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RESEARCH ARTICLE

Comparative Evaluation of the Remineralization Potential of Silver Diamine Fluoride (SDF) and Silver Diamine Fluoride with Potassium Iodide (SDF+KI) With or Without Laser Activation: An In-Vitro Study

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Abstract: Diamine Fluoride with Potassium Iodide (SDF+KI), Laser activated SDF and LASER activated SDF+KI. ***Method:*** Sample consisted of 40 freshly extracted healthy human premolar teeth, extracted solely for orthodontic purpose. The selected samples were randomly divided into four groups (n = 10): Group 1 (SDF); Group 2 (SDF+KI); Group 3 (LASER activated SDF) and Group 4 (LASER activated SDF +KI). All samples were evaluated for values of DIAGNOdent at baseline, after demineralization and after remineralization. They were further assessed for surface alterations using Polarized Light Microscopy and Morphometric analysis was also used to determine the amount of fluoride uptake. The statistical analysis was done using One Way ANOVA and post hoc Tukey's Test. ***Results:*** Highest remineralization potential was exhibited by Group 3. Polarized light Microscopic images of Group 3 shows the Greatest Surface alterations followed by Group 4 and Group 2 as the least at 10X magnification. Morphometric Analysis also reveals the highest fluoride uptake in Group 3 followed by Group 4. (Laser Activated SDF> Laser activated SDF with Potassium Iodide > SDF> SDF with Potassium Iodide). ***Conclusion:*** The use of laser-activated topical fluorides enhances fluoride uptake and offers superior caries prevention. Laser activated SDF with Potassium iodide can be used as an aesthetic alternative to SDF, providing comparable fluoride uptake without causing discoloration of the enamel surface.

Keywords: Remineralization, Silver Diamine Fluoride (SDF), Silver Diamine Fluoride, Potassium Iodide, Laser .

INTRODUCTION

Despite recent advancements in oral healthcare, dental caries remain a significant health problem for individuals of all age-groups.[1] The cycles of demineralization and remineralization continue with only one progressing at one time, depending on various pathological and protective factors.[2] With the paradigm shift for the management of dental caries, emphasis is now laid on remineralization therapies that can halt the progression of caries and repair the subsurface lesions rather than restoring the tooth and controlling the disease when obvious cavitation occurs.[3] Dental caries is one of the oldest and the most common diseases found in humans. The term "dental caries" is first describe in literature around 1634 years ago, and it is derived from the Latin word "caries," which means "decay".[4] In India, a studies done by Tyagi Ret al in 2008 shows prevalence rate of early childhood caries is 19.2%.[5] Caries occurs when the biofilm microbiota which are normally present in the oral cavity undergo homeostasis changes in to acidogenic, aciduric, and cariogenic due to the frequent consumption of sugars. [6] The result can be invisible clinically or lead to a net mineral loss in the tooth's hard structures, causing a visible carious lesion.[7] Thus, caries formation is not a continuous unidirectional process of demineralization. Instead, it is a cycle of demineralization and remineralization process with various stages being either reversible or irreversible. [8] Once this dynamic equilibrium shifts toward demineralization, the first sign of reversible white spot lesions occurs.[9] The demineralization process involves loss of minerals at the advancing front of the lesion, at a depth below the enamel surface, with the transport of acid ions from the plaque to the advancing front and mineral ions from the advancing front toward the plaque.[10] The remineralization process is a natural repair mechanism to restore the minerals again, in ionic forms, to the hydroxyapatite (HAP) crystal lattice.[11] There are various remineralizing agents for treatment of caries. The effectiveness and important of fluorides in treatment of caries has been well documented. Topical fluoride agents are commonly used to prevent caries formation especially in children.[7] Among the various professionally applied topical fluorides, Silver Diamine Fluoride (SDF) has significant antibacterial effect on streptococcus and actinomyces and gets precipitated in the biofilm. The application of silver diamine fluoride (SDF) leads to a significant increase in the microhardness of carious dentin.[12] Fluoride inhibits enolase and proton-translocating ATPase thereby inhibits the metabolic activity of bacteria and silver also reduces metabolic activity binding to the bacterial proteins. Both silver and fluoride hassynergestic effects.[13] But there are some adverse effects associated on application of SDF like oral pain, gum bleaching, and transient gum swelling can be seen in some children. Black staining is frequently found and it is directly proportional to concentration and frequency of application. [14] Silver Diamine Fluoride with Potassium Iodide reacts to form silver iodide (AgI) and Tripotassium phosphate (K_3PO_4). Tripotassium phosphate is a white powder, which masks the black stain caused by SDF alone. Zhao et al. reported that potassium iodide reduces discoloration by SDF and does not affect the bonding of glass ionomer cement (GIC) to dentin.[15]

