10.21608/ADJC.2023.196751.1271 Advanced Dental Journal
Volume 5| Issue 2| Pages 442-448

Original Article

Microhardness and Bacterial Inhibitory Effect of Riva Star versus Silver Diamine Fluoride on Carious Dentin of Primary Teeth: In-vitro study)

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Submitted: 10-3-2023
Accepted: 31-5-2023

Abstract

Aim: The aim of this study was to measure the microhardness and the antibacterial effect of Riva star on carious dentin of primary teeth versus those treated with SDF only.**Subjects and methods:** For microhardness testing: Thirty freshly extracted primary molars were collected. Occlusal enamel and soft carious dentin were removed. The teeth were randomly allocated to control (SDF) or intervention group (Riva star). Vicker's microhardness was measured at baseline and after treatment. For inhibition zone measurement: The standard strain of *Streptococcus mutans* was used to test the antibacterial effect. Four wells were incorporated with Riva Star, SDF, chlorhexidine and distilled water. The zones of inhibition were measured in millimeters in diameter around the material after 24 h. **Results:** Microhardness test: There was no statistically significant difference at baseline between (Riva Star) and (SDF) groups (p=0.050). After treatment, there was a statistically significant difference between (Riva Star) and (SDF) groups (p<0.001). Inhibition zone measurement: A statistically significant difference was found between (Distilled water) and each of (Riva Star), (SDF) and (Chlorhexidine) groups (p<0.001). **Conclusion:** SDF and Riva star both resulted in increased microhardness of carious dentin of primary teeth. SDF/KI had a relatively lower antibacterial effect in comparison with SDF alone.

Keywords: Silver diamine fluoride, Potassium iodide, Riva star, Microhardness, Carious dentin of primary teeth, Antibacterial effect.

I. INTRODUCTION

Dental caries is defined as a chronic disease which affects people all over the world of all ages and it is also a common dental health problem within school children globally. When a great amount of tooth structure is destroyed by caries, restorative treatment can restore function, improve esthetics or phonetics. As children are usually afraid of restorative treatment, restorative treatment may be deferred when caries arrest is achieved, especially in uncooperative children (Calatayud et al., 2009 and Naaman et al., 2017).

Silver diamine fluoride (SDF) is considered one of the most recent means of preventive and therapeutic caries management. It is

liquid that has the bactericidal effects of silver and the remineralization effects of fluoride; it is an excellent new agent for managing carious lesions and treatment of dentin sensitive surfaces in young children and those with special health care needs. When silver diamine fluoride is applied to a decayed surface, it increases its resistance to acid dissolution and enzymatic digestion and the treated lesion has higher mineral density and hardness and less lesion depth (Mei et al., 2013 and Crystal et al., 2019).

Silver diamine fluoride reduces the breakdown of the dentin organic matrix. The fluoride strengthens the tooth structure during the bacterial attack by its acid byproducts and decreases its solubility. Silver ions action is directly against bacteria in lesions; break membranes, denature proteins, and inhibit DNA replication. Silver diamine fluoride is the only anti-caries medicament that kills cariogenic bacteria in dentinal tubules (Nuvvula et al., 2019).

Despite its benefits, the major side effect for SDF is staining to the arrested carious lesions, skin, clothes and work surfaces. Tooth discoloration definitely cause patient dissatisfaction, although older adults rarely complained. Moreover, staining in posterior teeth was more acceptable than anterior ones (Zhao et al., 2017).

SDF only stains carious enamel and dentin, but not sound tooth structure. The darker the color, the more arrested the lesion is, especially by increasing the concentration and frequency of application. Thus, most studies recommend an informed consent; so that the patient can understand the benefits and drawbacks of the treatment

Recently, application of saturated solution of potassium iodide (KI) immediately after SDF has been suggested to overcome the black staining caused by SDF. It is postulated that KI prevents staining through the precipitation of excess silver ions as white silver iodide. However, till now there is contradictory evidence around the efficacy of KI to remove discoloration and also not to affect SDF to perform its actions (Espíndola et al., 2020). The aim of this study is to measure the microhardness and the antibacterial effect of Riva star on carious dentin of primary teeth versus those treated with SDF only.

