Turkish Journal of Medical Sciences

Volume 42 | Number 2 Article 22

1-1-2012

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ŞENTUT, TUBA; KIRZIOĞLU, ZÜHAL; GÖKÇİMEN, ALPASLAN; ASLAN, HÜSEYİN; and ERDOĞAN, YILDIRIM (2012) "Quantitative analysis of odontoblast cells in fluorotic and nonfluorotic primary tooth pulp," *Turkish Journal of Medical Sciences*: Vol. 42: No. 2, Article 22. https://doi.org/10.3906/sag-1007-964

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Original Article

Turk J Med Sci
2012; 42 (2): 351-357
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E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-1007-964

Quantitative analysis of odontoblast cells in fluorotic and nonfluorotic primary tooth pulp

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Aim: To determine the effect of fluoride ingested during the development period on the number of odontoblast cells using stereological methods.

Materials and methods: A total of 56 freshly extracted primary first and second molar teeth were used, obtained from children (aged between 9 and 11 years) requiring dental extractions for clinical reasons. Following histological procedures, longitudinal tooth sections, 25 µm thick, were stained with hematoxylin and eosin. The total number of odontoblast cells was estimated using the optical fractionator method with a stereological workstation. All of the data were expressed as means ± standard deviations for each group, and statistical analyses were performed using SPSS software.

Results: The mean number of odontoblast cells in fluorotic second molar teeth was significantly lower compared to nonfluorotic teeth in the maxillary jaw. Furthermore, a statistically significant difference was found in the mandibular jaw, and the mean number of odontoblast cells in fluorotic second molar teeth was significantly lower than in the nonfluorotic group.

Conclusion: Fluoride was found to decrease the number of odontoblast cells in fluorotic teeth, and this finding indicates that fluoride could affect the mechanism of pulpal recovery following operative procedures as a result of fewer odontoblast cells.

Key words: Dental fluorosis, odontoblast, primary tooth, stereology, tooth pulp

Florozisli ve florozisli olmayan süt dişi pulpalarında odontoblast hücrelerinin kantitatif analizi

Amaç: Bu çalışmanın amacı, gelişim döneminde alınan florun, odontoblast hücrelerinin sayısı üzerindeki etkisinin stereolojik metot kullanılarak belirlenmesidir.

Yöntem ve gereç: Bu çalışmada, klinik sebeplerden dolayı diş çekimi yapılmış olan (9-11 yaşları arasındaki) çocuklardan elde edilen toplam elli altı süt birinci ve ikinci azı dişi kullanılmıştır. Histolojik işlemlerden sonra, 25 µm kalınlıktaki dikey diş kesitleri hemotoksilen ve eozin ile boyandı. Toplam odontoblast hücre sayısı, optik parçalama metodu kullanılarak hesaplandı. Tüm veriler, her grup için ortalama ± standart sapma şeklinde ifade edildi ve istatistiksel analizleri SPSS istatistik yazılım programı kullanılarak gerçekleştirildi.

Bulgular: Üst çenede florozisli ikinci süt azı dişlerinde ortalama odontoblast hücre sayısı, florozisli olmayan dişlerle karşılaştırıldığında anlamlı biçimde düşük çıktı. Ayrıca alt çenede de istatistiksel olarak anlamlı bir fark bulundu, ortalama odontoblast hücre sayısı, florozisli ikinci süt azı dişlerinde, florozisli olmayan gruba göre daha düşüktü.

Sonuç: Florun, florozisli dişlerde odontoblast hücre sayısını azalmasına neden olduğu bulundu ve bu bulgular, florun, odontoblast hücre sayısının azalmış miktarı sonucu pulpal operatif işlemlerden sonra pulpal iyileşme mekanizmasını etkileyebileceğini göstermektedir.

Anahtar sözcükler: Dişsel florozis, odontoblast, süt dişi, stereoloji, diş pulpası

Received: 19.10.2010 – Accepted: 24.04.2011

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Introduction

Over the last few decades, fluoride has been used all over the world to prevent caries, in spite of its link with dental fluorosis when consumed in high concentrations (1). However, despite the preventive effectiveness of fluoride, not much is known about its effects on the tooth pulp cells.

Odontoblasts, highly differentiated postmitotic cells, are responsible for the regulation of dentine synthesis, secretion, and mineralization throughout life (2). They have been demonstrated to detect and respond to dentine injury following dental procedures, and the capacity to increase the barrier between odontoblasts and the stimuli depends on the vitality of these cells to secrete reactionary dentine (3-6). Dentinal repair following dental procedures is dependent on several parameters, including the numbers of odontoblasts (4).

