THE RELATIONSHIP BETWEEN DENTAL FLUOROSIS AND TOOTH FLUORIDE CONCENTRATION – A STUDY IN AN ENDEMIC AREA

Relação entre fluorose dental e a concentração de flúor no dente – um estudo em área endêmica

Artigo Original

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RESUMO

Objetivo: *Determinar a relação entre severidade de fluorose dental (FD), e concentração de flúor [F] no dente e água em áreas endêmicas de FD.* ***Métodos***: *Residentes de duas comunidades endêmicas de FD foram estudados. 45 dentes foram coletados e analisados para severidade de FD e concentração de flúor. O índice de Thylstrup-Ferjeskov (TFI) foi utilizado para medir a severidade de FD e análise por ativação com nêutrons (AAN) para concentração de [F] dental. Águas de poços da região foram coletadas (n=9) e analisadas para [F], utilizando eletrodo específico de flúor.* ***Resultados***: *Concentração de [F] da água variou entre 0,2 e 4,7ppm. Os escores do TFI variaram entre 0 e 6; [F] no esmalte entre 120 e 2140ppm e na dentina entre 304 e 4800ppm. Não se observou correlação entre severidade de DF e [F] no esmalte (r_s=0,22,p=0,15) e dentina (r_s=-0,11,p=0,56). Encontrou-se correlação fraca entre FD e [F] na água (r_s=0,38,p=0,04). Análise de regressão linear demonstrou que TFI não podia ser predito por uma combinação linear das variáveis (idade, e concentração de flúor no esmalte e dentina). Quando a concentração de flúor no esmalte, dentina e água foram analisadas de forma independente na regressão linear, apenas flúor na água demonstrou influência na severidade de FD (p=0,013;t=2,67).* ***Conclusão***: *Mesmo em áreas endêmicas de FD, concentração de flúor dental não se correlaciona com severidade de FD, e a relação entre este e a concentração de flúor nas águas foi fraca. Assim, concentração de flúor dental pode não ser um bom preditor/indicador de severidade de FD.*

Descritores: *Flúor; Fluorose Dentária; Dentina; Esmalte Dentário; Água.*

ABSTRACT

Objective: *To determine the relationship between dental fluorosis (DF) severity and fluoride [F] concentration in tooth and water in DF endemic areas.* ***Methods***: *Life-long residents from two DF endemic communities were studied. Forty-five extracted teeth were collected and analyzed for DF severity and tooth [F]. Thylstrup-Ferjeskov Index (TFI) was used to measure DF severity and instrumental neutron activation analysis (INAA) for tooth [F] concentration. Water from regional wells was also collected (n=9) and analyzed for F content using specific ion F electrode.* ***Results***: *Water [F] varied between 0.2ppm and 4.7ppm. TFI scores ranged from 0 to 6; [F] from 120ppm to 2,140ppm in enamel and 304ppm to 4,800ppm in dentin. No correlation was found between DF severity and [F] in enamel (r_s=0.22,p=0.15) and dentin (r_s=-0.19,p=0.20), nor between water [F] and [F] in enamel (r_s=-0.09,p=0.65) and dentin (r_s=-0.11,p=0.56). Weak correlation between DF severity and water [F] (r_s=0.38,p=0.04) was found. Linear regression analysis showed that TFI couldn't be predicted from a linear combination of the independent variables (age, enamel and dentin [F]). When enamel, dentin and water [F] were used as independent variables in the linear regression (predict DF severity), only water [F] showed influence in DF severity (p=0.013;t=2.67).* ***Conclusion***: *Even in areas of endemic DF, tooth [F] didn't correlate with DF severity and the relationship between water [F] and DF severity was very weak. Therefore, tooth [F] may not be a good predictor/indicator of DF severity.*

Descriptors: *Fluorine; Dental Fluorosis; Dentin; Dental Enamel; Water.*

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INTRODUCTION

Fluoride has been topically[1] and systemically[2,3] used all over the world. An association between increasing drinking water fluoride concentration ([F]), decreasing caries experience and increasing severity of dental fluorosis (DF) has been shown[4-6].

DF is a tooth malformation believed to be produced by chronic ingestion of excessive amounts of fluoride during tooth development that primarily affects enamel, but can also affect dentin[7]. DF has a wide range of clinical presentation, going from narrow enamel white lines to almost complete loss of enamel. Premolars are most severely affected teeth, followed by the upper incisors, canines, first molars and lower incisors[8,9]. In Brazil, there are communities where the water has high levels of fluoride and in such area the prevalence of DF in school children can reach 23% of moderated and severe cases[10], which can influence in the population health and quality of life.

