Frailty of Human Enamel in Dental Pieces with and without Fluorosis: An *in Vitro* Study

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**Abstract:** Hardness is considering an essential physiological property of human dental enamel. Much more serious is the fluorosis, teeth pieces are subject to extensive mechanical failures of the surface. Objective: To compare relationship between force and hardness (F-H) in samples of human dental enamel with and without fluorosis. Material and Methods: *In vitro* experimental study. Dental pieces of adult humans derived from extractions due to periodontal problems or orthodontic treatment were used. Teeth with fluorosis (n = 11) and without fluorosis (n = 11) were included (beta = 10% and alpha = 5%, maximum error admitted at simple size calculation). From each tooth, 4 blocks were cut, each of one catted parallel to respective tooth surface (vestibular, mesial, palatine-lingual, distal); those little pieces were included into acrylic resin and polished. A Vickers microhardness test was used, consisting in measuring the diagonal length of an indent left by indentation of a diamond pyramid with a given load into the sample material. The F-H relationship was measured with Spearman coefficient (Sp), adjusting by group (fluorosis: yes-no) and by dental surface (palatine/lingual, mesial, distal, vestibular); statistical significance level: \( p < 0.05 \). Results: F-H relationship showed statistically associated in the group of teeth with fluorosis (Sp = 0.774, \( p = 0.009 \)) but not in the group without fluorosis (Sp = 0.249, \( p = 0.586 \)). Adjusting by dental surface, F-H relationship was statistically significant in fluorosis group (Sp = 0.699, \( p = 0.024 \) in mesial side; Sp = 0.964, \( p = 0.001 \) in distal side; Sp = 0.701, \( p = 0.036 \) in palatine-lingual side and Sp = 0.617, \( p = 0.077 \) in vestibular side), but not in the group without fluorosis. Conclusion: Evidence suggested that F-H relationship was statistically significant only in dental pieces with fluorosis, suggesting that dental fluorosis involves a weakening of complete dental structure.

**Key words:** Dental enamel, fluorosis, dental enamel resistance, dental enamel hardness.

1. Introduction

Dental fluorosis is the first sign of fluor excess due to chronic exposition/intake during the stage of dental formation. Much more serious is the fluorosis; teeth are subjected to serious mechanical failures of their surface [1].

The most probably cause of sub-superficial porosity is the delay of hydrolysis and removal of enamel proteins—amelogenins, particularly during maturation of enamel [2].

From definition, the method to measure emerges: it consists of penetrating a sample of study material by means of a defined indentation, applying one pre-established charge over that element [3]. The hardness of material is quantified using a variety of scales that, direct or indirectly, relate the pressure of contact needed to deform the surface.

We hypothesize that fluorosis promotes a loss of relationship between indentation and hardness of dental enamel. The objective of this paper was to compare the fracture threshold of dental enamel in permanent teeth of adult patients with and without fluorosis, expressed in terms of resistance/hardness when they underwent to increase and continue indentation/force.

2. Materials and Methods

It was an *in vitro* experimental study. Adult teeth
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products of exodontia for periodontal disease or for orthodontic causes were used. Twenty-four teeth of patients with fluorosis (12) and without fluorosis (12) were included (β: 10%, α: 5%, maximum errors admitted to sample size calculation).

From each tooth, four parallel blocks to respective surface (vestibular, mesial, palatine/lingual and distal) were cut, accounting $12 \times 4 \times 2 = 96$ samples which were included and polished into acrylic blocks.

To determine the fracture threshold, the Vickers method of microhardness assay was used; it consisted in using an indentation machine to introduce perpendicular to sample (dental enamel) a pyramidal-shape diamond penetrator, and pushing growing pressures up the fracture is done. A microscope added to indentation machine kept measure the resistance/hardness (H) averaging the two diagonals generated by the action of applied indentation/force (F).

From each sample the pair $(F_i, H_i) (i = 1, 2 \ldots 96)$ was registered, where $F_i$: indentation/force applied at moment of fracture—measured in Newtons (N)- and $H_i$: average of two diagonals generated at moment of fracture, interpreted as the maximum value of resistance/hardness to penetration—measured in hardness Vickers (HV)-.

Statistical analysis included the comparison of medians of F and H of groups with and without fluorosis with Mann-Whitney test. Correlation between F and H was estimated with the Spearman coefficient ($Sp$), adjusting by group with/without fluorosis and by tooth surface; their statistical significanation was measured respect $Sp = 0$ (no relation). In all test, a $p$-value < 0.05 was considered with statistical significance.

3. Results

Three samples of each group were excluded from analysis because they were not broken at different force levels that indentation machine offered.

Medians of F at moment of fracture were similar between groups with and without fluorosis: 0.1287 N (range: 0.0736-4.9280) and 0.8213 N (range: 0.0613-2.9445) respectively ($p = 0.337$ of Mann-Whitney test). But median of H was significantly lower in group with fluorosis: 0.2270 HV (range: 0.0981-4.1678) and 1.5016 HV (range: 0.2084-5.6387), respectively ($p = 0.036$ of Mann-Whitney test).