The amount of fluoride uptake is limited to the surface layer. Thus, to increase the fluoride uptake and to improve the long-term effectiveness of the treatment a dental LASERs in conjunction with topical fluoride is used.[16] Laser technology and its applications in the dental field have recently emerged as a non-invasive modality and are considered a less painful approach, making it a good alternative to conventional treatment, particularly in children.[17] Diode laser have been shown to exhibit a significant antibacterial action against Streptococcus mutans.[18] Thus the purpose of this study is to compare the efficacy/ remineralization potential of SDF and SDF with Potassium Iodide with or without laser activation.

Materials And Methods

The present study was conducted in the Department of Pediatric and Preventive Dentistry. Prior to the study, approval was taken from the Institutional Ethical Committee. The sample size for each group was determined using the following formula: $N = [(4\sigma^2) (Z(1-(\alpha/2)) + Z(1-ß))^2] \div E^2$ where N = total sample size, σ = assumed standard deviation of each treatment response, Z(1-(α/2))= related to the chosen significance criterion α; can be found in normal distribution tables, or calculated in Microsoft Excel using the formula = NORM.S.INV(1- (α/2), Z(1-ß)= related to the chosen power, or sensitivity of the experiment; can be found in normal distribution tables, or calculated in Microsoft Excel using the formula = NORM.S.INV(1-β), E = minimum detectable difference between treatment mean. Using the above formula in SPSS statistical software 27.0 Version the sample size for each group was calculated to be 10, giving a total sample size of 40 for four groups. With the Inclusion criteria of freshly extracted healthy human premolar teeth and Exclusion criteria of teeth with caries, attrition, abrasion, erosion, fracture and teeth with any developmental anomalies. A consort showing the study design is presented in Fig 1.40 healthy human premolar teeth extracted for orthodontic purpose, free from caries, fracture or any other developmental anomalies were included in the study. Teeth exhibiting caries, attrition, abrasion, erosion, fractures, or any developmental anomalies were excluded from the study. The samples were subjected to sterilization according to CDC Guidelines (2003) and maintained in a hydrated state in a well constructed closed container. Each of the samples were decoronated by sectioning approximately 2 mm below the cement enamel junction and perpendicular to the long axis of the tooth, using carborandum disc. The coronal part of each tooth was embedded in self-cured acrylic resin in square moulds, keeping the buccal surface exposed. Specimens were stored in deionized water at room temperature till they were evaluated. Baseline DIAGNOdent values were taken before demineralization using Kavo DIAGNOdent™ pen.

The samples were subjected to demineralization for 1h in an acidic solution of 37% Phosphoric acid. Surface DIAGNOdentTM values were taken to calculate the amount of demineralization. After demineralization, all samples were randomly divided into the following 4 groups and subjected to remineralization as mentioned below.

Group 1: Treatment with silver diamine fluoride (SDF): Following demineralization, 38% (w/v) SDF (e-SDF, Kids-e-Dental LLP) solution was topically applied to the sample using a micro brush and then left for 1-3 min as per manufacturer’s instructions.

Group 2: Treatment with silver diamine fluoride with potassium iodide (SDF+KI): Following demineralization, SDF+KI (Riva Star, SDI) was also topically applied to the sample using a micro brush and then left for 1-3 min as per manufacturer’s instructions.

Group 3: Treatment with LASER activated silver diamine fluoride: Following demineralization, 38% (w/v) SDF (e-SDF, Kids-e-Dental LLP) solution was topically applied to the sample using a micro brush. Activation of SDF was done by Low level LASER Therapy with 980 nm Diode LASER (i-laser 2, Shenzhen Soga Technology Co. Ltd, Guangdong

Province, China) at 0.5 W, in continuous non-contact mode, keeping the tip 3-4mm away from the tooth surface for 1 min.

Group 4: Treatment with LASER activated silver diamine fluoride with potassium iodide. Following demineralization, SDF+KI (Riva Star, SDI) was also topically applied to the sample using a micro brush. Activation of SDF+KI was done by Low level LASER Therapy with 980 nm Diode LASER (i-laser 2, Shenzhen Soga Technology Co. Ltd, Guangdong Province, China) at 0.5 W, in continuous non-contact mode, keeping the tip 3-4mm away from the tooth surface for 1 min. Following application of respective remineralizing agents, DIAGNOdent values of all samples were taken, and further assessed under Polarized Light Microscopy (Fig. 2-5) and Morphometric analysis values (Fig. 6) were recorded. In Polarized light microscopy (Olympus BX53), an image was taken using a digital camera (Olympus EPL3) connected to the microscope, when examined in quinoline, a 30-40-µm surface zone displayed clear positive birefringence, suggesting that the outermost enamel functioned as a molecular sieve. Because of the lower content of minerals, the interpretation of this zone differed from that of similar “dark zones” observed in hypomineralized enamel.

