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FLUORIDE CONCENTRATION IN DENTIN OF HUMAN PERMANENT TEETH

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ABSTRACT: Among various types of biological materials used in the assessment of the risk of fluoride (F) exposure, special importance is given to mineralized tissues which reflect long term accumulation of F. The aim of the present paper is to investigate F concentration in dentin of permanent teeth of residents of Poland in relation to environmental factors. The study material consisted of teeth (incisors, canines, premolars, and molars) from 74 residents of Poland, extracted due to medical reasons with the exception of dental caries. Following teeth extraction, the patients were asked to complete a questionnaire concerning date of birth (before or after the cessation of drinking water fluoridation in Poland in 1995), use of F-containing toothpaste in oral hygiene, and consumption of F in certain food products. Determination of F content in teeth was conducted using the potentiometric method. There were significant differences in F concentration in dentin of patients born before or after the year 1995. The use of F-containing toothpaste was found to have a significant effect on F content in dentin. The study did not confirm significant differences between frequency of marine fish and tea consumption and an increase of F content in dentin.

Keywords: Dentin; Fluoride; Marine fish; Tea; Toothpaste; Water fluoridation.

INTRODUCTION

The composition of dentin is 70% nonorganic compounds and, approximately, 30% organic compounds and water.[1] The share of apatite crystals is lower in dentin than in enamel. It is more hydrated and consequently its ability to accumulate fluoride (F) is greater. Dentin, particularly the coronal dentin, can be the best indicator of total F load. It contains only F accumulated in the tissue as a result of systemic intake.[2] Moreover, dentin does not undergo resorption, and is easier to obtain than bone. It is also protected against F exposure from the oral cavity and the surrounding bone by the layer of enamel and cementum.[2]

According to Vieira et al., the F concentration in the outer layers of dentin is greater than in the inner ones.[3] Dentin F levels decline progressively from the pulpal surface to the dentin-enamel junction (DEJ). Bulk enamel (all the enamel from a tooth) F concentrations mainly reflect the level of F exposure during tooth formation, while dentin and bone F concentrations are generally proportional to the long-term level of intake.[4] In many cases, dentin is an accessible tissue, and, as discussed above, its F concentration profile and changes with age appear to be similar to those of cortical bone.[4] However, bone biopsies are invasive and not practical for epidemiological studies. As dentin is accumulated throughout the entire life span of an organism, F concentration increases with age.[3,4] Consequently, dentin can constitute a useful tissue for the purpose of assessment of long-term F accumulation in the human body.[4]

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The aim of the present paper is to investigate F concentration in dentin of permanent teeth extracted from residents of Poland born before or after the cessation of drinking water fluoridation in Poland in 1995, and the determination of the presumed relationships between F concentration in dentine and F-rich components of diet as well as the use of F-containing toothpaste by patients.

MATERIAL AND METHODS

All procedures were in accordance with the ethical standards of the Institution and/or the National Research Committee and with the 1964 Helsinki declaration. The patients received a thorough explanation of the experimental rationale, clinical procedures, and possible complications and informed consent was obtained. All experimental protocols were approved by the Local Ethics Committee of the Pomeranian Medical University, Szczecin, Poland (approval number KB-0012/57/16).

The study material consisted of permanent teeth (incisors, canines, premolars, and molars) extracted due to medical reasons (e.g., orthodontic, post-traumatic, lack of conservative treatment options) from 74 residents of Poland. Following extraction, the patients were asked to complete a questionnaire regarding: date of birth (before/after the cessation of drinking water fluoridation in Poland), use of F-containing toothpaste, and frequency of tea and marine fish consumption (Figure).

PATIENT QUESTIONNAIRE

1. Date of birth (before or after the cessation of drinking water fluoridation in Poland)
2. Eating habits:

Consumption of marine fish
- Never ☐
- 1-2 times a month ☐
- 1-2 times a week ☐
- More often ☐

Consumption of tea
- Never ☐
- 1-2 times a month ☐
- 1-2 times a week ☐
- More often ☐

3. Do you use F-containing toothpaste in oral care?
   - No
   - Yes

Figure. The patient questionnaire used in the study.

