Environ Health Prev Med (2015) 20:404–409
DOI 10.1007/s12199-015-0482-2

REGULAR ARTICLE

Caries prevalence and manganese and iron levels of drinking water in school children living in a rural/semi-urban region of North-Eastern Greece

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Received: 18 March 2015 / Accepted: 6 July 2015 / Published online: 19 July 2015

Abstract
Objective The aim of this study was to correlate different combinations of manganese (Mn) and iron (Fe) concentration in drinking water with prevalence of dental caries in both primary and permanent dentition, among school children with similar socio-demographic characteristics.
Methods Evros region, in North-Eastern Greece, was divided into four areas, according to combinations of levels of Mn and Fe in drinking water (High Mn–high Fe; High Mn–low Fe; Low Mn–high Fe; Low Mn–low Fe). Children of similar socio-economic background, attending either first or sixth grade (primary or permanent dentition, respectively) of elementary schools, were clinically assessed for caries by three dentists. Caries was defined by the use of dmft/DMFT index. A questionnaire answered by the parents was also analysed.
Results 573 children were included. Caries prevalence was high in both age groups (64.2 % with mean dmft 3.3 ± 3.6 in primary and 60.7 % with mean DMFT 2.3 ± 2.5 in permanent dentition, respectively). Residence in a high Mn–low Fe area was associated with a significant OR for caries in both age groups [OR (95 % CIs) for primary and permanent dentition was, respectively, 3.75 (1.68–8.37), $p = 0.001$ and 3.09 (1.48–6.44), $p = 0.003$], independently of factors like sugar consumption or brushing frequency.
Conclusion Prevalence of caries was high in general, and was associated with the combination of high Mn/low Fe levels in drinking water, independently of various socio-demographic factors.

Keywords Trace elements · Manganese · Iron · Drinking water · Dental caries

Abbreviations

dmft	Decayed, missing, filled teeth (for deciduous teeth)
DMFT	Decayed, missing, filled teeth (for permanent teeth)
Fe	Iron
Mn	Manganese

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Introduction

Dental caries is a common disorder, which negatively affects the quality of life, causing pain, chronic infections, eating disorders and sleep disturbances that may result in diminished growth. Despite the advances in oral health, dental caries is the most prevalent chronic condition among school children and is high elevated even in developed countries consisting a major public health issue [1].

The exact etiological agent of caries is multifactorial, resulting from metabolic activities of bacteria that form a dental biofilm, mainly *Streptococcus mutans*. At the same time, caries development is influenced by various environmental factors, with trace metal exposure being one of them [2]. Actually, it has been demonstrated a long time ago that differences in caries prevalence, observed in similar populations with low fluoride uptake, may occur due to the effect of other trace elements in the environment, and especially manganese (Mn) [3]. On the contrary, it was demonstrated that iron (Fe) has anticarietic properties [4].

The aims of this study were to assess the prevalence rates of caries and to describe behaviours and habits regarding oral hygiene, in children with similar socio-demographic characteristics, living in a large region of North-Eastern (NE) Greece and to define those factors that are associated with caries prevalence, focusing especially on the effect of different combinations of levels of Mn and Fe in the drinking water.

Methods

Study area and selection of communities

The region of Evros is located in the NE part of Greece, next to the Turkish and Bulgarian borders with a total population of 147,530 inhabitants. It comprises mainly semi-urban (towns) and rural settings and one major city (capital). The selection of communities was carried out in order to cover all possible concentrations of Fe and Mn in drinking water, as monitored by state authorities between 2000 and 2012.

After data processing and systematisation, the region (prefecture) of Evros was divided into four areas, according to the standards on Mn and Fe concentration in water intended for human consumption, set by EU Council Directive 98/83/EC and the Joint Ministerial Decision Y2/2600/2001 which incorporated the above Directive into the Greek legal order. Thus, areas were classified into the following categories: Area A: High exposure to both Mn and Fe. [Average concentrations of both trace elements exceed regulatory standards (Mn >50 mg/L, Fe >200 mg/L)]; Area B: High exposure to Mn. Fe concentration does not exceed regulatory standards. (Mn >50 mg/L, Fe < 200 mg/L); Area C: High exposure to Fe. Mn concentration does not exceed regulatory standards. (Mn <50 mg/L, Fe >200 mg/L); Area D: Average levels of both Mn and Fe do not exceed regulatory standards. (Mn <50 mg/L, Fe <200 mg/L). It should be noted that conducted measurements in all communities of Evros region have shown low fluoride concentrations (<0.1 mg/L) in drinking water.