Statistical Analysis

The data for the present study was entered in the Microsoft Excel 2010 and analyzed using the SPSS statistical software 27.0 Version. The descriptive statistics included frequency and percentage. The level of the significance for the present study was fixed at 5%.The inter group comparison of the quantitative data was done by OneWay ANOVA test and post hoc Tukey’s test

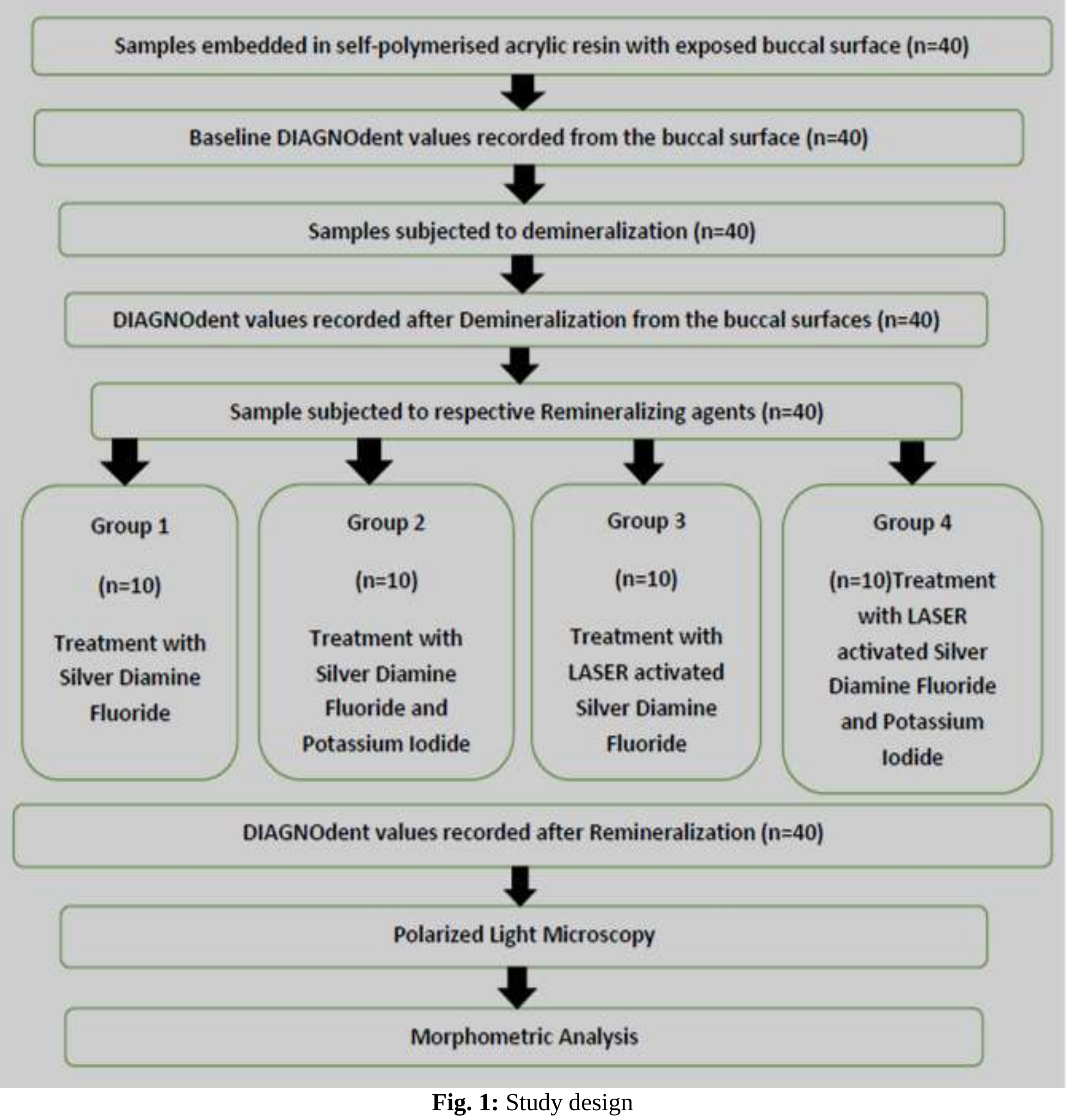

Fig. 1: Study design

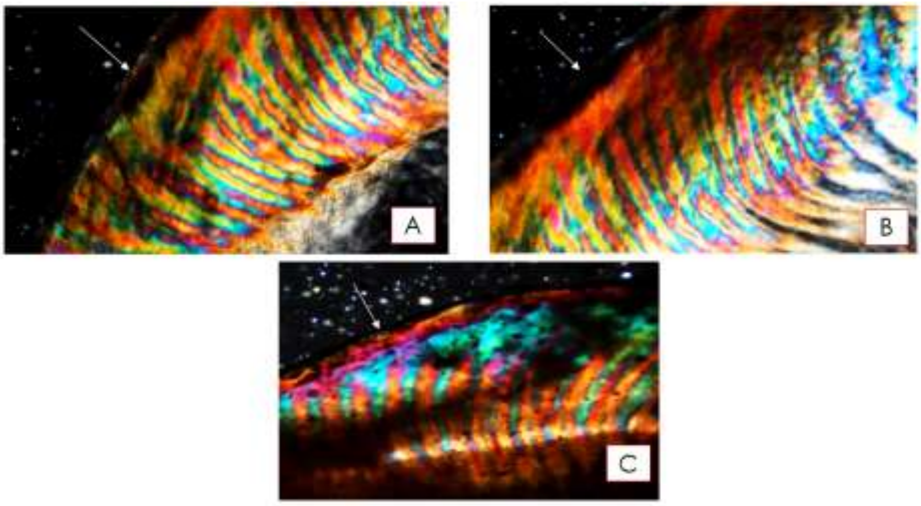