The teeth selected for the analysis were stored in a fridge at a temperature of 4ºC until the moment of the analysis. Then, the teeth were cleansed, dried, and degreased with acetone. In the next stage, using a dental drill, coronal dentin including the chamber and pulp residue was prepared from each tooth. Each tooth fragment

selected for the analysis was weighed (with an accuracy of 0.0001 g) to identify air-dry weight which was determined by means of the weighing method. Next, the teeth were dried to a constant weight in a laboratory drier at 105ºC for 4 days. The teeth were then ground in an agate mortar and test portions of approximately 1 g each were prepared. Each sample was weighed with an accuracy of 0.0001 g, treated with 1 mL of perchloric acid, and shaken at the temperature of 90ºC for 1 hr. When cooled, a 0.5 mL sample was transferred to a plastic container and supplemented with 2 mL of sodium citrate solution and 2.5 mL of TISAB II solution. After mixing, the potential was measured for 10 min in each sample: 5 min before and 5 min after the addition of the respective standard. The F concentration in a sample was calculated by means of the differences between the determined potentials, the mass of the sample, and the concentration of the respective standard added. The correctness of the analytical procedure was controlled by determining the F ions in reference materials of known concentration, i.e., standard NaF solutions at concentrations of 0.1, 1, and 10 mg/L by Orion (USA).

Statistical analysis was conducted using Statistica 12. The concentrations of F in dentin are summarized in Table 1. Using the Shapiro-Wilk test, the normality of the distribution of the analyzed variables was not confirmed. A non-parametric U-Mann Whitney test was used to compare the two independent groups. The test included determination of the mean F concentration in dentin of residents of Poland born before or after the cessation of drinking water fluoridation in Poland, comparison of results with respect to frequency of marine fish and tea consumption, and the use of F-containing toothpaste. The significance level of the test (p) was 0.05.

RESULTS

The concentration of F in dentin was found to be varied and ranged from 40.57 to 393.76 mg/kg dry weight (dw). There were statistically significant differences in the F concentration in the dentin of patients born before and after the year 1995—the year of complete cessation of drinking water fluoridation in Poland. In patients born before the year 1995, the median concentration of F in the dentin was higher and amounted to 128.1 mg/kg dw. The corresponding concentration in the patients born after the year 1995 was 94.06 mg/kg dw (Table 1). Significant differences were found between the median F concentration in the dentin of patients using F-containing toothpaste in comparison with those who did not use such toothpaste (124.57 and 91.12 mg/kg dw, respectively) (Table 1). The study also investigated the relationship between the frequency of fish and tea consumption and the changes in the F concentration in the dentin. Despite the lack of a statistically significant relationship ($p>0.05$), it was found that the F concentration was higher in the patients declaring a consumption of marine fish with the frequency of more than 2 times a week as compared to those who declared they did not eat marine fish (median=124.45 vs. median=97.91). In this regard, the correlation was found to be very weak and positive (rho=0.204; p=0.082). Smaller differences were found between the patients who declared a tea consumption with a frequency of more than once a week and those who declared that they drank tea less frequently than once a month (median=104.38 vs median=117.13). In this respect no correlation was identified ($p>0.05$).

Table 1. Descriptive statistics, including selected variables, concerning fluoride (F) concentration (mg/kg) in dentin

Variable	Total	Use of F-containing toothpaste		Date of birth — before or after the cessation of drinking water fluoridation in Poland (1995)	
		No	Yes	After 1995	Before 1995
n	74	20	54	36	38
%	96.10	25.97	70.13	46.75	49.35
X (Mean F) (mg/kg)	130.29	99.14	141.83	99.81	159.18
SD	71.25	45.75	75.76	39.40	82.38
Median F (mg/kg)	114.32	91.12	124.57	94.06	128.10
Q_1	87.84	69.34	104.05	69.87	112.73
Q_3	150.25	104.35	158.46	121.98	167.37
Minimum F (mg/kg)	40.57	40.57	55.33	40.57	58.70
Maximum F (mg/kg)	393.76	236.34	393.76	232.23	393.76
Mann-Whitney test		–3.079		–4.110	
p		0.002		0.000	

DISCUSSION

Various factors may affect F concentration in human dentin: age, sex, metabolism, concomitant dental diseases, F supplements, F in drinking water, and the type of dentition (primary, permanent) as well as the duration and level of exposure to F.[5,6] Table 2 shows the identified value ranges of the average F concentration in human dentin (healthy individuals, without F supplementation, and residents of areas not polluted with F).