The next step was the selection of villages and communities from each category. Since differences in caries prevalence between urban and rural areas are reported [5], urban settings (i.e. one capital city) were excluded. Therefore, only small, neighbouring, non-coastal, rural (villages) or semi-urban (towns) communities with the same geographical and climatological conditions, with primary school settings in operation were included. Additionally, since the Evros region is characterised by significant cultural diversity [6], all areas inhabited by religious or cultural minorities were excluded, in an effort to include communities inhabited by families of the same socio-economical status and background, as possible.

Drinking water exposure assessment

All municipal Water Supply and Sewage Companies of Evros prefecture were contacted and information regarding drinking water supply was obtained. It appeared that the residential population received drinking water from the taps, which was supplied by the main system via pipes that pump groundwater, using a water well. It is worth mentioning that until nowadays, all municipal water supply companies do not use a technique to eliminate Mn and Fe from water supplies.

Selection of subjects and exclusion criteria

Primary schools attended by children who resided in the selected communities were considered as potential study sites. Consent for inclusion in the study was obtained from the principals of the selected schools, who were also requested to obtain informed consent from parents and guardians prior to the beginning of the study. Children of elementary schools with either primary dentition (i.e. children of the first grade, aged 5–6 years) or permanent dentition (i.e. sixth grade, aged 11–12 years old) were included. Children whose parents did not fill the distributed questionnaire or did not consent to participating in the study were excluded from the study. Moreover, children who were not lifelong residents of the area and children whose parents reported everyday use of bottled water or water from a private well were also excluded. The clinical examination of children took place from September 2010 to May 2011. Experimental procedures were approved both by the Ethical Committee of our Institution and by the Ministry of Education and Religious Affairs of Greece. (Registration number 1850/C7-09.01.2009).

Questionnaire

Parents were asked to complete a detailed questionnaire on children's demographics (gender, residence), social status of the family (i.e. parents' education, current occupation) along with habits, attitudes and behaviours, (i.e. water consumption, tooth brushing frequency, visits to the dentists, attitude towards oral hygiene, etc.).

Dental examination

Dental examination was performed by three dentists (one of them a specialised orthodontist), on school premises. Prior to this study, the examiners had been calibrated against each other, with good to excellent kappa values. The children were examined in a sitting position under natural light or with a portable LED head light lamp with a use of standard mouth mirror and a periodontal probe applied (WHO CPI probe) in order to remove any food debris. A new probe was used for each examination. Any fissure or enamel surface in which the explorer stuck was regarded as carious; other surfaces were listed as sound.

Caries was assessed by the decayed, missing, and filled teeth index, abbreviated as dmft for deciduous and DMFT for permanent dentition, as established by WHO guidelines [7]. Dental caries was defined by the presence of a dmft/DMFT value >0, while children with dmft/DMFT = 0 were characterised as caries free.

Statistical analysis

Analyses were performed separately for the two age groups. Descriptive statistics were used. For comparisons between subgroups, either Student *t* test or ANOVA (for more than two subgroups) was used, while for differences in prevalence, Pearson's χ^2 test was applied.

For the main outcome (i.e. factors affecting caries prevalence), binary logistic regression analysis was applied in order to define the caries-promoting factors among examined children. The following parameters were examined: area according to Mn/Fe concentration (area D used as control), gender (females used as controls), brushing frequency (times/day), consumption of sugar products frequency (number of sweets/week), child's awareness of oral hygiene importance (full understanding of hygiene importance was used as control), parents' attitude towards oral hygiene (those considering it important were used as control). Statistical significance was set at 0.05 level.

Results

In the selected communities, 629 children studying either in first ($n = 317$) or the sixth grade ($n = 312$) of the primary school were invited to attend, and finally 573 were included in the study, since they filled all inclusion criteria and provided parents' consent. (Response rate 91.1 %).

142 children (76 with deciduous and 66 with permanent dentition) from area A, 145 children (70 with deciduous and 75 with permanent dentition) from area B, 141 children (83 with deciduous and 58 with permanent dentition) from area C and 145 children (63 with deciduous and 82 with permanent dentition) from area D were included.

Regarding gender distribution, caries prevalence among girls was 68 % ($n = 104$) and 63.6 % ($n = 140$), for deciduous and permanent dentition, respectively. In boys, prevalence was 60 % ($n = 84$) and 57.9 % ($n = 140$), respectively. However, these differences were not statistically significant ($p = 0.155$ and $p = 0.328$).

