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Relationship between enamel fluorosis severity and fluoride content

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ABSTRACT

Objectives: Enamel fluorosis is a hypomineralization caused by chronic exposure to high levels of fluoride during tooth development. Previous research on the relationship between enamel fluoride content and fluorosis severity has been equivocal. The current study aimed at comparing visually and histologically assessed fluorosis severity with enamel fluoride content.
Methods: Extracted teeth ($n = 112$) were visually examined using the Thylstrup and Fejerskov Index for fluorosis. Eruption status of each tooth was noted. Teeth were cut into 100 μm slices to assess histological changes with polarized light microscopy. Teeth were categorized as sound, mild, moderate, or severe fluorosis, visually and histologically. They were cut into squares (2×2 mm) for the determination of fluoride content (microbiopsy) at depths of 30, 60 and 90 μm from the external surface.
Results: Erupted teeth with severe fluorosis had significantly greater mean fluoride content at 30, 60 and 90 μm than sound teeth. Unerupted teeth with mild, moderate and severe fluorosis had significantly greater mean fluoride content than sound teeth at 30 μm; unerupted teeth with mild and severe fluorosis had significantly greater mean fluoride content than sound teeth at 60 μm, while only unerupted teeth severe fluorosis had significantly greater mean fluoride content than sound teeth at 90 μm.
Conclusions: Both erupted and unerupted severely fluorosed teeth presented higher mean enamel fluoride content than sound teeth.
Clinical significance: Data on fluoride content in enamel will further our understanding of its biological characteristics which play a role in the management of hard tissue diseases and conditions.

1. Introduction

Enamel fluorosis is a hypomineralization of dental enamel characterized by chronic exposure to high levels of fluoride during tooth development [1,2]. Fluoride interacts with mineralized tissues and when present in excess disturbs dental enamel development. As severity of fluorosis increases, changes in the porosity of subsurface enamel extend deeper into the tissue resulting in hypomineralized areas covered by a defined zone of highly mineralized tissue that may affect the entire enamel surface [3].

An analysis comparing fluorosis prevalence data in the United States from the 1930s and 1980s indicated an increase in enamel fluorosis in children with a prevalence 27% in children residing in areas with optimal fluoride (0.7–1.2 μg/ml) and 15% in children from areas with suboptimal fluoride (<0.7 μg/ml) in the 1980s versus a prevalence of 12–25% for those living in areas with optimal natural fluoride content (ranging from 0.09 to 1.3 μg/ml) and 7% in children in areas with suboptimal fluoride (<0.7 μg/ml) in the 1930s [4]. Comparison of the data from 1986–1987 and a survey conducted in 1999–2002 identified a 9% increase of fluorosis prevalence in children and adolescents aged 6–19 years (from 22.8% in 1986–1987 to 32% in 1999–2002). Although the increase in prevalence has occurred primarily in very mild and mild forms of enamel fluorosis, between 3 and 4% of children and adolescents had moderate or severe fluorosis in 1999–2002 [5].

Studies into the relationship between enamel fluorosis severity and fluoride content have been equivocal. Some studies have reported a positive correlation between the clinical severity of enamel fluorosis and enamel fluoride content [6–8]. Brudevold et al. [9] reported a positive trend between enamel fluorosis severity and fluoride content within rat incisors. However, each

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fluorosis category had a large standard deviation and overlapping fluoride content. Therefore, teeth that were categorized visually as sound may have had the same fluoride content as those teeth that presented with severe forms of fluorosis. Furthermore, it has been reported that enamel fluoride content vary depending on the location within the oral cavity with central incisors having significantly lower fluoride [10].

On the other hand, some studies have reported that enamel fluorosis severity is independent of enamel fluoride content. Olsen and Johansen [11] studied human teeth and found that enamel surface appearance was independent of the enamel fluoride content. Furthermore, Vieira et al. [12] reported a correlation between dentin fluoride concentration and the presence of enamel fluorosis in unerupted third molars. With regards to enamel; however, the authors found no such correlation.

Despite conflicting results, no studies have thoroughly examined the correlation between enamel fluorosis severity and enamel fluoride content. Additionally, to the authors' knowledge, no studies have been reported investigating fluoride content at different enamel depths in relation to fluorosis severity. Previous studies have relied on visual examinations to classify the severity of enamel fluorosis' clinical signs and none have related the fluoride content of enamel to the histological severity of fluorosed enamel changes. Therefore, the aim of this study was to investigate the relationship between fluoride content in dental enamel at different depths with the presence of enamel fluorosis detected visually and using histological assessments.

2. Materials and methods

Extracted teeth with fluorosis, free of caries, including incisors, premolars and molars (total of 120, 44 erupted – 8 incisors, 5 premolars and 31 molars; and 76 unerupted – 11 incisors, 9 premolars and 56 molars), were collected after approval was obtained from the Indiana University Institutional Review Board. Teeth were stored in deionized water saturated with 0.1% thymol. The teeth were collected from dental offices and transported to the study site in the saturated 0.1% thymol solution. Upon receipt, the teeth were sorted, cleaned and the root removed.