Although some believe in a strong correlation between DF severity and tooth [F], contradictory data has been published[11-13]. In unerupted teeth from areas with optimum and sub-optimum levels of fluoride in the water, the correlation between tooth [F] is either non-existent (enamel) or very weak (dentin)[14]. However, there is a lack of data regarding erupted teeth from endemic areas of DF. This information could provide a better understanding of the influence of fluoride in DF severity. This is important due to influence of this tooth malformation in people's self perception and in their quality of life.

Fluoride can be incorporated into teeth systemically and topically (toothpaste, mouthwashes, fluoride application, etc.). Unerupted teeth can only incorporate fluoride systemically[15,16]. In erupted teeth, enamel [F] corresponds to fluoride incorporated during tooth formation (systemically) and after tooth eruption (topically), while dentin [F] corresponds only to fluoride incorporated systemically, (unless exposed to the oral environment[17], since this tissue is protected by enamel and cementum.

Some communities in the Northeastern part of Brazil have been identified as DF endemic areas due to their high levels of fluoride in the drinking water[18-20]. Dental treatment is normally limited to dental extraction and the use of fluoridated home care dental products is limited in these areas. Therefore, life-long residents in these areas can provide interesting data related to [F] (dentin and enamel).

The main objective of this study was to evaluate the relationship between tooth [F] (dentin and enamel) and DF severity in erupted teeth from DF endemic areas. The correlations between drinking water [F] and enamel [F], dentin [F] and DF severity were also evaluated.

METHODS:

Descriptive and analytical study developed in a partnership between Brazil and Canada. The teeth were collected in Brazil and analyzed at the university of Toronto-Canadá between 2003 and 2005. Two Brazilian rural communities were included in the study: Catolé do Rocha (Genipapeiro)-PB and Sobral (Rafael Arruda)-CE. Theses two villages are known to suffer of endemic dental fluorosis, due to naturally fluoridated water, are located at very low altitude (almost at sea level) and have annual temperature varying between 25 and 35°C. The overall socio-economical status of these communities is low and the use of fluoridated home care dental products is limited.

Teeth (n=111) were collected from life-long residents in the two communities. A questionnaire was used to determine patients' drinking water source and their period of residency in the area. These data allowed further correlation between drinking water [fluoride], tooth [fluoride] and DF severity. The teeth were extracted by local dentists at the public health care units, mainly due to periodontal or aesthetic problems and carious lesions. Only after the local dentists had decided the need for tooth extraction, were the patients asked to participate in the research, by reading and singing an informed consent form in order to donate their teeth. Therefore, there was no interference by the members of this study in the decision to extract or not an individual tooth. The University of Toronto ethical committee approved this research in 2003 – protocol number 9118.

The inclusion criteria were: **a**) teeth originated from a patient who had lived in the region and who drank water from the same well for the majority of his/her life (90% or more) and **b)** teeth were either caries-free or had minimal dental decay. From the initial 111 teeth, 45 teeth met these criteria and were analyzed. Water from the wells in the region was collected (which accounts for the drinking water sources of 70% [31 out of 45 teeth] of the analyzed population). Water from each well was collected at two time points (raining and non-raining seasons) and the average [F] of these two samples was used as the overall [F] for the each well. An electrode specific for F ions - Model 96-09 (Thermo Orion, Beverly, CA, USA) was used to evaluate drinking water [F].

The extracted teeth were washed in running water and kept in wet gauze immersed in thymol in the health care unit and within a couple of days sent to the University of Toronto-Canada (Dr. Marc Grynpas laboratory), where they were immediately frozen (-4^{o}C freezer) and later analyzed. This method of storage has been proven to be adequated for this type of analysis[21]. Teeth were defrosted prior to tooth preparation for approximately 12 hours at room temperature (21ºC), washed in running water, quickly dried (5sec.) and analyzed for DF severity using the Thylstrup-Fejerskov Index (TFI)[22] by one of the authors (AV). AV was calibrated to use the TFI by co-author HL, and the inter-examiner weighted kappa value was excellent (k>0.90). The kappa intra-examiner reliability (for AV) was 0.88.

After the DF severity assessment, teeth were transferred to molds and embedded in epoxy resin (Epoxycure resin, Buehler, Markham, ON, Canada). The blocks were sectioned using a low speed saw (Isomet, Buehler ltd., Lake Bluff, IL, USA) as previously described[14].

The dentin samples from the buccal and lingual sides of each tooth were collectively analyzed for [fluoride] using instrumental neutron activation analysis (INAA). The same procedure was used for the enamel samples. In INAA, each sample is bombarded with thermal neutrons that produce short-lived radioisotopes from the elements in the sample. These radioisotopes decay with specific half-lives, emitting gamma rays of discrete and characteristic energies. The relative amounts of gamma rays detected are proportional to the concentrations of the elements in the sample[23]. INAA gives the average [F] of the sample and it is capable of measuring [F] above 50ppm (0.05%) with an error of 10ppm (0.01%). All statistical analysis (descriptive and analytical) was done using statistical analysis software (SPSS for Windows, SPSS Inc., Chicago, IL, USA).

