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Relationship between fluoride intake in Serbian children living in two areas with different natural levels of fluorides and occurrence of dental fluorosis

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A B S T R A C T

The amount of fluoride present naturally in drinking water is highly variable, being dependent upon the individual geological environment from which the water is obtained. Chronic exposure to exceeding fluoride doses induces set of toxic effects, i.e. fluorosis. The aim of this study was to examine fluoride content in water and in the most frequently used vegetables, potato and bean, grown in two different Serbian regions, i.e. control region (Valjevo) and high naturally occurring fluoride region (Vranjska Banja), and moreover, to correlate estimated daily intake with dental fluorosis occurrence as an adverse effect of fluoride exposure of schoolchildren in Serbia. Study confirmed significant difference in fluoride content in water, potato and bean, consumed by 12-year-old children in two investigated municipalities. Results of the study indicated positive and statistically significant correlation between daily intake of fluoride and dental fluorosis level in the fluorotic municipality of Vranjska Banja ($r = 0.61$; $p = 0.000017$). Obtained relationship could be evaluated by means of binary logistic regression analysis, whereas probability for fluorosis occurrence could be predicted using the following equation: fluorosis occurence (%) = $(34.852 \times C_{water} - 12.644 \times C_{potato} - 9.362 \times C_{bean} - 7.673) \times 100$ (Chi-Square (3) = 33.033; $p < 0.001$).

1. Introduction

From the first reports by Dean and colleagues published in the 1930s, fluorides are still considered as the most effective mean in reducing dental caries. However, assessment on the quantitative relationship between dental fluorosis and fluoride intake conducted by WHO (1994), initiated a large number of further studies on this topic. Studies in the 1950s and 1960s estimated that 7–16% of children reared in an optimally fluoridated area showed signs of mild or very mild fluorosis in the permanent dentition, which is confirmed by, recently performed meta-analysis (McDonagh et al., 2000; Whelton et al., 2004). Public health programmes, seeking to maximize the beneficial effects of fluoride on dental health through the introduction of fluoridated drinking water have, at the same time, strived to minimize its adverse effects on teeth (WHO, 1987; Petersen, 2003; Jones et al., 2005). Dental fluorosis is hypomineralisation of the enamel caused by intake of an amount of fluoride that is above optimal levels during enamel formation (Pendrys, 2000). From various publications, it became clear that dental fluorosis varies within the population. Factors responsible for these variations could be fluoride intake by drinking water, dietary intake, especially intake of food grown in soil or irrigated with water rich in fluoride. However, systemic and topical fluoride administration, could also contribute to fluorosis occurrence (WHO, 2002).

The aim of this study was to examine fluoride content in water and in the most frequently used vegetables, potato and bean, grown in two different Serbian regions, *i.e.* regions with low and high naturally occurring fluoride, and moreover, to correlate estimated daily intake with dental fluorosis as an adverse effect of fluoride exposure of schoolchildren in Serbia.

2. Materials and methods

2.1. Subjects and sample collection

This study was conducted in two areas in Serbia with significantly different levels of fluoride in drinking water, municipalities of Valjevo and Vranjska Banja. Region of Vranjska Banja is well documented particularly in national literature as fluorotic endemic area, whereas region of Valjevo as non-fluorotic area (Ivanovic et al., 1991). It should be added that in these areas there are no other relevant sources of exposure, *i.e.* industries that cause the pollution of the environment by fluoride emission.

Abbreviations: CFI, community fluorosis index; DI, daily intake; EPA, environmental protection agency; FAO, Food and Agricultural Organisation; FE, fluoride exposure; NOAEL, non-observed adverse effect level; TISAB, total ionic strength adjustment buffer; WHO, World Health Organisation.

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Seventy six healthy, 12-year-old schoolchildren, both gender (33 boys and 43 girls) were participated in our study. Prior to the study, the objectives of the study and entire procedure were thoroughly presented to their parents and after that written consent was obtained. Ethical Committee of School of Dentistry, Belgrade University, approved this study (No. 727/1; 2006).

Samples of water were collected from the community water supplies and wells in spring season in 2006. Portions of soil were taken near well springs from the depth of 20–30 cm. Vegetable items used in this study (potato and bean) shown to participate predominantly in schoolchildren diet in both regions, were purchased from the local markets (FAO, 2006). Until analysed, samples of water were stored in polyethilen bottles, whereas samples of soil and vegetables were stored in plastic bags in dry and dark place at the temperature of 4 °C.

2.2. Sample preparation and fluoride determination

In water samples, fluoride was determined directly using ion-selective electrode (Orion 9609, Cambridge Mass, USA). Soil, potato and bean samples were previously homogenised, weighted and than mineralised. Complete analytical procedure for fluoride determination in either water, vegetable or soil samples were published by Nedeljkovic et al. (1991). A portion of homogenised samples of 2–3 g was transferred into the diffusion cell and after that dried in a laboratory oven at 55 °C. On the diffusion cell cover, thin layer was formed by the evaporation of 0.5 mL 1 mol/L NaOH in ethanol in laboratory oven at 55 °C. Mineralisation was done using 1.5 mL 40% $AgClO_4$ and 1.5 mL 70% $HClO_4$. The diffusion cell was immediately covered. During the micro diffusion process (for 24 h at 55 °C), the fluoride, released under the influence of 70% $HClO_4$, reacted with the NaOH to form NaF. The constituents of the thin layer coating the diffusion cell cover was dissolved in 5 mL of de-ionised water, than quantitatively transferred into a polyethylene dish and mixed with the TISAB buffer solution in ratio 1:1. TISAB was made of 57 mL of glacial acetic acid, 58 g NaCl, 300 mg of sodium citrate and water up to 500 mL. After dissolution, the solution was neutralized to pH 5–5.5 with 5 mol/L NaOH, while immersed in a cooling water-bath. The buffer was than diluted to 1 L with water.