Prevalence ratios of dental caries and dmf/DMFT scores in the four examined areas

Table 1 displays prevalence of included children with dmft/DMFT > 0 and calculated prevalence ratios. As seen in this table, the prevalence ratio of dental caries was significantly higher for children of both age groups in area B (who consumed water containing high Mn/low Fe concentrations), compared to area D. Accordingly, dmft and DMFT values in area B were higher. According to ANOVA analysis, this difference was statistically significant among the 6-year-old group ($p = 0.004$), while for 12-year-old group difference in DMFT was not ($p = 0.138$).

On the other hand and contrarily to what initially expected, no cariostatic effect of Fe was observed in the presence of low Mn concentrations (prevalence ratios of area C/D) in both age groups. Actually, no difference in caries prevalence in the deciduous teeth was observed between children from communities in area C (high Fe–low Mn concentration), which was 53.3 % and children from area D (control group), which was 53.1 %. Similarly, in permanent dentition of children in area C, caries prevalence was 53.4 %, while in area D, this was 54.3 %. As seen in Table 1, there was also no appreciable difference in the caries prevalence of deciduous and permanent teeth between children who consumed water with combined high Mn–Fe concentrations (area A), and those from communities with low Mn–Fe content in drinking water (area D).

Caries prevalence and trace elements concentration

Binary regression analysis was designed in order to define the parameters that promote caries among examined children, as described in the "Methods" section. This analysis was applied twice for children with deciduous and permanent dentition and is displayed in Table 2.

As seen in this table, for the group of the 6 years old, the odds for caries were higher in area B (high Mn–low Fe concentrations in drinking water) in comparison to the control area (area D: low Fe–low Mn): OR 3.750 (95 %

Table 1 Values of dmft/DMFT, dental caries prevalence and prevalence ratios (area D used as control) of children with primary and permanent dentition

	Children with primary dentition (1st grade of elementary school) dmft (mean ± SD)	Children with permanent dentition (6th grade of elementary school) DMFT (mean ± SD)
Total	2.3 ± 2.6	3.3 ± 3.6
Area A	3.2 ± 3.7	2.0 ± 2.4
Area B	4.5 ± 3.9	2.9 ± 2.2
Area C	3.0 ± 3.4	2.0 ± 2.6
Area D	2.3 ± 2.9	2.3 ± 2.7
p	0.004	0.138
	Children with primary dentition (1st grade of elementary school) dmft >0 [*n* (%)]	Children with permanent dentition (6th grade of elementary school) DMFT >0 [*n* (%)]
Total	188 (64.2 %)	170 (60.7 %)
Area A	48 (63.2 %)	36 (54.5 %)
Area B	56 (80 %)	59 (78.7 %)
Area C	50 (53.3 %)	31 (53.4 %)
Area D	34 (53.1 %)	44 (54.3 %)
p	0.011	0.003
	Children with primary dentition (1st grade of elementary school) Prevalence ratio vs. Area D (95 % CIs)	Children with permanent dentition (6th grade of elementary school) Prevalence ratio vs. Area D (95 % CIs)
Area A	1.19 (0.892–1.584)	1.00 (0.75–1.35)
Area B	1.51 (1.16–1.95)	1.45 (1.15–1.83)
Area C	1.13 (0.85–1.51)	0.98 (0.72–1.34)

Area A High Mn–high Fe area, *Area B* High Mn–low Fe area, *Area C* Low Mn–high Fe area, *Area D* Low Mn–low Fe area

CIs 1.680–8.372); $p = 0.001$. On the contrary, no favourable effect in a statistically significant way was detected in area C (high Fe–low Mn): failing to demonstrate the anticarietic properties of Fe in the absence of Mn. Behavioural factors, like brushing frequency or consumption of sugar-containing products, did not predict this outcome (χ^2 3.243 and $p = 0.356$; χ^2 2.276 and $p = 0.518$ respectively). Likewise, none of the rest of the examined socio-demographic or behavioural factors produced a significant effect.

The same statistical analysis in children with permanent dentition revealed that only exposure to high Mn/low Fe concentration in drinking water (children living in area B) was associated with higher OR for caries prevalence. Likewise, all other parameters did not produce a significant effect.

Discussion

To the best of our knowledge, this is the first study addressing the combined effect of Mn and Fe on dental caries development. High Mn concentration in drinking water was found to increase dental caries prevalence in children of the same socio-economic background, with either deciduous or permanent dentition, in line with previous works, [3, 8]. On the other hand, Hussein et al. [9], which studied salivary levels of various trace elements concluded that Mn concentrations did not differ between children with or without caries. Likewise, an older work by Duggal et al. concluded that Mn did not have a consistent relationship with caries prevalence [10].