II. SUBJECTS AND METHODS

A. Microhardness Testing

A total of 30 extracted primary molars with occlusal caries were collected from outpatient clinic of Pediatric Dentistry Department, Faculty of Dentistry, Cairo University. Teeth were stored in distilled water (Abrams et al.,2017) and numbered from 1 to 30. Excavation of all soft caries was done using a sharp excavator. To expose an area of flat carious dentin surface, 2-4 mm of the crown was removed horizontally from all teeth using diamond disks (MANI, Inc. Japan) under copious water irrigation. The roots were cut using a diamond disc under copious water irrigation (Alulaiyan et al., 2019). The teeth were embedded half-way in fast set cold cure acrylic resin (Acrostone, Egypt). The specimens were divided randomly into 2 equal groups: control group (SDF) and experimental group (Riva Star).

Control Group (SDF):

After air dryness, one drop of SDF (Riva star, SDI Limited, Australia) was applied to the dentin surface, and actively rubbed for 10 seconds. The material was then left untouched for 1 minute to be absorbed within the tooth. Excess material was removed by a cotton Q-tip (blot dryness) (Alulaiyan et al., 2019).

Test Group (Riva star):

The first step was performed typical to SDF application followed by immediate application of generous amount of KI solution (Riva star, SDI Limited, Australia) to treatment site using a micro brush until creamy white precipitate turned clear then blot dryness, (Alulaiyan et al., 2019). Digital Vickers hardness tester (Tukon 1102 Wilson microhardness tester, Buehler, Germany) was used in this study for assessment of the microhardness of carious dentin before and after application of the two materials, using a

microscope of X100 magnification.

Vickers hardness number (VHN) was measured at three points of each specimen. The mean and the standard deviation of the three values were obtained. Each measurement was obtained by applying 100 grams load for 5 seconds oriented perpendicularly to the dentin surface.

B. Measurement of Bacterial Inhibition Zone

The microorganisms used in this study were S. mutans. The culture medium used to isolate the strains of S. mutans was Mitis Salivarius agar.The steps of agar diffusion test as suggested by Scarpelli et al., 2017 were employed.

a) Saliva samples obtained were grown in brain heart infusion (BHI) broth for isolation of S. mutans and incubated anaerobically for 18 hours at 370c.

b) The obtained growths were subcultured on Mitis Salivarius agar and incubated anaerobically for 48 hours at 370c.

c) The pure bacterial strains isolated from Mitis Salivarius agar plates were again transferred into broth under sterile conditions and incubated for 18 hours anaerobically at 370c.

d) Four wells of 5 mm diameter and 2 mm depth were prepared in agar plates with agar punchers. These wells were incorporated as follows: the two test materials which are SDF and Riva star, in addition to, chlorhexidine as positive control and distilled water as negative control.

e) The plates were incubated anaerobically for 24 h at 37°C under 5% CO2.

The antimicrobial properties of materials were assessed by the diameters of these zones of bacterial inhibition were measured in millimeters (mm) using ruler after 24 hours (from the edge of the zone from one end to the next edge).

III. RESULTS

A. Microhardness testing results:

Effect of time:

The results of microhardness testing before and after material application within each group (intragroup comparison) were as follows:

a) Riva Star:

There was a statistically significant difference between (Baseline) and (After 24hrs) groups where ($p<0.001$). The highest mean value was found in (After 24 hrs), while the least mean value was found in (Baseline) group.

b) SDF:

There was a statistically significant difference between (Baseline) and (After 24hrs) groups where ($p<0.001$). The highest mean value was found in (After 24 hrs.), while the least mean value was found in (Baseline) group as shown in table (1) and figure (1).

Effect of groups:

The results of comparing microhardness testing before and after material application between the different groups (intergroup comparison) were as follows:

a) Baseline:

There was no statistically significant difference between (Riva Star) and (SDF) groups where ($p=0.050$). The highest mean value was found in (Riva Star), while the least mean value was found in (SDF) group as shown in table (1) and figure (1).

b) After 24 hours:

There was a statistically significant difference between (Riva Star) and (SDF) groups where ($p<0.001$). The highest mean value was found in (SDF), while the least mean value was found in (Riva Star) group as shown in table (1) figure (1).

Two-way ANOVA:

Data shows the results of Two-way ANOVA analysis for the interaction of different variables. The results showed that different groups had a statistically significant effect. Also, time between baseline and after application of the materials (24 hours) had a statistically significant effect. The interaction between the two variables also had a statistically significant effect.