Fig.2: Group 1 SDF, Polarized light microscopic photographs at baseline (A), after demineralization (B) and after remineralization (C) at the middle of the third crown.

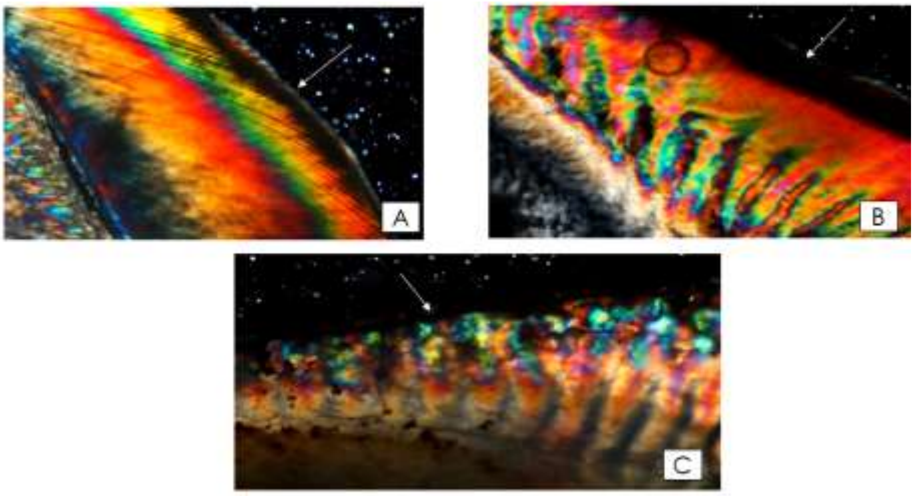

Fig.3: Group 2 SDF with Potassium iodide, Polarized light microscopic photographs at baseline (A), after demineralization (B) and after remineralization (C) at the middle of the third crown.

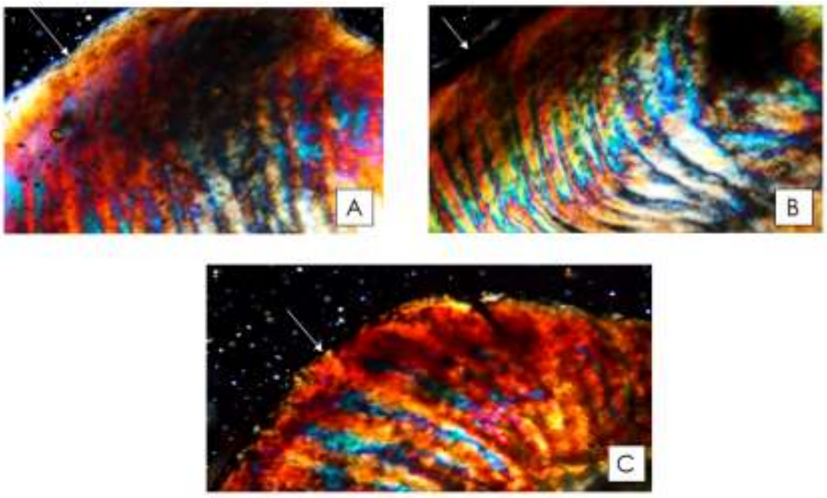

Fig.4: Group 3 Laser Activated SDF, Polarized light microscopy photographs at baseline (A), after demineralization (B) and after remineralization (C) at the occlusal third of the crown