It should be noted that although the mean F concentration in dentin identified by the aforementioned authors showed a high variability, none of these studies identified cases of fluorosis. Therefore, as can be assumed, these values are well tolerated by dentin and have no toxic effect. The high variability of the aforementioned F

concentration levels in dentin can be connected with various environmental conditions as well as the origin and method of sample preparation.

At the same time, there is a noticeable shortage of studies on F content in human dentin and the existing, most recent data concern the period of at least 10 years ago. That is why the present study has considerable value and contributes to the issue of the assessment of the present exposure to F of the population of Poland. According to the report by the Polish Ministry of Health, as compared to other countries, the population of Poland tops the rankings in terms of the highest frequency of dental caries. Regrettably, such a high incidence of dental caries recorded in Poland, i.e., 86.9%, is exceptional—compared with 60.3% recorded in South Africa and 30% in the USA. In Poland, the percentage of edentulous adult people between 35–44 and 65–74 years of age is rising rapidly. Additionally, in these periods of life, the average number of preserved natural teeth is decreasing sharply and there is a very high incidence of periodontal diseases. The main reason behind these health problems may be a general low health awareness in the population in Poland.[12]

Table 2. Mean fluoride (F) concentration (mg/kg) in dentin of primary and permanent human teeth not exposed to fluoride

Mean F concentration (mg/kg)	Type of denitn	Country	Source
130	Permanent teeth	Poland	Present paper
792	Primary incisors	USA	De la Cruz et al.[7]
768	Primary molars	North Carolina	
378	Primary teeth	Brazil	Sá Roriz Fonteles et al.[8]
99	Primary teeth	Finland	Lakomaa et al.[9]
76	Permanent teeth		
133.5	Permanent teeth	USA Virginia	Derise et al.[10]
500	Permanent teeth	South Africa	Ockerese et al.[11]

Nowadays, there is a worldwide trend of decreasing the admissible F concentration in water and air. In the 1990s, in many countries (including Poland), water fluoridation ceased. In Poland, the admissible F content in water is 1.5 mg/L, and the reference dose for F is 0.06 mg/kg.[6] Similar norms are applied in other European Union countries. The present study found that there is a statistically significant difference in the F concentration in the dentin of patients born before the year 1995, i.e., in the period of drinking water fluoridation in Poland, in comparison with patients born after 1995. This is in line with findings by Ockerese et al. who established the connection between F concentration in drinking water and dentin.[11] A higher concentration of F in dentin was found in individuals drinking artificially fluoridated water as compared to those who did not. However, it must be taken into

account that the established differences may also result from the age of the individuals and, consequently, the fact that F tends to accumulate in mineralized tissues with age.[4]

F enters the human body with water (60%), food (35%), and inhaled air (5%). The digestive and respiratory systems play a significant role in the uptake of F. The daily dose of F absorbed by the human organism depends on the nature of the diet and the climate with an approximate range of 0.6–2 mg.[13] Ninety % of ingested fluoride is absorbed into the blood stream with 10% excreted in the faeces.[14] Nearly half of the absorbed fluoride is quickly taken up by bone and growing teeth while 45% is filtered in the kidneys, stored in the bladder and eventually excreted.[14] Only a small amount of fluoride, 0.05%, is excreted in other bodily fluids (breast milk, sweat, and saliva).[14] Ninety-nine % of the F accumulated in the human body is present in bone and other mineralized tissues.[3,4] The F ions are easily accumulated in the mineralized tissue due to the exchange of hydroxyl ions (a component of apatite forming the structure of bones and teeth) for F ions which are incorporated into the apatite crystals in the exchange process.[4] Different sources of F as well as an appropriate diet and the selection of proper oral care products can result in significant variations in the level of accumulated F. This individual variability can lead to an excessive accumulation of F in an organism. These factors pose a considerable challenge to the assessment of F exposure. Caries preventing products (toothpaste, dental sealant, varnish, and mouthwash) are an abundant source of F pollution. These products may contain 1,000–1,500 mg F/kg in the form of NaF or sodium monofluorophosphate.[4]