B. Measurement of Inhibition Zone:

There was a statistically significant difference between (Riva Star), (SDF), (Chlorhexidine) and (Distilled water) groups ($p<0.001$) regarding the diameter of inhibition zones. A statistically significant difference was found between (Distilled water/Negative control) and each of (Riva Star), (SDF) and (Chlorhexidine/Positive control) groups ($p<0.001$). No statistically significant difference was found between any other groups. The highest mean value was found in (SDF) followed by (Riva Star) and (Chlorhexidine), while the least mean value was found in (Distilled water) as shown in table (2) and figure (2).

Table (1): The mean, standard deviation (SD) values of Micro-hardness of different groups

Variables	Micro-hardness				p-value
	Riva Star		SDF		
	Mean	SD	Mean	SD	
Baseline	34.50	5.27	30.75	4.59	0.050ns
After 24 hours	55.84	7.59	69.01	4.95	<0.001*
p-value	<0.001*		<0.001*		

Table (2): The mean, standard deviation (SD) values of inhibition zone of different groups

Variables	Inhibition zone	
	Mean	SD
Riva Star	2.47	0.78
SDF	2.55	0.58
Chlorohexidine	2.19	0.20
Distilled water	0.00	0.00
p-value	<0.001*	

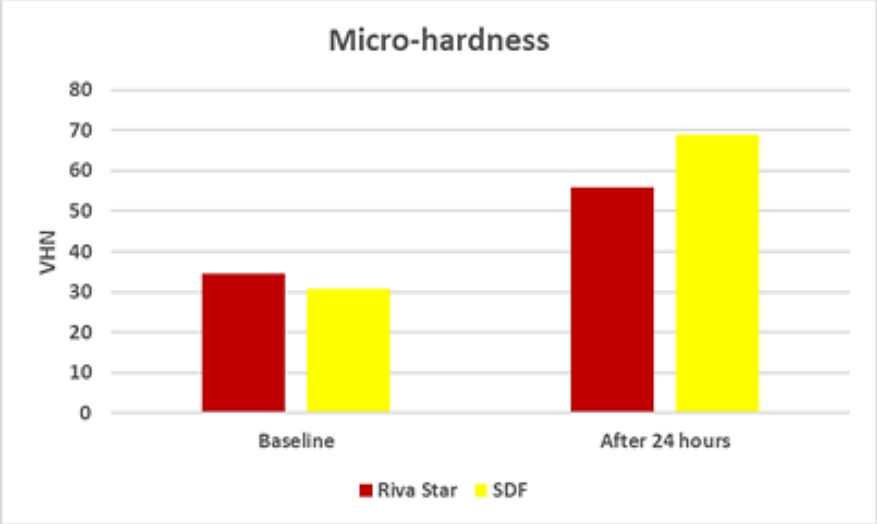

Figure (1): Bar chart representing microhardness

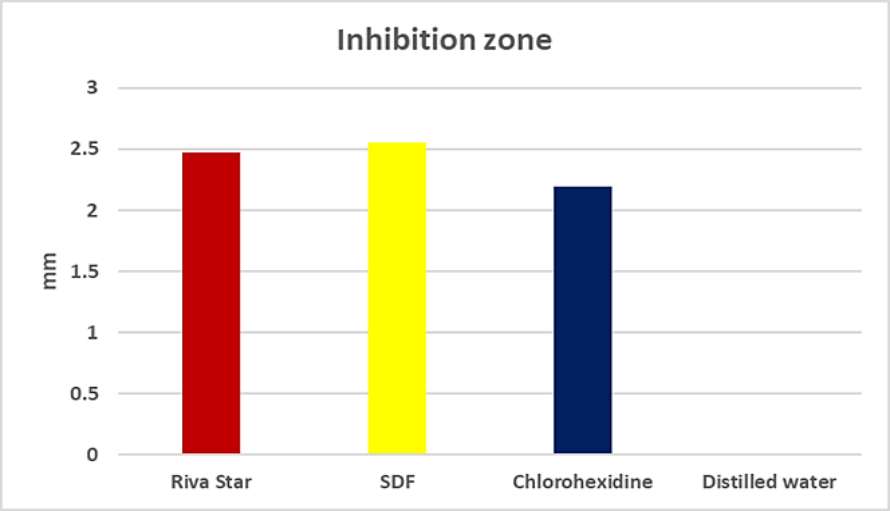

Figure (2): Bar chart representing inhibition zone for different group

IV. DISCUSSION

The commercial product of SDF + KI used in the current study was Riva star from SDI Limited as it is the only commercial product of SDF + KI available in the market. Thus, this study evaluated the microhardness and bactericidal potential using Vickers microhardness testing and inhibition zone measurement by agar diffusion method.