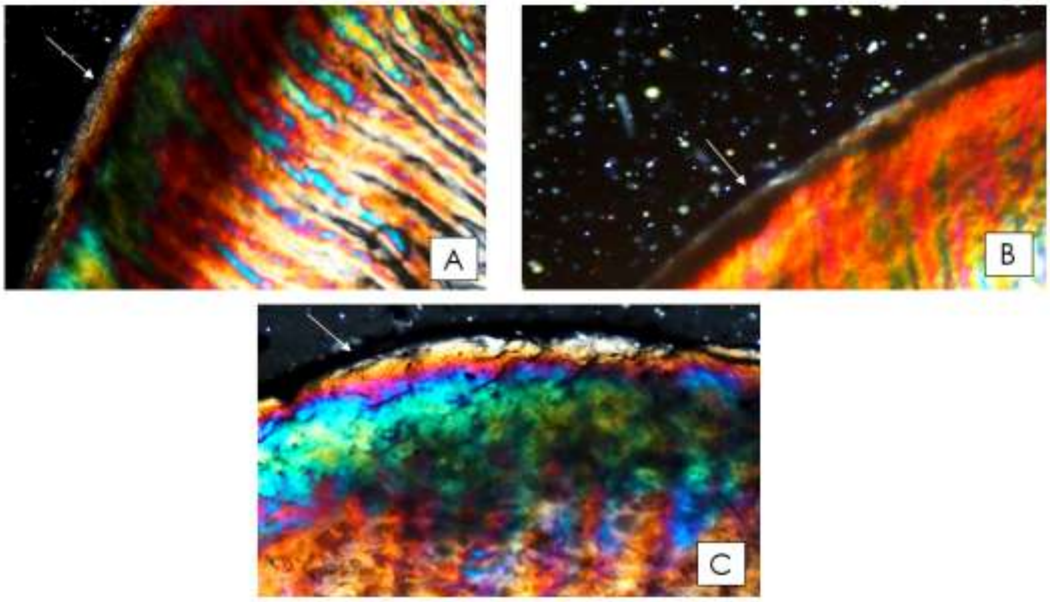

Fig.5: Group 4 Laser Activated SDF with Potassium iodide, Polarized light microscopy photographs at baseline (A), after demineralization (B) and after remineralization (C) at the middle third of the crown

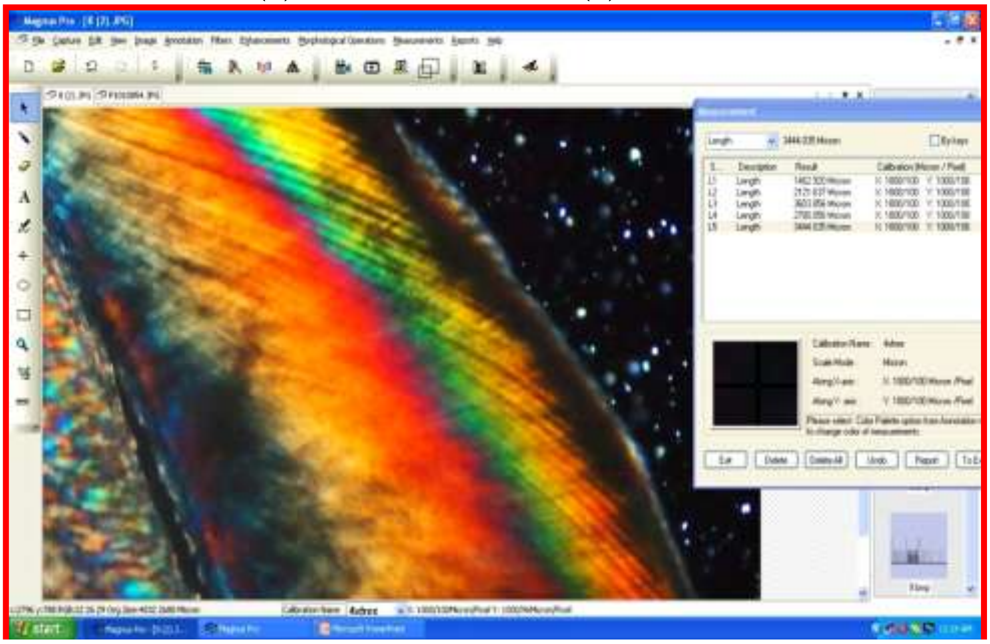

Fig.6: Linear depth measurement of demineralization zone in enamel under polarizing microscopy using morphometric analysis (Magnus-Pro image analysis software)