F and H showed significantly associated in group without fluorosis ($Sp = 0.405$, $p = 0.006$), but not in group with fluorosis ($Sp = 0.087$, $p = 0.574$). Fig. 1 shows that samples from group without fluorosis tolerated higher hardness values, and therefore their F-H respective pairs were more distributed to the right side of picture (F data were graphed in logarithmic scale to appreciate their distribution).

Adjusting by dental surface, no F-H relationship was observed in group with fluorosis but in group without fluorosis that relation showed statistical significanice in palatine/lingual and borderline in mesial surfaces (Fig. 2).

![Fig. 1 Relationship between indentation/force and resistance/hardness in samples with and without fluorosis.](image)

![Fig. 2 Intensity of relationship between force and hardness in samples with and without fluorosis, adjusting by dental surface.](image)
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Taking into account the correlation between F and H in group without fluorosis, a multiple regression analysis was applied. Taking as response variable the H value at fracture time, and taking as potential explicative variables the applied F, dental surface of samples, age and sex of patient, the linear model was the best adjusted model to explain that relationship ($R^2 = 0.376$), and the applied indentation (F) was the only one explicative variable with statistical significance ($\beta = 0.614, p = 0.034; \alpha = 206.33$ HV, $p = 0.0001$) (Fig. 3).

4. Discussion

One of the most important characteristics of teeth is their capacity of supporting a wide range of charges, until up 1,000 N, in a contact area between 0.45 and 2.5 mm$^2$, without showing failures; that is why dental enamel is the hardest tissue of human being, and highly mineralized [4].

4.1 Indentation/Force

In our study, the F registered at the moment of fracture in dental enamel without fluorosis ranged from $0.0613$ to $2.9445$ N (equivalent to $0.0063$ kgf/m/s$^2$ and $0.3003$ kgf/m/s$^2$, respectively) and those with fluorosis ranged from $0.0736$ to $4.9280$ N (equivalent to $0.0075$ kgf/m/s$^2$ and $0.5025$ kgf/m/s$^2$, respectively). Both indentation values could be low, but it is necessary to consider that we did not use constant values because previously we did not know the hardness value in pieces with fluorosis group.

4.2 Resistance/Hardness

In our experience, the range of H was significantly lower in group with fluorosis (from $0.0981$ to $4.1678$ HV) and the group without fluorosis (from $0.2084$ to $5.6387$ HV). When comparing our experience with others authors, wide variations in hardness value of dental enamel without fluorosis were revealed: although some studies have done more than 60 years ago, Caldwell and Muntz [5] reported hardness values from $357$ to $399$ HV; Craig and Peyton [6] do it in a range from $344$ to $478$ HV; and Collys et al. [7] from $344$ to $466$ HV.

Using nano-indentation Vickers’ technology, Xu et al. [8] registered a range between $3.23$ and $2.94$ GPa (equivalent to $330.44$ and $300.77$ HV respectively). Gutierrez-Salazar and Reyes Gasga [9] revealed that enamel hardness ranged among $270$ to $360$ HV, and cervical level showed the lowest value ($230$ HV). Rivera Velázquez et al. [10] analyzed the hardness of third molars indenting forces showing that low Fs between $0.1$ and $100$ N obtained H values equivalent to $716.13$ HV, but forcing upper $3$ N, hardness showed a relatively stable value, $50\%$ lower than maximum measured in other studies. Mahoney et al. [11] carried out a microindentation study to analyze hardness in enamel of hypomineralized permanent first molars, comparing them with normal pieces. They showed that range of hardness of affected group ranged from $2.03$ to $4.99$ GPa (equivalent to $207.68$ and $510.49$ HV, respectively), similar to cervical hardness of control group: from $2.71$ to $4.15$ GPa (equivalent to $277.24$ and $424.56$ HV, respectively).

Unfortunately, those experiences did not referred to dental pieces with fluorosis, impeding the respective comparisons; we did not found experiences using indentation technology to measure hardness in dental pieces with fluorosis.
Were there relation between F values and the respective ones of H in pieces without fluorosis? Our regression model showed that linear model was the best adjusted to dependence relationship, expressing that just 40% of H variability could be explained only by the respective F.

Conventional and static proof methods, like the microhardness test, have several limitations when they use to measure mechanical properties of dental enamel and dentin, due to the complex structure of material and small pieces of samples. Other issues to consider for discussing are the measure units. The majority of Vickers indentation machines use forces in N units (equivalent to 1, 2, 5, 10, 20, 50 and 100 kg/f) and calculate their hardness respective values in HV or GPa; to transform GPa to HV, it is necessary to factor by 102.3 [12].

5. Conclusions

As a conclusion, the knowledge of the indentation/force cutoff to destroy the enamel in dental pieces with and without fluorosis contributes to being alert at clinical considerations to warrant the permanence of dental structures, offering them the best biomechanical conditions.

Statement of Ethics

This study was approved by Ethic Committee of Facultad de Odontología, Universidad Nacional de Rosario (Argentina), with Resolution Code: 117/010 CD.

References