Odontoblast survival is a multifactorial process, and factors such as cavity remaining dentine thickness (3,7), presence of bacteria (4), method of placement of the restorative material (8), pulpal inflammation (9), and restoration material toxicity (10) may individually or cumulatively play a role in the quantity of odontoblasts.

Until recently, methods for obtaining reliable measure estimates of the accurate total number of odontoblast cells in the tooth pulp were insufficient. The application of stereological methods that have the potential for forming the basis of a reliable method for estimating cell number can be used to evaluate the total number of objects in a defined region of biological tissue (11).

The potential effects of fluoride on tooth pulp cells have been investigated in a limited number of studies. Although the results of these studies are controversial, most of them concluded that it is important to protect the dentine-pulp complex from excessive exposure to fluoride, particularly during stages of development (12-15). In vitro studies that investigated the effects of fluoride on human pulp cell cultures (12) and the cellular morphology and synthetic activity of the rat dentine-pulp complex (13) found that fluoride is a cytotoxic agent to pulp cells, inhibiting cell growth, proliferation, mitochondrial activity, and protein synthesis. However, Nakade et al. (14) stated that fluoride, at micromolar concentrations, can stimulate the proliferation and alkaline phosphatase activity of dental human pulp cells.

It is crucial to clarify the effects of fluoride on tooth pulp cells, especially the odontoblasts, because this agent is widely used for the prevention of dental caries. The aim of this study was to determine the number of odontoblast cells in fluorotic primary teeth using stereological methods.

Materials and methods

A total of 56 freshly extracted primary first and second molar teeth were used, obtained from children (28 males and 28 females, aged between 9 and 11 years) requiring dental extractions for clinical reasons. Patients and their parents were informed that the extracted teeth would be used in an in vitro study, and their informed consent was obtained under protocol approved by the Süleyman Demirel University Ethical Committee. Teeth were excluded from the study if they had caries lesions beyond superficial enamel caries and physiological root resorption of more than one-third of the root length. The total number of teeth that were investigated according to groups and tooth types is shown in the Table.

Patient records were evaluated in order to determine the study population. Fluorotic and healthy teeth were obtained from 2 sets of patients: patients who had been living in Isparta, an endemic fluorosis area in southern Turkey, and a second set of patients who had been living in a nonendemic area since birth, with water fluoride levels of >0.79 mg/L and <0.3 mg/L, respectively. The dental fluorosis index created by Thylstrup and Fejerskov (16) was used to classify the primary molar teeth as fluorotic and nonfluorotic.

The extracted teeth were immediately fixed in neutral-buffered formalin (10%) for 24 h, decalcified in nitric acid solution (4%), dehydrated in a graded alcohol series, cleared in xylenes, and embedded in paraffin. The paraffin blocks were cut in longitudinal sections with a thickness of 25 µm using a rotary microtome (RM 2125 RT, Leica, Nussloch, Germany). The average number of tooth sections with pulp tissue that were obtained from the first and second

Table. Number of teeth according to groups and tooth types.

	Tooth type	Fluorotic	Nonfluorotic	Total
Maxillary	1. Molar	7	7	14
	2. Molar	7	7	14
Mandibular	1. Molar	7	7	14
	2. Molar	7	7	14
Total		28	28	56

primary molars was 130 and 190, respectively. The sections were mounted onto gelatinized glass slides and stored for 24 h at 37 °C in a thermostatically controlled oven, and they were later stained with hematoxylin and eosin (Figure 1).

The total number of odontoblast cells in the coronal part of each tooth in both groups was estimated using an optical fractionator method (a combination of optical dissector and fractionator) (Figure 2) (17). The coronal part of the tooth pulp (above a line across the most superior part of the pulpal floor) was determined according to the criteria described by Chandler et al. (18). A stereological workstation composed of a digital camera (Nikon E 4500, Nikon Co. Ltd., Tokyo, Japan), a personal computer, a computer-assessed motorized specimen stage (Prior, Rockland, MA, USA), a microcator (Heidenhain, Traunreut, Germany), and a light microscope (Nikon Eclipse E600, Nikon) was used for estimation of the total number of odontoblast cells. Odontoblasts were identified as being the outermost stratum of columnar cells, adjacent to the predentine and