RESULTS AND OBSERVATIONS:

Remineralizing Potential of Group 1–4

All samples were evaluated with DIAGNOdent at baseline, following demineralization, and after the remineralization phase. Following the application of remineralizing agent, the surface of each sample was assessed using DIAGNOdent. Mean DIAGNOdent value after application of remineralizing agent in descending order was as follows: Group 2 (5.7±2.311)> Group 4 (4.3±0.948)> Group 1(3±1.154)> Group 3 (1.8±0.788). A statistically highly significant difference was observed in all the groups ($p<0.001$) (Table 1).To assess the remineralising potential of each remineralising agent, the difference between the DIAGNOdent values after demineralisation and subsequently after remineralisation were analysed for Group 1—4. The mean remineralising potential of the remineralising agents was in the following descending order and showed statistically highly significant difference in remineralising potential ($p < 0.001$): Group 3 (14.8 ±1.059)> Group 1(11.6 ±1.032)> Group 4 (6.7±1.197) > Group 2 (6.6±0.699). (Table 2).On intergroup comparison of the remineralising potential of the groups using post hoc Tukey's test, a statistically highly significant difference was observed between the entire Group 1-4 ($p <0.05$).(Table 3).

Morphometric Evaluation of the Remineralisation Potential of Group 1–4

The Mean Morphometric Comparative Evaluation of the Remineralization Potential using polarised Light microscopy in descending order was as follows: Group 2 (1315.9±536.87)> Group 1(1280.03±281.45) > Group 4 (1220.8±215.80)> Group 3 (984.7±530.90).A statistically significant difference was observed in all the groups (p = 0.011) (Table 4).The mean remineralising potential of the remineralising agents was in the following descending order and showed statistically significant difference in remineralising potential (p = 0.011): Group 3 (3049.7±530.9)>Group 4 (2936.8±215.8)> Group 1 (2087.9±281.45) > Group 2 (1789.03±536.8) (Table 5). Intergroup comparison of the remineralizing potential among the groups using the post hoc Tukey's test revealed a highly significant statistical difference between Group 1 and Group 2, as well as between Group 1 andGroup 4, Group 2 and Group 3, Group 3 and Group 4 (p < 0.05). Also, no statistically significant difference was observed in the colour changes of Group 1 and Group 3, Group 2 and Group 4, (p = 0.011) (Tables 6).

Table 1: Mean comparison of baseline DIAGNOdent values, after demineralization and after remineralization using One Way ANOVA tes

Group	N	Baseline Mean ± SD	p-value	After Demineralization Mean ± SD	p-value	After Remineralization Mean ± SD	p-value
SDF without Laser	10	4.9±1.814	0.987, NS	14.6±1.349	<0.001, HS	3±1.154	<0.001, HS
SDF + KI without Laser	10	4.9±1.912		12.3±0.823		5.7±2.311	
SDF with Laser	10	5.1±1.792		16.6±1.776		1.8±0.788	
SDF + KI with Laser	10	4.8±1.813		11±1.054		4.3±0.948	
p ≤ 0.05 – Significant, CI = 95 %							

Table 2: Mean comparison of remineralisation Potential of all groups using One Way ANOVA test

Group	N	Remineralization Mean ± SD	Minimum	Maximum	p-value
SDF without Laser	10	11.6 ±1.032	7.00	13.00	<0.001,HS
SDF + KI without Laser	10	6.6±0.699	5.00	7.00	
SDF with Laser	10	14.8 ±1.059	8.00	16.00	
SDF + KI with Laser	10	6.7±1.197	4.00	8.00	
p ≤ 0.05 – Significant, CI = 95 %					

Table 3: Inter comparison of remineralising potential of all the groups using post hoc Tukey's Test

