



Effect of Radiotherapy on Microhardness and Surface Morphology of Pretreated Primary Teeth with Silver Diamine Fluoride: An *In vitro* Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Study Protocol

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ABSTRACT

Background: Radiation therapy has proven to be an effective modality in treating children suffering from cancer. High prevalence of dental caries is seen in children undergoing radiation therapy. This is primarily due to the direct effects of these harmful radiation on the enamel and not as a consequence to the reduced salivary flow. Mechanical properties of the enamel have been seen to be affected by radiation according to various studies conducted.

Objectives: The objective of this study is to evaluate whether radiotherapy causes changes in the microhardness and surface morphology of enamel in primary teeth pretreated with silver diamine fluoride.

Methodology: 22 primary teeth samples will be divided into two groups, 11 samples each-study group which will be pretreated with silver diamine fluoride (SDF) and control group. Both groups will be subjected to radiotherapy of 10 Gy radiation for a duration of 6 days. The samples will be tested for Vickers microhardness test and SEM analysis for surface morphological changes post

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radiotherapy. Statistical analysis will be done using “chi square test, paired and unpaired student t - test”. Surface microhardness and morphological changes of the study and control group compared.

Expected Results: It is expected that SDF application will be effective modality in prevention of caries due to frequent radiation exposure in children suffering from cancer and undergoing radiotherapy.

Conclusion: The study will conclude that SDF will cause a reduction in the microhardness of the surface and lesser morphological changes in the enamel of the primary teeth post radiation therapy in children suffering from cancer.

Keywords: Radiotherapy; silver diamine fluoride; primary teeth.

1. INTRODUCTION

Cancer is one of the deadliest diseases known to mankind resulting in around 4 lakh children being diagnosed each year, of which a large number around the globe do not have proper access to health care [1].

Radiation therapy has been used extensively in the field of medicine, for the treatment of various kinds of cancers but its margin of safety is still low. Underdosage of radiation may result in missing parts of the tumor. While, increased radiation dose may result in intolerable complications. Both leading to massive treatment failures.

Head and neck region undergoing radiation therapy has various adverse effects on the oral cavity like changes in the saliva production which leads to xerostomia, radiation caries and also changes in the oral flora these effects are even more profound in children who are not able to maintain good oral hygiene [2].

In addition, as seen in various literature review articles, the increased amount of caries incidence observed in patients undergoing radiation therapy especially children are primarily due to the direct effects of these harmful radiation on the enamel and not as a consequence to the reduced salivary flow. The main content of the tooth structure that is the organic and inorganic content is affected during radiation therapy. Various studies have proved that there is destruction of the prismatic structure of enamel, decrease in mechanical properties such as tensile strength, wear resistance, and acid attack resistance all these changes have been seen in the permanent teeth of patients who have undergone radiation therapy [3]. However, destruction of the collagen fibrils is also seen, which results in decrease in the hardness of dentin, tensile strength, and stability of the DEJ. Primary teeth when compared to

permanent teeth have thinner and higher density of rods, large number of microporosities, exposed rods in some areas, and a lot of carbonate addition. All these factors make primary teeth more susceptible to radiation caries and more extensive carious lesions [2,4-5].

The use and applications of different concentrations of silver diamine fluoride (SDF) can stop and obstruct the progression of carious process. Various studies have shown that SDF has a positive antibacterial effect on *Streptococcus mutans* and *Lactobacilli* which proves its anticariogenic properties. DNA replication is impaired and cell wall damage occurs when Silver nitrate ($AgNO_3$) nanoparticles pierce the bacterial cell wall. Not only with the presence of bacteria but also bacteria-free conditions, it has been seen that it prevented further demineralization of the already very carious lesion [6-7].

Rationale: Children having cancer and undergoing radiation therapy have compromised oral health. Such children have to undergo a series of invasive treatment approaches for the management of caries which are not readily acceptable by them. Evaluating the effect of radiotherapy on the enamel of primary teeth treated with SDF and if it is effective in prevention of development of caries.

Aim: To evaluate the effect of radiotherapy on microhardness and surface morphology of pretreated primary teeth with silver diamine fluoride.

1.1 Objectives

- To evaluate the microhardness of pretreated enamel of primary teeth with SDF before and after radiotherapy.
- To evaluate the microhardness of the enamel of normal primary teeth before and after radiotherapy.

- To compare the microhardness of the pretreated enamel of primary teeth with SDF and normal primary teeth after radiotherapy.
- To evaluate and compare the surface morphology of pretreated enamel of primary teeth with SDF and normal primary teeth after radiotherapy.

Specimens will be divided into two groups:

1. Study group: Deciduous teeth pretreated with SDF.
N=11
2. Control group: Normal deciduous teeth.
N=11

2. MATERIALS AND METHODS

2.1 Materials

- Mouth mirror
- Straight probe
- Kidney tray
- Gloves
- Mouth mask
- Head cap
- Disposable cups
- Primary teeth slabs (3x3x2 mm)
- Kids E SDF (Silver diamine fluoride)

2.2 Methods

2.2.1 Study design

This in vitro study will be conducted in the "Department of Pediatric and Preventive Dentistry, Sharad Pawar Dental College, Sawangi, Wardha".

Inclusion criteria: Anterior or posterior caries free primary teeth.

2.2.2 Exclusion criteria:

- (1) Carious teeth.
- (2) Primary teeth with any developmental defect.
- (3) Primary teeth with any cracks

Sample size: 11 samples will be taken in each group.

2.3 Sample Preparation

The selected teeth will be sectioned into crown and root, with the help of cutting machine which has a double-sided diamond disc. The buccal and lingual surfaces of the crown would be separated, the final specimens will have dimensions of 3 × 3 × 2 mm. Specimen will then be grinded and polished. Sandpaper grains of 600 and 1,200 will be used for polishing the enamel. The specimens will be numbered.