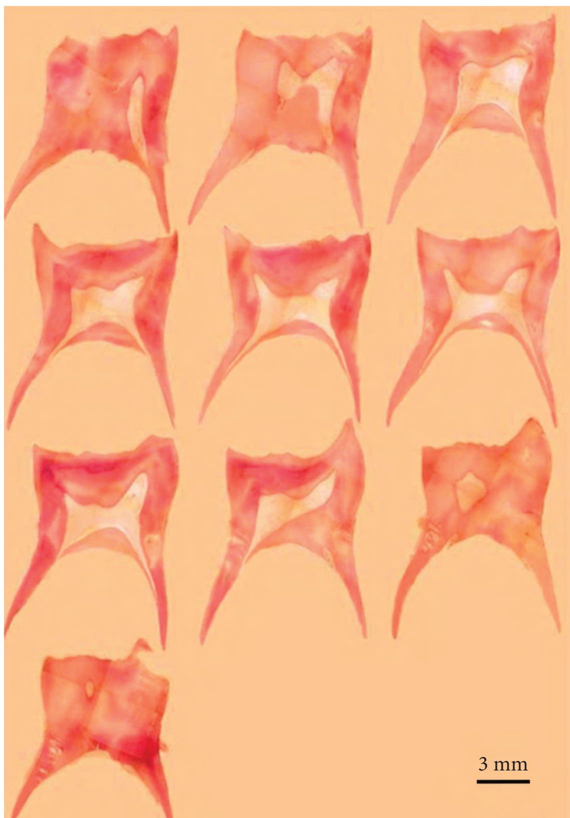

Figure 1. Systematically and randomly sampled cross-sections of a tooth.

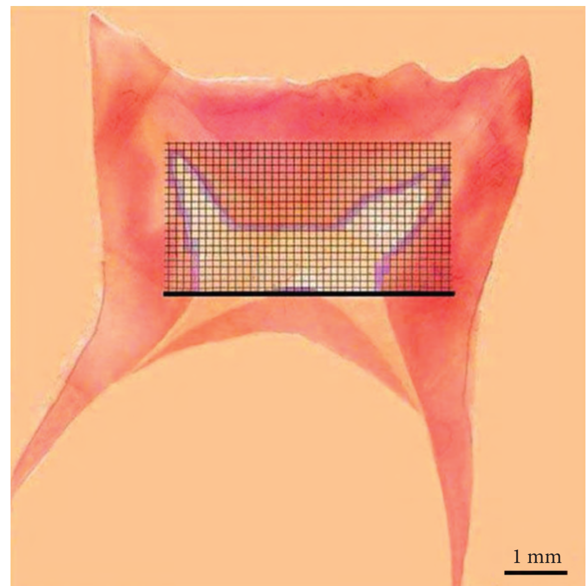

Figure 2. Using the optical fractionator method, a systematically and randomly sampled cross-section of a tooth. Drawn and painted areas represent the steps used in the optical fraction.

displaying eosinophilic cytoplasm and a nucleus located in a basal polarized position (19,20). Odontoblasts were counted using the widest profile of the nucleus that came into focus within the optical dissectors systematically, randomly spaced throughout the odontoblast layer (Figure 3). The estimated total number of cells was calculated from the number of counted cells and the sampling probability (17). The total number of odontoblast cells in the coronal part of tooth pulp was estimated according to the following formula:

$$N = \Sigma Q . \frac{1}{ssf} . \frac{1}{asf} . \frac{1}{tsf}$$

where N represents the total number of odontoblasts, ΣQ is the total dissector odontoblast number, ssf is the section sampling fraction, asf is the area sampling fraction, and tsf is the thickness sampling fraction (21). The values of the section sampling fraction, area sampling fraction, and thickness sampling fraction were differentiated due to the tooth types.

Homogeneity of the variables was controlled by Levene's test, and it was observed that all of the group variances were homogeneous. All of the data were expressed as means ± standard deviations for each group. Statistical significance of the difference between groups was analyzed with a parametric test of the independent-samples t-test. Mean values were considered significantly different when $P < 0.05$. Statistical analyses were performed using SPSS software (13.0 standard version).