The separated fluorides were subsequently determined by composite fluoride ion-selective electrode (Orion 9609, Cambridge Mass, USA). All chemicals, obtained from commercial sources, were of analytical grade purity.

2.3. Examination of dental fluorosis

Randomised sampling was performed for examination of dental fluorosis occurence expressed as CFI score.

$$\mathrm{CFI} = \frac{\sum(\text{frequency} \times \text{statical weight})}{\text{number of individuals}}$$

Dental examinations were made using dental mirrors and probes, under indirect sunlight by one calibrated and well trained dentist. Except for third molars, all partly or fully erupted teeth were examined. The teeth of the schoolchildren were assessed for fluorosis, using Dean's criteria according to the WHO guidelines (WHO, 1987). Criteria for Dean's fluorosis index are described as follows. Normal (0): the enamel represents the usual translucent semivitriform type of structure, and the surface is smooth, glossy, and usually of a pale creamy white color. Questionable (1): the enamel discloses slight aberrations from the translucency of normal enamel, ranging from a few white flecks to occasional white spots. This classification is utilized in those instances where a definite diagnosis of the mildest form of fluorosis is not warranted and a classification of "normal" is not justified. Very mild (2): small opaque, paper white areas scattered irregularly over the tooth but not involving as much as 25% of the tooth surface. Frequently included in this classification are teeth showing no more than about 1–2 mm of white opacity at the tip of the summit of the cusps of the bicuspids or second molars. Mild (3): The white opaque areas in the enamel of the teeth are more extensive but do not involve as much as 50% of the tooth. Moderate (4): all enamel surfaces of the teeth are affected, and the surfaces subject to attrition show wear. Brown stain is frequently a disfiguring feature. Severe (5): includes teeth formerly classified as "moderately severe and severe." All enamel surfaces are affected and hypoplasia is so marked that the general form of the tooth may be affected. The major diagnostic sign of this classification is discrete or confluent pitting. Brown stains are widespread and teeth often present a corroded-like appearance (WHO, 1987). The kappa index for repeated scores was 0.82, when considering all levels of fluorosis (0–5).

2.4. Exposure assessment

In order to evaluate apparent risk of dental fluorosis on the bases of estimated dietary exposure, two different sets of data were used in the present study: fluoride concentration database and consumption database. Fluoride content in water and vegetables was originally measured in this study. Estimated daily intake of fluoride was calculated using formula based on daily intake data for potato and bean taken from the GEMS/Food Consumption Cluster Diets built up by the FAO (FAO, 2006). Integration of food consumption database and contaminant concentration data has been done by the use of a simple, deterministic procedure. Average body weight of 12-year-old children was obtained from the scale (Fisher et al., 2001), which recommends that body weights of 12-year-old children at 50th percentile are 37.7 kg and 40.5 kg, for boys and girls, respectively, and at 90th percentile are 49.0 kg and 54.7 kg, for boys and girls, respectively.

The following formula was used for calculation of FE

$$\mathrm{DI} = \text{daily intake (kg)} \times \text{fluoride concentration (mg/kg)}.$$

$$\mathrm{FE} = \mathrm{DI}/\text{body weight at 50th or 90th percentile (mg/kg bw)}.$$

2.5. Statistical analysis

Statistical analysis was performed using a Chi-squared test. Pearson's correlation was performed for the relationship between fluorosis score and fluoride content in water and vegetable items. Fluoride content in all samples was correlated with prevalence of dental fluorosis and consequently binary logistic regression was used for probability of fluorosis occurrence. Values of $p < 0.05$ were considered significant. All statistical calculations were performed using STATISTICA version 5.0.

3. Results

Approximately 230 km far from Valjevo municipality, region of Vranjska Banja is well known as fluorotic endemic area (Fig. 1). Soil fluoride contents in Valjevo and Vranjska Banja regions reach the values of 9.83 ppm and 29.63 ppm, respectively, indicating significant difference between these two regions with respect to natural resources of certain minerals that are principal donors of fluoride.

Regarding to the region and gender, results of Pearson's Chi-Square statistical analysis, did not show statistically significant differences (Table 1).

Results of fluoride content in water indicated significant difference between two regions included in the study: in the region of Vranjska Banja, fluoride levels in water were more than 100 times higher than in Valjevo region, which was considered as control one. Consequently, fluoride intake by water was higher in Vranjska Banja area. Concerning consumption rate of potato and been, there is significant difference between daily intakes of these two vegetables. High value for potato daily intake of 244 g could be ascribed to the certain socio-economic status and to the specific dietary habits due to intake of vegetable carbohydrates are assured predominantly by potato consumption. Therefore, potato intake contributes to total daily intake of fluorides largely (Table 2).

Total daily intake of fluoride was further recalculated taking into account body weight. In comparison with NOAEL value of 0.06 mg/kg bw/day (EPA, 2002), obtained values for fluoride exposure in the region of Vranjska Banja were higher, whereas values at 50th percentile representative for the population of schoolchildren in Valjevo, were slightly higher than NOAEL (Fig. 2). The NOAEL value of 0.06 mg/kg bw/day, given by EPA was calculated on the basis of results obtained in a study, conducted among children, consuming fluoride via drinking water, where dental fluorosis was used as critical effect (EPA, 2002).

Fig. 1. Sampling locations: fluorotic region of Vranjska Banja and control region of Valjevo.