2.4 Sample Size Justification

Sample size formula for difference between two means

$$n = \frac{(z\alpha + z\beta)^2 \left[\frac{\delta_1^2}{k} + \frac{\delta_2^2}{k} \right]}{\Delta^2}$$

Where,

Z α is the level of significance at 5% level of significance i.e 95% confidence interval =1.96

Z β is the power of test=80%=0.84

δ_1 =SD of control group=53.48

δ_2 =SD of 20 Gy group=26.31

Δ =204.19-152.34=51.84

$$n = \frac{(1.96+0.84)^2 [(53.48)^2 + (26.31)^2 / 1]}{(51.84)^2}$$

$$=10.35$$

$$=11 \text{ samples in each group}$$

SDF application before radiation: SDF will be applied on the specimens of the study group as per the manufacturer's instructions.

2.5 Enamel Irradiation

Specimens of both the study and control group will be subjected to 10Gy radiation per day for 6 days. Children suffering from head and neck cancers are given a radiation therapy of 45-60 Gy radiation over a stipulated time, so this protocol has been replicated. The specimens will be kept in individual wells immersed in artificial saliva of pH7.0 containing 0.9 mM PO₄, 1.5 mM Ca & "150 mM KCl incorporated into 20 mM Tris buffer". SDF will be reapplied on the samples of the study group after the 2nd and 5th cycle of radiation.

2.6 Enamel Microhardness Analysis

For evaluation of microhardness after irradiation, three indentations will be created on the central areas of the enamel specimens, using a Vickers microhardness tester. The average will be calculated of all the three indentations for each specimen of the study, and the final median

microhardness's of enamel will be calculated and expressed as the Vickers hardness number.

2.7 SEM Analysis

Two specimens each of the study and control group will be kept in silica for at least 48 h for dehydration while double-sided carbon tapes will be used to fix the stubs. Then, scanning electron microscope will be used for examination of the specimen. Standardized images will be acquired of the enamel surfaces.

2.8 Statistical Analysis

"Chi square test" and "paired and unpaired student t -test" will be used for statistical analysis.

Hypothesis: It is expected that SDF application will be effective modality in prevention of caries due to frequent radiation exposure in children suffering from cancer and undergoing radiotherapy.

3. REVIEW OF LITERATURE

Lenita Marangoni-lobes et al. [2] In their study evaluated the "microhardness, mineral content, organic composition and morphological changes" in the primary teeth after radiotherapy. Thirty prepared samples of deciduous teeth were exposed to radiotherapy. At different amounts of radiation, the samples had to undergo various tests for evaluation. They were immersed in artificial saliva so its pH was determined along with the calcium and phosphate concentrations of the solution. The outcomes suggested that the microhardness of the study specimen reduced after 2nd dose of radiation which was 2,160 cGy. However, for dentin, the surface hardness reduced after the 1st dose of radiation. The mineral and organic content of enamel reduced after 3rd dose of radiation. For dentin, they noticed and could see a rising increase in the amount of "phosphate v2, amide, and hydrocarbon content" after 1st and 2nd and a reduction after 3rd dose of radiation. Results showed enamel cracks and destruction of peritubular dentin. The study inferred there was an all over decrease in "surface hardness, altered mineral and organic composition, and changes in the morphology are seen on the primary teeth after radiation therapy".

C.J. Soares et al. [8] evaluated the therapeutic outcome of using mouthwash in preventing

radiation therapy damage to the "ultimate tensile strength (UTS)" of the hard tissue of the teeth. 120 teeth were separated into 2 groups. The first group was exposed to "60 Gy" of radiation in daily additions of "2 Gy" and the second group was not exposed to any radiation. Further categorized into "2 mouthwash protocols used 3 times per day": First, CHX of 0.12% second, sodium fluoride of 0.05% and third was control group (n =10). Microtensile testing was performed on the samples. The UTS was significantly reduced after radiation therapy. SEM analysis detected structural alterations. It was concluded that mouthwash with 0.12% CHX partly helped in preventing harm to the "mechanical properties" of the tooth subjected to radiation.

Franzel et. al. reported on Effect of tumor therapeutic irradiation on the mechanical properties of teeth tissue [9].

Sura A. Abdil-Nafaa et al. [10] compared and evaluated the result of "fluoride varnish application and SDF application" on the "surface microhardness" of deciduous teeth. 150 deciduous anterior teeth were selected in the study population. Three groups were made of: Fluoride varnish, SDF and the control group of 50 samples then underwent PH cycling. Vickers tester machine was used to measure the microhardness once before and after the PH cycle. Results presented with a reduction in surface microhardness in all samples because of demineralization, but the minimum decrease in surface microhardness was seen with SDF group followed by varnish group. SDF showed better results with preserving the surface hardness of the enamel of the tooth structure.

Few of the studies on primary teeth were reported by Singh et al. [11] Teni et al. [12] Khubchandani et al. [13] Rathi et al. [14] and Baliga S [15] Studies by Prabhakar A [16] [17] were reviewed.

4. CONCLUSION

Children suffering from cancer undergo frequent radiotherapy and this radiation exposure to the enamel makes it more prone to radiation caries. The present study will suggest pre-treatment of the primary teeth with SDF for prevention from the harmful effects of radiation.

SDF application may prove as an effective and affordable prevention measure in children who

are frequently undergoing radiation therapy for cancer.

5. LIMITATIONS

In radiation therapy, the salivary glands are dysfunctional which leads to decrease in the salivary flow which was not taken into consideration in this study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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