ground water.



1-Pre-natal enamel

2-Post-natal enamel

3-dentin

Figure 1: Location of the areas on which chemical analyses were performed.

Table 1: Results of the analyses of enamel chemical components (C- carbon, O- oxygen, Mg- Magnesium, P- phosphate, Ca- calcium) pre and post birth (Post=post-natal, Pre=Pre-natal) in primary teeth of children exposed to desalinated water (termed here "Desalination") vs. primary teeth of children drinking ground water ("Control").

		Number of teeth	Mean Mol. wt%	Std. Deviation Lower Bound	95% CI		
					Upper Bound	P-value	
C pre	Control	7	12.6214	5.03266	7.9670	17.2759	0.878
	Desalination	7	12.9857	3.57057	9.6835	16.2879	
C post	Control	7	14.1443	6.02860	8.5688	19.7198	0.291
	Desalination	7	17.5329	5.43517	12.5062	22.5596	
O pre	Control	7	17.8500	1.37182	16.5813	19.1187	0.985
	Desalination	7	17.8200	3.87666	14.2347	21.4053	
O post	Control	7	17.1200	1.43612	15.7918	18.4482	0.489
	Desalination	7	17.9229	2.60402	15.5145	20.3312	
Mg pre	Control	7	0.1957	0.10293	0.1005	0.2909	0.840
	Desalination	7	0.1829	0.12906	0.0635	0.3022	
Mg post	Control	7	0.1543	0.06133	0.0976	0.2110	0.655
	Desalination	7	0.1357	0.08791	0.0544	0.2170	
P pre	Control	7	19.3229	1.32335	18.0990	20.5468	0.537
	Desalination	7	18.8600	1.39893	17.5662	20.1538	
P post	Control	7	19.1414	1.47771	17.7748	20.5081	0.298
	Desalination	7	18.1229	1.98482	16.2872	19.9585	
Ca pre	Control	7	50.0086	2.89540	47.3308	52.6864	0.864
	Desalination	7	49.7200	3.27563	46.6905	52.7495	

Table 2: Results of the analyses of dentin chemical components (C- carbon, O- oxygen, Mg- Magnesium, P- phosphate, Ca- calcium) pre and post birth (Post= post-natal, Pre= pre-natal) in primary teeth of children exposed to desalinated water (termed here "Desalination") vs. primary teeth of children drinking ground water ("Control").

		Number of teeth	Mean Mol. wt%	Std. Deviation Lower Bound	95% CI		
					Upper Bound	P-value	
Dentin C	Control	7	36.6500	9.68300	27.6947	45.6053	0.935
	Desalination	7	37.0486	8.10917	29.5488	44.5483	
Dentin O	Control	7	15.1500	2.03781	13.2653	17.0347	0.607
	Desalination	7	15.7271	2.04728	13.8337	17.6206	
Dentin Mg	Control	7	0.3486	0.18676	0.1758	0.5213	0.526
	Desalination	7	0.3986	0.07798	0.3265	0.4707	
Dentin P	Control	7	13.2714	2.45407	11.0018	15.5411	0.916
	Desalination	7	13.1386	2.17389	11.1281	15.1491	
Dentin Ca	Control	7	35.0100	5.63133	29.8019	40.2181	0.681
	Desalination	7	33.6886	6.09528	28.0514	39.3258	

Enamel and dentin are composed of biological apatite, which is not pure hydroxyapatite (HA), but rather a carbonate containing apatite together with other elements such as fluoride and magnesium. The distribution of the different ions throughout the crystal matrix affects the physical diffusion and dissolution characteristics of the apatite. In the present study we did not detect any significant differences in the enamel and dentin mineral content of primary teeth collected from children living in areas with exclusively desalinated water, except for higher calcium levels in the post-natal enamel of the control group. Our hypothesis that limited exposure to calcium and magnesium would affect the mineral content of enamel and dentin in primary teeth of children that were exposed to desalinate water was refuted.

Although calcium and magnesium levels in the enamel were higher in the control group, the results were statistically non-significant. Calcium borderline significant result was observed only in the post-natal enamel. These finding can be related to the fact that in uterus the mother provides the fetus with minerals essentials for tooth development[14], therefore the pre-natal enamel did not present significant difference between the groups. Calcium levels were slightly affected by drinking water in the post-natal period, a finding that may be associated with tooth resistance to the caries progression process. This should be further studied in epidemiological studies.

Unlike enamel, there is no simple way to distinguish between pre- and post-natal dentin. All dentin samples were from areas near the DEJ that probably developed prenatally; perhaps samples from other areas, closer to the pulp or more apically located, would have yielded different results.

In some areas of Denmark, the drinking ground water contains considerable amounts of calcium. Schroder et al. found strong associations between the incidence of sialolithiasis and the drinking water concentration of calcium and magnesium. Others have also confirmed correlations between drinking water calcium and magnesium and their concentration in saliva[15]. Sejdini *et al* found that saliva calcium level significantly influenced hard dental tissues defense mechanism and suggested that calcium molality decrease in saliva might play a significant role in caries occurrence. Magnesium levels correlated with calcium levels, favoring basic caries resistance[15]. Lin *et al* examined daily intake of calcium and phosphorus (P) and its association with primary caries index and concluded that the daily Ca/P ratio intake is an important factor for caries resistance[16].

According to the present study lower levels of calcium and magnesium may affect post- eruption maturation of the enamel but during the essential process of enamel mineralization the body compensates for the lack of minerals in the water.

In the last decade water quality standards and post treatment process are being designed by Israeli authorities to encounter the diminished amounts of minerals in the desalinated drinking water[17]. This process will supply water with alkalinity, Ca^{2+} and calcium carbonate precipitation potential values as required in the standard criteria, along with the addition of a threshold Mg^{2+} concentration recommended by the WHO[1]. The amount of calcium added will be 25 mg/l as Ca^{2+}, providing an additional 50 mg/day of calcium for a two-liters intake of drinking-water, either as beverage or in water absorbed into food during cooking[2]. These future changes may affect post-natal calcium levels in the enamel of primary teeth.

CONCLUSION

Due to the growing exposure to desalinated water, further large-scale studies are required to examine possible effects on the dental tissues both in the primary and permanent dentition.

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