The teeth were visually examined using the Thylstrup and Fejerskov Index for fluorosis (TFI) by two trained and calibrated examiners (AESR and EAMM). In those cases where there was disagreement, the examiners discussed their findings and reached an agreement [13]. Using the buccal and occlusal aspects of posterior permanent teeth and labial aspects of anterior permanent teeth, *in vitro* scores were assigned to each aspect. Fluorosis severity in this study was rated as mild, moderate, or severe. Thirty sound teeth were included as negative controls. Four categories were therefore created: mild, moderate, severe and sound. Thirty teeth from each of the four categories, as determined visually, were analyzed in this study.

After visual scores were assigned, specimens were cut into halves. From one half of each tooth, bucco-lingual tooth sections of 100 ± 20 μm were cut longitudinally in the midline of the fluorosis area using a Series 1000 Deluxe Hard Tissue Microtome (SciFab, Lafayette, CO, USA), and an average of 2 sections per tooth were obtained. Sections were imbibed in water and examined using a polarized light microscope (Orthoplan, Leitz, Wetzlar, Germany). Digital images were taken for qualitative evaluation of the hypomineralized area. A demineralized area in enamel or dentin with positive birefringence was defined as a lesion. Using the results from polarized light microscopy, each tooth was assigned as sound, mildly fluorosed, moderately fluorosed, or severely fluorosed based on histological characteristics described by Fejerskov et al. [14].

The other half of each tooth was mounted onto an acrylic plate (Total Plastics, Indianapolis, IN, USA) using sticky wax (Kerr Corporation, Orange, CA, USA) with an area of flat enamel facing up. Using an Isomet low speed saw (Buehler, Lake Bluff, IL, USA) with a 2 mm spacer between two blades, 2×2 mm square pieces were cut from each tooth. Each piece was then waxed with enamel facing down to another acrylic block, and the dentin was sanded away using a RotoPol-31 machine (Struers, Cleveland, OH, USA) with 1200 grit laboratory grade SiC abrasive paper (Struers, Cleveland, OH, USA) until a flat dentin surface remained. Pieces were then glued onto steel drill rods (Grainger, Inc., Indianapolis, IN, USA) measuring ~3.0 mm in diameter using Duro Quick Gel No-Run Super Glue (Loctite, Plainfield, IL, USA) with enamel facing up and parallel to top of drill rod. Clear, fluoride-free fingernail polish (Del Laboratories, Inc., Farmingdale, NY, USA) was applied to the rods to prevent rust, and they were individually stored in vials (7 ml-vials, Fisher Scientific Co., Itasca, IL, USA) with enough water to cover each specimen. Finally, 4.0×5.0 mm pieces of 1200 grit laboratory grade fluoride-free SiC abrasive paper were cut and attached to $3/4 \times 1 \times 1$ in acrylic blocks (Total Plastics, Indianapolis, IN, USA) with a light application of sticky wax.

2.1. Fluoride microbiopsy

Fluoride microbiopsies were conducted using a modification of the procedure described by Hellwig et al. [15]. Each drill rod with the attached specimen was inserted into the chuck of a microdrill machine (Stellar Systems, Vienna, VA, USA). Acrylic blocks were secured onto the microdrill stage, and the spinning specimen was slowly lowered until initial contact with the abrasive paper was made. A digital micrometer attached to the microdrill was zeroed, and the specimen was lowered in small increments while using the stage controls to navigate over the entire abrasive surface until 30 μm of enamel had been removed. The acrylic block was removed from the stage, and an open vial was placed on top of the abrasive paper. The block was turned upside-down, and the bottom was tapped to remove the lose powder into the vial. Next, the vial was placed under the specimen in the microdrill, and the rod was tapped to remove lose powder attached to the specimen. The abrasive paper with enamel powder was removed from the acrylic block and placed in the vial. Finally, the vial was capped tightly, and the specimen was brushed to prevent carry-over of enamel powder to succeeding samples. This process was repeated twice on each specimen, resulting in samples at depths of 30 μm, 60 μm, and 90 μm for each tooth.

Due to the tooth morphology, the employed abrasion technique did not always collect powder from 100% of the enamel surface within the first 30 μm. Therefore, after collection of the 30 μm sample, a picture of the 2×2 mm specimen was taken on a Nikon SMZ1500 Stereomicroscope (Nikon Instruments Inc., Elgin, IL, USA) with attached NI-150High Intensity Illuminator (Nikon Instruments Inc., Elgin, IL, USA). Using this photograph, a percent volume of abrasion was calculated. This percent volume was used to adjust observed fluoride concentrations to an estimated fluoride concentration value if 100% of the 2×2 mm surface was subjected to abrasion within the first 30 μm sample.

2.2. Fluoride content analysis

Eighty μL of 0.5 M perchloric acid ($HClO_4$ Fisher Scientific Co., Itasca, IL, USA) were added to a vial containing the enamel powder collected though the microbiopsies. Vials were capped tightly, vortexed for approximately 10 s, and centrifuged in a micro 18R microcentrifuge (VWR International, Germany) at 15000 rpm for 5 min to ensure settling of enamel powder and abrasive paper. Vials were then placed in a rack and secured to a CH-4103 rotary