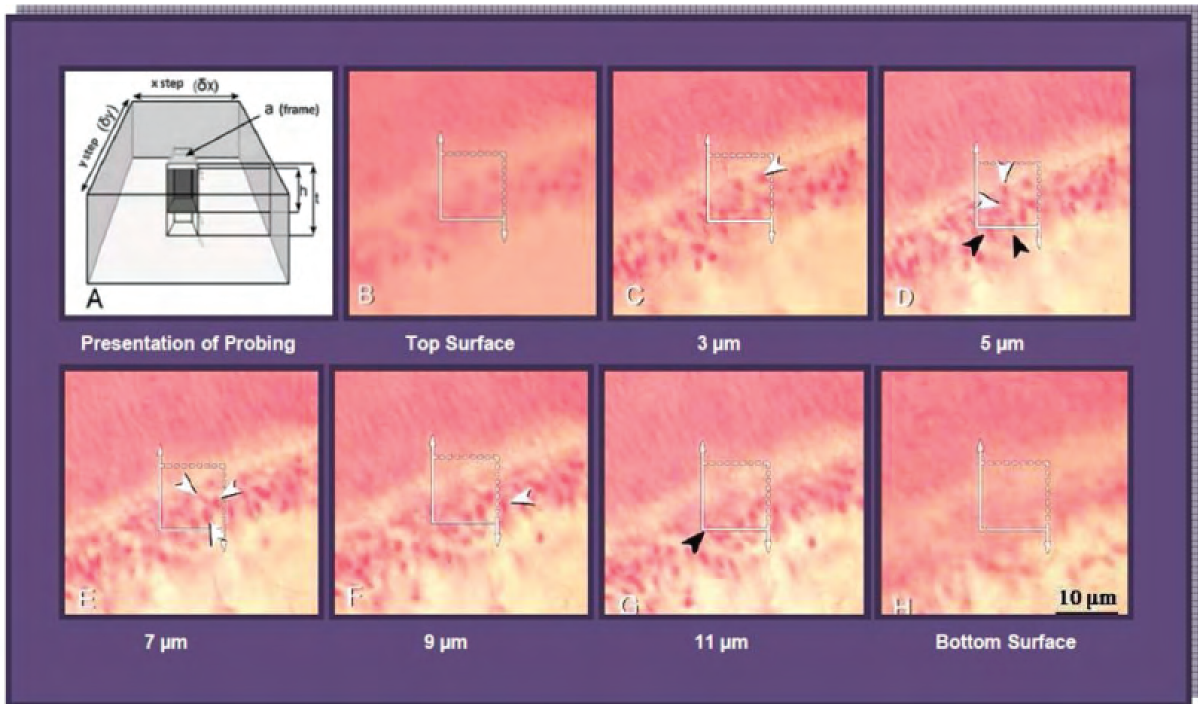

Figure 3. Counting with the optical dissector method. Presentation of probing (A); counting has been done on subsurfaces (in µm) from the bottom to top surface by leaving security zones (h: the height of dissector, t: the thickness of the fraction). Photographs of optical fractions on a 2-µm space were taken throughout the dissector height, from the top surface (B) to the bottom surface (H). The forbidden corners of the objective count framework used in the count and the limbs of these corners are shown with a straight line, whereas the free corners are shown with dashed lines. While the optical platform approached the fraction from the top of the fraction, the place where the tissue was first seen clearly was determined as the fraction top surface (B) and the microcator gauge was reset. By proceeding 3 µm into the fraction from the top surface of the fraction, the top security zone was established. In the odontoblast count, levels where the nucleuses had the widest diameter were taken as the criteria. Nucleuses (shown with white arrow) lower in the framework and not overlapping with the forbidden corner were included in the count. The nucleuses that overlapped with the forbidden corner (shown with black arrow) were not included in the count. With the G fraction, we have come to the end of the dissector height; the distance from here to the fraction bottom surface was maintained as the bottom security zone. The final clearly seen image surface is the bottom surface of the fraction. Hematoxylin and eosin staining was used.

Results

The mean number of odontoblast cells in fluorotic second molar teeth (n = 630,775.0) was significantly lower compared to nonfluorotic teeth (n = 665,200.9) (P = 0.025) in the maxillary jaw. Similarly, a statistically significant difference was found in the mandibular jaw, and the mean number of odontoblast cells in fluorotic second molar teeth (n = 630,758.9) was significantly lower than in the nonfluorotic group (n = 679,726.4) (P = 0.048). There was no significant difference between fluorotic and nonfluorotic first molar teeth in both jaws (maxillary, n = 370,203.9/399,594.9; mandibular, n = 522,077.0/534,534.4) (P > 0.05) (Figure 4).

Discussion

Accurate information on odontoblast cell numbers can provide information on the dentinal repair capacity of the pulp tissue following restorative treatment. This is because the number of odontoblast cells is a critical factor mediating the scale of pulp reparative responses (3,4).