RESULTS

Different types of teeth (incisors [n=16; 35%], canines [n=8; 18%], pre-molars [n=8; 18%] and molars [n=13; 29%]) were collected from male (n=16; 35%) and female (n=29; 65%) patients (42±15 years-old). TFI scores ranged between 0 and 6 (n=45). Fluoride concentrations ranged between 120ppm and 2,140ppm in enamel and 304ppm and 4,800ppm in dentin. Water [F] varied between 0.2ppm and 4.7ppm in the different wells. DF severity, enamel and dentin [F] information was available for all patients (n=45) while patient age information was available for 76% (n=34) and drinking water [F] for 70% (n=31) of subjects. As shown in the Figure 1, a large range of [F] at each level of DF was found. Teeth with no DF (TFI=0) have both enamel and dentin [F] with the largest range (going from almost no [F] to the highest levels of [F] presented in this data set).

Spearman correlation tests showed no correlation between dentin [F] and DF severity (r_s=-0.193,p=0.204); enamel [F] and DF severity (r_s=0.221,p=0.154); dentin [F] and drinking water [F] (r_s=-0.109,p=0.560); and between enamel [F] and drinking water [F] (r_s=-0.089,p=0.648). However, a weak correlation was seen between DF severity and drinking water [F] (r_s=0.378,p=0.036).

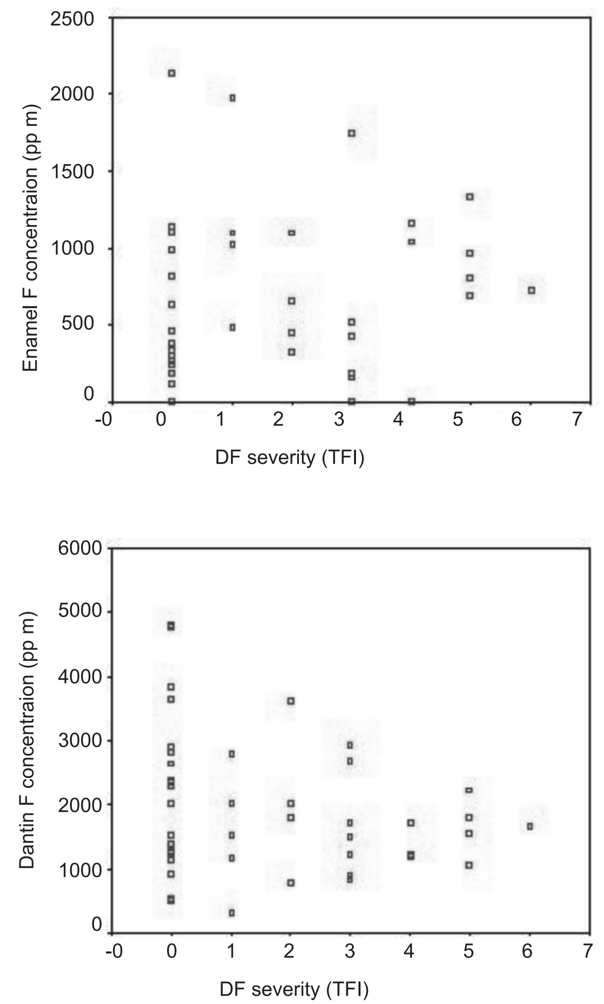

Figure 1 - Enamel F concentration vs. DF severity (left) and Dentin F concentration vs. DF severity (right) – erupted teeth (n=45).

Similar results were seen when the different types of teeth (incisors, canines, pre-molars and molars) were analyzed separately. No correlation was seen between dentin [F], enamel [F], DF severity and water [F] in any of the 4 teeth type groups. Therefore, further analysis was performed combining all 4 different teeth types together. No association was noted between DF and gender.

Analysis of variance (Kruskall-Wallis H) showed no difference between dentin (p=0.480) and enamel (p=0.135) [F] in the 6 different DF groups (TF score 0; TF score 1; TF score 2; TF score 3; TF score 4; TF score 5 and 6) (Table I).

Table I - Fluoride concentration in enamel and dentin related to dental fluorosis (DF) groups.