Contrarily to previous studies reporting a cariostatic effect of Fe [4, 11], the findings of the present study did not confirm the negative correlation between caries prevalence and increased Fe concentration in drinking water. One possible explanation of these findings is that when groundwater is pumped up to the surface, soluble Fe^{2+} compounds contact air and transform into the insoluble Fe^{3+}, which poses difficulties in microbial Fe uptake [12]. Hence, poor solubility of Fe^{3+} hinders its participation in bacterial metabolism and the elaboration of its cariostatic properties. In line with this, both works by Hussein et al. and Duggal et al. [9, 10] reported that Fe concentrations in saliva have no association with caries prevalence. Finally, no significant differences were found in dmft/DMFT between children exposed to high concentration of both Mn and Fe in drinking water and those of the control group. This effect can probably be explained by findings of

Table 2 Logistic regression analysis predicting caries occurrence depending on area of residence, gender, brushing frequency, consumption of sugar-containing products and parents' attitude in children with primary and permanent dentition

Predictor	B	Wald	OR	95 % CI	*p*
Children of the first grade					
Area		10.528			
Area A vs. D	0.447	1.514	1.564	0.767–3.190	0.218
Area B vs. D	1.322	10.405	3.750	1.680–8.372	0.001
Area C vs. D	0.576	2.555	1.779	0.878–3.603	0.110
Gender	−0.392	2.252	0.676	0.405–1.127	0.133
Brushing frequency (times/day)	0.223	1.813	1.250	0.903–1.730	0.178
Sugar consumption (sweets/week)	0.157	1.032	1.170	0.864	1.583
Parents' attitude on oral hygiene		3.916			0.141
Not important vs. important	−0.081	0.047	0.922	0.444–1.913	0.828
Of medium importance vs. important	0.470	1.635	1.600	0.779–3.288	0.201
Children of the sixth grade					
Area					
Area A vs. D	0.082	0.056	1.086	0.549–2.149	0.813
Area B vs. D	1.127	9.045	3.087	1.481–6.437	0.003
Area C vs. D	−0.004	0.000	0.996	0.495–2.004	0.992
Gender	−0.236	0.833	0.790	0.476–1.310	0.361
Brushing frequency (times/day)	−0.253	2.378	0.776	0.563–1.071	0.123
Sugar consumption (sweets/week)	−0.008	0.003	0.992	0.748–1.317	0.957
Parents' attitude on oral hygiene		5.714			0.057
Not important vs. important	−0.036	0.010	0.965	0.470–1.982	0.922
Of medium importance vs. important	0.603	2.957	1.828	0.919–3.634	0.086

previous scholars, which noted that Fe competitively inhibits microbial Mn uptake.

A major finding in our study is that the combination of high Mn and low Fe in drinking water is certainly associated with higher prevalence of dental caries in children with either primary or permanent dentition. This finding was confirmed with two different types of statistical analysis and was independent of different other confounders, since included children were of the same socio-economic background.

Other findings of this study that certainly need to be highlighted are the following: Caries prevalence was, in general, high in our cohort, estimated at 64.2 % for 6-year olds, which is significantly higher to previous reports from Greece [13, 14], where caries prevalence was estimated at 54 % for 6-year olds and 46 % for 5-year olds. Regarding children with permanent dentition, the prevalence in our studied population (60.7 %) was higher than that reported by Chatzistavrou et al. [13] (54 %), but similar to that recently reported by Oulis et al. [14] (63 %). A study conducted also in our area revealed a falling trend for caries prevalence from 81 to 50 % in a study of consecutive records of 11.5-year olds between 1989 and 2001, in a public health centre of the area [15].

A certain limitation is the ecological design of the study, which limits our ability to exclude inter-individual variability, and apply our findings to an individual level. Nevertheless, this study is important in highlighting the cariogenic potential of Mn in combination with low Fe concentration, as was proved by two different statistical analyses and motivate for further analytical exploration.

In conclusion, dental caries remains globally a major problem of public health and genuine efforts should be made, in order to find an effective measure to control its expansion. Despite the fact that Mn seems to have a cariogenic potential, scientific attention focuses mainly on its neurological effects [16] and tends to neglect its impact on dental health. In the light of the above, the results of the present study have a practical significance, since they demonstrate that environmental differences in trace metal exposure between various communities are associated with an increased risk of caries in the deciduous and permanent dentition of children. Nevertheless, no definite indications that Fe decreases caries development were found, and we believe that additional studies are necessary in order to determine the cariostatic properties of Fe in drinking water.

Acknowledgments We would like to thank Mr. Panagiotis Michilidis for his help in carrying out the statistical analysis of this study and all children, parents and school staff of the participating communities for making this work successful.

Conflict of interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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