The effects of dental restorative procedures on pulp tissue and odontoblasts were previously investigated by counting the number of odontoblasts in a pulpal unit area using an ocular graticule (3,4,6,20). Quantitative information about 3-dimensional structures was obtained from observations made on 2-dimensional histological sections; however, counting the profile numbers per unit area of a section will never give a reliable estimate of number (22). According to stereological principles, the 3-dimensional dissector probe, with its unbiased counting rule, is used systematically to estimate the number of cells. The technique for estimating the total number of odontoblasts in a defined region is free of assumptions about the size, shape, orientation, and distribution of odontoblasts; is readily performed; and is intuitively easy to understand (23).

It was reported that stereological techniques may provide quick, unbiased, and reproducible estimations, and they have to be performed to obtain reliable results in spite of their more time-consuming features (24-26). The widespread application of stereological principles can contribute to elimination of the controversy about age-related odontoblast loss (27), or any other process in which the quantitation of the structural dynamics may be of interest, to which the stereological tools can make a contribution.

In a previous study, it was found that pulpal recovery after experimental injury was delayed following fillings that contained 30% NaF (by weight), and in the postoperative period, full recovery was incomplete even at day 32 (28). The reaction of the pulp to the application of fluoride in deep cavities was studied by Brännström and Nyborg (29), and they stated that an application of 8% stannous fluoride for 5 min could result in local necrosis and moderate inflammation.

Although odontoblast cell numbers have been found to be critical to accomplish dental repair (3), the number of studies that have investigated the effect of fluoride on the odontoblast cell numbers is

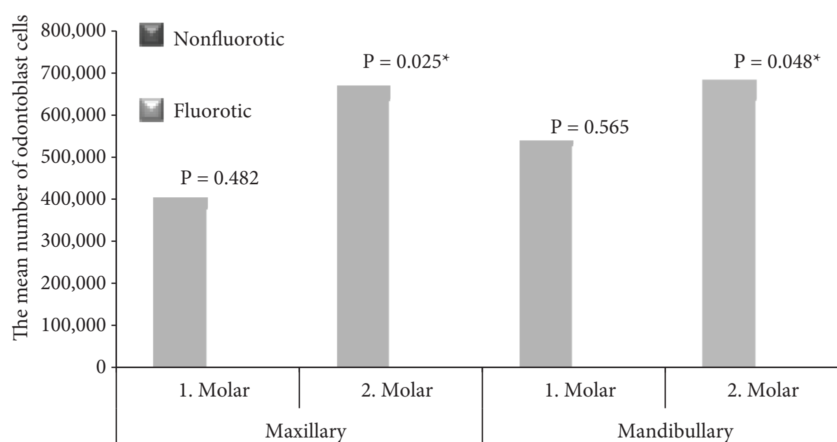

Figure 4. Number of odontoblast cells according to groups.

limited. In an earlier report, fluoride was found to change the number of odontoblast and cementoblast cells. Investigators determined that the number of odontoblast cells was increased on day 30 and decreased on day 60 of the experiment (15). Distinctly, Moseley et al. (13) observed that there were no significant differences in odontoblast numbers throughout the culture period, both in the absence and presence of sodium fluoride. A literature search showed that the number of studies that investigated odontoblast cells in human teeth is limited; the results of these studies revealed no significant difference in odontoblast cell numbers found according to patients' sex, which is in accordance with the results of the present study (3,27).

Despite the limited number of studies aimed at revealing the effect of fluoride on the odontoblast cells, in the present study, the relationship between fluorosis and the odontoblast cell numbers in human teeth was investigated. To the best of our knowledge, this relationship had not been studied until now. In the current study, the number of odontoblast cells in fluorotic second molar teeth was significantly lower than in the nonfluorotic group, in both the maxillary and mandibular jaws. However, the odontoblast numbers in the first molar teeth groups were not significantly different in the presence or absence of fluorosis. This may be due to the longer development period of second molar teeth than first molars; the prolonged fluoride exposure to second molar teeth during development stages can increase harmful effects. Furthermore, in understanding the pre- and postnatal development effects of fluoride on the first and second primary molars, it should be considered that the first primary molar starts mineralizing before birth.

This study's finding that the number of odontoblast cells in the fluorotic second primary molar teeth was lower than that in nonfluorotic teeth indicates that fluoride could affect the number of odontoblast cells and the mechanism of pulpal recovery following operative procedures as a result of fewer odontoblast cells. In conclusion, operative dental treatments of fluorotic teeth should be performed in consideration of reduced pulpal repair capacity. Clinicians, especially in endemic areas for dental fluorosis, should place importance on extended periods of follow-up visits.

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