DF Groups (Based on TF score)	n	Enamel F concentration (ppm)			Dentin F concentration (ppm)		
		Range	Mean	SD	Range	Mean	SD
TF 0	20	120 - 2140	560	515	502 - 4800	2250	1268
TF 1	5	489 - 1980	1149	617	304 - 2810	1566	934
TF 2	4	320 - 1100	630	341	768 - 3620	3620	1180
TF 3	8	160 - 1750	610	655	835 - 2950	1836	892
TF 4	3	1046 - 1160	1103	81	1195 - 1713	1374	293
TF 5 and 6	5	698 - 1335	904	262	1044 - 2230	1657	430

SD = standard deviation.

Linear regression analysis showed that TFI could not be predicted from a linear combination of the independent variables (enamel [F] [p=0.61], dentin [F] [p=0.33] and age [p=0.11]). When enamel, dentin and water [F] were used as independent variables in the linear regression (to predict DF severity), only water [F] was shown to influence DF severity (p=0.013;t=2.67). The *t* statistics provide information regarding the relative importance of each variable in the model. Because the *t* value for water [F] is greater than 2, this variable is a useful predictor in this model[24].

DISCUSSION

The present study represents the only data available on the correlation between dentin [F] and DF severity in erupted fluorotic human teeth. No correlation was seen between dentin [F] and DF severity nor between enamel [F] and DF severity. One can argue that the absence of correlation may be due to the use of different types of teeth in this study (incisors, canines, pre-molars and molars) or due to the wide range of patient age (42±15 years-old), as those variables have the potential to influence tooth [F]. However, in our data, age showed no effect on DF severity (linear regression) and no significant correlation was seen when the different types of teeth (incisors, canines, pre-molars and molars) were analyzed separately from each other. Additionally, as in other studies[25], no association was noted between DF and gender.

The data presented here do not support the hypothesis that tooth [F] and DF severity correlate. This is in agreement with an abstract presented by Olsen and Johansen[11] using enamel samples, but is not in agreement with the claims made by Richards *et al*.[12,13], who only used enamel samples (not dentin). Other authors, using different erupted teeth types (incisors, canines, pre-molars and molars), concluded that an increasing severity of fluorotic lesions was associated with increasing enamel [F].

However, no difference was seen between the TF0 and TF1 groups, nor between the TF (5 and 6) and the TF (7,8 and 9) groups. The group TF (2 and 3) was only different than TF (0 and 1) group in the superficial enamel layer[12].

The ranges of enamel and dentin [F] in subjects exposed to drinking water [F] of 1ppm or less were larger than those previously seen in patients living in non-endemic DF areas with similar water F intake[14]. It seems, therefore, that subjects from DF endemic areas concentrate more F in their dental tissues than do patients in non-endemic areas. The explanation for this is not clear. Additionally, it is not clear if this observation is due to the type of teeth analyzed in the two groups (unerupted vs erupted teeth), from F ingestion from other sources (food produced in areas with higher [F]) or other factors, such as tooth porosity. Teeth with more severe DF have more porous (hypomineralized) enamel[26], which may permit more ion exchange with the environment. Nonetheless, this information, together with the fact that the correlation between water [F] and DF severity is weak, may indicate that other factors, such as genetic susceptibility to fluoride, influence DF severity.

It is clear that fluoride needs to be present in teeth for the development of DF; but DF severity does not seem to be dictated by tooth [F]. Genetic susceptibility may play an important role in the way a tooth develops in the presence of F. A study using different mice strains ingesting high levels of F showed that some strains were more susceptible to F than others[27]. The Grand Rapids fluoridation study, showed that DF is more prevalent among African-american children than white children[28]. The odds ratio for African-American children having DF, compared with Hispanic and non-Hispanic white children was 2.3[29,30]. Nevertheless, due to the relative limited literature on the subject, further studies are needed regarding genetic susceptibility to F.

The correlation between drinking water [F] and DF severity found in this study agrees with findings from the beginning of last century (Dean's studies in the 1900's)[6]. However, the *coefficient of determination* (r^2), which expresses the proportion of variance in the dependent variable explained by the independent variable[24], is very low for the correlation between water [F] and DF severity (r^2=0.14), indicating that only 14% of DF severity can be explained by water [F]. The absence of a correlation between drinking water [F] and dentin and enamel [F] was expected, as DF severity did not correlate with dentin nor enamel [F]. Thus, it is possible that some patients may be affected by DF without a strong history of F ingestion, whereas a strong history of F ingestion may not necessarily result in a severe DF.

CONCLUSION

In conclusion, this study showed that even in areas of endemic DF, with a wide range of F ingestion, tooth [F] did not correlate with DF severity and that the relationship between water [F] and DF severity was very weak. Therefore, tooth [F] may not be a good predictor/indicator of DF severity. Finally, the dental practitioner cannot only rely on levels of water fluoridation and/or tooth [F] in order to diagnose DF.

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