The present study identified a statistically significant difference in the median F concentration in dentin in people using a F-containing toothpaste in comparison to those who did not use such oral hygiene products (124.57 and 91.12 mg/kg dw, respectively). Arguably, it is connected with F permeating the mucus membrane, and reaching the bloodstream, the pulp and consequently the dentin.[4] The analysis of F concentration was performed on teeth with coronal dentin and the chamber with the pulp, which may have affected the obtained result, as the dentin areas localised near to the pulp show a higher content of F.[7] The excessive accumulation of F can cause dental fluorosis, and in extreme cases skeletal fluorosis and tumours of the skeletal tissue.[3] The study on human teeth has shown a relationship between the F content in dentin and the occurrence of dental fluorosis.[3] It was observed that high F doses in rat feed resulted in a severe disturbance of the dentin mineralization process, manifested by the occurrence of extensive interglobular spaces filled with the proteins of organic matrix of dentin and a blurred interface between unmineralized predentin and mineralized dentin.[1] Apart from the extensive interglobular space, there were wide decalcification stripes which identified the regions of poor mineralization.[1] In addition, Maciejewska and Bereznowski state that the study on the effect of F on the organic matrix of dentin showed changes both in collagen type I expression—the main organic component of dentin— and in the non-collagen proteins synthesized in dentin during its formation and mineralization.[1]

The supply of F to the enamel and dentine is one of the factors of caries prevention and, as can be observed, it may have a negative effect on the structure of dentin. Consequently, there arises an issue of whether the indiscriminate use of F-containing toothpaste, varnish, and mouthrinses is appropriate. In caries prevention plans,

particular attention should be paid to constantly raising the awareness of the society of oral hygiene, which involves correctly performing mechanical cleaning of teeth of dental plaque by using a toothbrush and interdental cleaning tools, cleaning of the tongue, and avoiding a high carbohydrate diet, particularly by avoiding the so called hidden sugars in the diet. Preventive dental care should not consist in applying F compounds to all patients indiscriminately. There is a need for a tailor-made attitude to dental caries meeting the individual needs of the patients and taking into consideration the multifactorial character of caries. Owing to this, the use of exogenous F for caries prevention should be limited with the use of F-containing toothpaste being considered only for special needs patients (at high and very high risk of caries according to CAMBRA).

Most food products contain F at concentrations lower than 0.5 mg/kg. The exception is marine fish, which contains F at a concentration of 6–27 mg/kg.[4] Some food products and beverages are rich in F compounds, for example rice, corn, wheat, carrot, garlic, tea, wine, juice and carbonated drinks as well as meat (mutton and beef), seafood, and bony fish (sprat, pilchard, salmon).[5,13] The frequent consumption of fish and tea, possibly high in F concentration, were self-reported in the brief dietary survey included in the questionnaire. Our own studies did not show any statistically significant differences between the F concentration identified in the dentin of the people who declared a frequent consumption of fish and tea as compared to those who do not include such products in their diet. These findings are in line with the results by Cruz et al.[7] However, these authors also showed a relationship between frequency of tea consumption and accumulation of F in teeth.[7] This may be connected with the concentration of F in the water used to prepare tea—a factor which was not taken into account in the present study.

CONCLUSIONS

Using F-containing toothpaste results in an increase of the content of this element in dentin. This may lead to impaired mineralization of the tissue and, consequently, cause an increased susceptibility to dental caries. Therefore, F-containing toothpastes and mouthwashes should be used prudently. Priority, in terms of caries prevention, should be particularly assigned to the correct technique and frequency of tooth brushing. Effective mechanical removal of dental plaque combined with the use of F-containing toothpaste is effective in reducing the incidence of dental caries. Each dental visit should include controlling and giving feedback on oral hygiene and the means and efficacy of tooth brushing, as well as educating the patients in terms of possible dangers resulting from the improper use of F-containing oral hygiene products.

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