(I)	(J)	Mean Difference (I-J)	p-value	95% Confidence Interval	
				Lower Bound	Upper Bound
SDF without Laser	SDF + KI without Laser	-3.2	< 0.000	-4.421	-1.979
	SDF with Laser	0.9	0.212NS	-0.321	2.121
	SDF + KI with Laser	-2.9	< 0.000	-4.121	-1.679
SDF + KI without Laser	SDF without Laser	3.2	< 0.000	1.979	4.421
	SDF with Laser	4.1	< 0.000	2.879	5.321
	SDF + KI with Laser	0.30	0.911,NS	-0.921	1.521
SDF with Laser	SDF without Laser	-0.90	0.212,NS	-2.121	0.321
	SDF + KI without Laser	-4.1	< 0.000	-5.321	-2.879
	SDF + KI with Laser	-3.8	< 0.000	-5.021	-2.579
SDF + KI with Laser	SDF without Laser	2.9	< 0.000	1.679	4.121
	SDF + KI without Laser	-0.30	0.911,NS	-1.521	0.921
	SDF with Laser	3.8	< 0.000	2.579	5.021
p ≤ 0.05 – Significant, CI = 95 %					

Table 4: Mean comparison of Morphometric analysis using one way ANOVA test

Group	N	Baseline Mean + SD	p-value	After Demineralization Mean + SD	p-value	After Remineralization Mean + SD	p-value
SDF without Laser	5	2321.9+690.30	0.948, NS	3367.6+539.05	0.011, S	1280.03+281.45	0.011, S
SDF + KI without Laser	5	2211.9+702.87		3104.8+629.42		1315.9+536.87	
SDF with Laser	5	2492.1+915.67		4033.6+84.608		984.7+530.90	
SDF + KI with Laser	5	2468.2+651.09		4156.8+537.48		1220.8+215.80	
p ≤ 0.05 – Significant, CI = 95 %							

Table 5: Mean comparison of remineralization Potential of all groups using One Way ANOVA test

Groups	N	Mean	Minimum	Maximum	p-value
SDF without Laser	4	2087.9+281.45	1023.5	1616.2	0.011 , S
SDF + KI without Laser	4	1789.03+536.8	802.1	2026.5	
SDF with Laser	4	3049.7+530.9	506.3	1720	
SDF + KI with Laser	4	2936.8+215.8	926.2	1423.2	
p ≤ 0.05 – Significant, CI = 95 %					

Table 6: Inter group comparison of Morphometric analysis using post hoc Tuckey's test

(I)	(J)	Mean Difference (I-J)	p-value	95% Confidence Interval	
				Lower Bound	Upper Bound
SDF without Laser	SDF + KI without Laser	66.9	0.001	808.6	942.5
	SDF with Laser	332.2	0.681,NS	-543.3	1207.8
	SDF + KI with Laser	96.1	0.001	779.4	971.7
SDF + KI without Laser	SDF without Laser	-66.9	0.001	-942.5	-808.6
	SDF with Laser	-265.2	0.001	-1140.9	-610.3
	SDF + KI with Laser	29.1	1.000,NS	-846.4	904.8
SDF with Laser	SDF without Laser	-332.2	0.681,NS	-1207.8	543.3
	SDF + KI without Laser	265.2	0.001	610.3	1140.9
	SDF + KI with Laser	236.09	0.001	639.5	1111.7
SDF + KI with Laser	SDF without Laser	-96.1	0.001	-971.7	-779.4
	SDF + KI without Laser	-29.1	1.000,NS	-904.8	846.4
	SDF with Laser	-236.09	0.001	-1111.7	-639.5
p ≤ 0.05 – Significant, CI = 95 %					

Discussion

The basic mechanism of remineralization involves the diffusion of calcium and phosphate ions from saliva and other topical sources to build a hypermineralized, acid-resistant, fluorapatite like layer on the existing crystal remnants, which act as remineralization nuclei. In this study, the Remineralization of white-spot lesions is done using SDF, SDF with Potassium Iodide, Laser activated SDF and Laser activated SDF with Potassium