Microhardness analysis has been considered as a method to assess loss and reincorporation of minerals to the dental tissue because the reduction in the numerical hardness value presents a linear relation to mineral loss. The dentin hardness has been reported as an indicator for caries removal because it is associated with the relative infectivity of carious dentin, helping the dentist to distinguish between either heavily infected (soft) or minimally affected (hard) dentin as stated by Anwar et al., 2017 and Valian et al., 2017.

Regarding the results of the microhardness test, there was a statistically significant difference between (Riva Star) and (SDF) groups. The highest mean value was found in (SDF), while the least mean value was found in (Riva Star) group.

The study of Chu et al., 2008 was in agreement with the result of this study. Chu's clinical trials on primary teeth reported microhardness to be higher in the outermost dentinal surface of arrested carious lesions receiving regular application of 38% SDF for 30 months compared to active carious lesions. Prakash et al., 2021 investigated the effect of SDF versus Riva star on microhardness of carious and sound dentin in permanent teeth with results similar to this study. They explained the higher remineralization effect by SDF alone because the application of KI may reduce the antimicrobial efficacy of SDF by reducing the concentration of silver ions.

This result was in disagreement with the result of the study of Briseño Marroquín et al., 2021. This conflict may be due to the small sample size in their study of less than ten individuals in each study group. Since the composition of plaque biofilm varies within individuals and between individuals, a larger sample size is required to illuminate the true antimicrobial effect of SDF/ KI as reported by Haiat et al., 2021.

Regarding the result of inhibition zone measurement there was a statistically significant difference between (Distilled water/Negative control) and each of (Riva Star), (SDF) and (Chlorhexidine/Positive control) groups. No statistically significant difference was found between any other groups. The highest mean value was found in (SDF) followed by (Riva Star) and

(Chlorhexidine), while the least mean value was found in (Distilled water), which means that there was no statistically significant difference between the antibacterial effect of SDF and that of Riva star.

The present study showed results similar to the result of a study done by Abdullah et al., 2020, where they found that KI did not have any antibacterial effect while both SDF and SDF/KI had potent antibacterial efficacy without any statistically significant difference between the two. Thus, they concluded that KI does not modulate the antibacterial efficacy of SDF.

The results of this study were in disagreement with Craig et al., 2012. They found that SDF has a significantly higher antibacterial effect compared with SDF combined with KI. However, (Hamama et al., 2015), has assumed that the original experimental SDF/KI solution used in the study by (Craig et al., 2012) consisted of 38% SDF; while the currently available commercial product 'Riva Star' consists of 30–35% silver fluoride and >60% ammonia solution. Thus the difference in SDF composition may have affected the antibacterial efficacy of SDF and the outcomes of this study. However, this assumption is not applicable in the present study as the concentration of the SDF used is identical to that used in Riva star group.

In contrast, Turton et al., 2020 has found that there is a difference in arrest rate according to the use of different silver fluoride solutions with and without the use of KI. There was no difference between the AgF and SDF groups. However, there were greater odds of caries arrest when KI was also applied over the SDF and the AgF group which indicates additional antibacterial activity of KI.

V. CONCLUSIONS

Within the limitations of this in vitro study, the following conclusions can be drawn:

(1) Silver diamine fluoride and Riva star both increased microhardness of carious dentin of primary teeth.

(2) Riva star has lower antibacterial effect against S. mutans than SDF.

(3) The addition of KI to SDF did not increase microhardness of dentin than SDF alone.

(4) SDF alone is more efficient in increasing microhardness of dentin than Riva star.

Conflict of Interest:

The authors declare no conflict of interest.

Funding:

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethics:

This study protocol was approved by the ethical committee of the faculty of dentistry-Cairo university on 30 /4 / 2020, approval number 12420.

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