Iodide. Topically, fluoride inhibits demineralization of sound enamel and enhances remineralization of demineralized enamel as well as inhibits dental caries by affecting the metabolic activity of cariogenic bacteria (AAPD 2021). Silver diamine fluoride (SDF) was first introduced 80 years ago as a formulation containing ionic silver, ammonia, water, and fluoride; its use was approved by the US FDA in 2014[19]. It is effective in slowing the progression of dental caries in both primary and permanent teeth, managing deep coronal lesions, and reducing root caries in older adults.[20,21] and alleviating the symptoms of sensitivity and sore teeth through the obturation of dentinal tubules [22]. Various studies are underway to investigate the role of SDF in inhibiting the spread of oral bacteria in dentinal tubules. A study found that SDF decreases the levels of *S. mutans*[23]. However, a critical disadvantage of a single application of SDF is the discoloration of teeth. Yee et. al. reported that enamel and dentin, including carious tissues, are decolorized into dark brown or black after SDF application[24]. Recently, the supplementation of SDF with potassium iodide (KI) was proposed to address the issue of discoloration [25]. It is hypothesized that KI will be extremely beneficial if it can prevent the staining associated with SDF without reducing its effectiveness in preventing caries [26]. Riva Star (SDI Limited, Bayswater, Australia) is one such product in which the excess silver ions from the SDF solution react with KI ions to form silver iodide, helping to minimize discoloration caused by SDF[27]. Furthermore, the present study showed that regardless of the application of Diode LASER, SDF topical fluoride application showed higher remineralizing potential than that of SDF with Potassium iodide. These results are in accordance with a study conducted by Sorkhdini et al[28] in which they concluded that, SDF appears to be an effective agent for preventing and treating dental caries through its ability to inhibit demineralization and promote remineralization. Additionally, treating the enamel surface with a LASER either before or after applying topical fluoride has been shown to further improve the enamel's surface properties. In the present study, the mean remineralization value of Laser activated SDF is found to be the highest and SDF with Potassium iodide to be the least (i.e. Laser Activated SDF> Laser activated SDF with Potassium Iodide>SDF>SDF with Potassium Iodide).These findings can be explained by the heat produced by the LASER, which enhances the enamel's fluoride uptake. Thermal effect of LASER has been found to enhance the uptake of fluoride to form fluor-apatite. As with all chemical reactions, temperature increases the kinetic energy, speed and collision rate of the molecules; thus, it increases the rate of reaction end product in the given amount of time. Al-Maliky MA et al[29] recorded surface temperature change of 6.26 °C with irradiation time of 1 min which leads to increase in the reaction rate between the topical fluoride and enamel. They also proposed the potential involvement of photochemical interactions, in addition to photothermal effects, which promote the formation of fluorohydroxyapatite by substituting carbonate or hydroxyl groups with fluoride, resulting in a more stable molecular structure. Thus, in the present study the highest remineralization potential is found in Laser activated SDF which is in accordance with the study done by K.Singh et al[30] in which their result shows highest remineralizing potential and maximum color changes of surface enamel was exhibited by Laser activated SDF. The results of the present study were also in accordance with a study done by Mei et al.[31], who demonstrated a significant increase in fluoride uptake after application of LASER activated SDF as compared to SDF alone. Similarly, Vitale et al.[32] concluded that there is an enhanced fluoride uptake of enamel and protection of enamel surface from acid attack when enamel surface was irradiated with Diode LASER after application of Fluoride gel. Villalba-Moreno et al.[33] through the results of their study concluded that the LASER treatment significantly increased the binding of fluoride to the enamel surface without damaging it. Polarized light microscope was used for qualitative assessment of lesion and microradiography as a quantitative measure of lesion mineralization as it shows more highly demineralized areas of the lesions as radiolucent areas and mineralized area as a radiopaque surface layer.However,polarized light microscope can give high degree of differentiation between demineralized area and normal area of tooth sample. This differentiation is better visualized in polarized light microscope than microradiography as described byWefel JS et al.[34]. In this study, as we were concerned with surface alterations, the polarized light microscope was supposed to be best method of choice. In this study, Polarized light microscope shows Laser activated SDF is best in reduction of surface alterations which correlates with the findings from previous studies on Effect of Silver Diamine Fluoride and Potassium Iodide Solution on Enamel Remineralization and Discoloration in Artificial Cariesdone by Lee KE et al[35], in which they concluded that, the surface alterations ions, the higher mineral density and remineralization rate in the Laser activated SDF is attributed to the existence of calcium fluoride and silver phosphate. The present study also shows that, the remineralization commences from the outside and proceeds toward the inside. Similarly, Romão DA et al[36] tested SDF using polarized light microscopy and they concluded that, SDF reduced the depth of non-cavitated carious lesions in primary enamel.

Clinical Significance

Out of the four modalities used for topical fluoride application, LASER activated topical fluorides (i.e. LASER activated SDF and LASER activated SDF with Potassium iodide) showed higher fluoride uptake as well as enhanced remineralising potential when compared to application of topical fluorides alone (i.e. SDF and SDF with Potassium iodide), hence enhancing the caries preventive effect against dental caries. Thus, the authors of the current study recommend the use of LASER activated topical fluorides in clinical pediatric practice to achieve superior caries prevention. Furthermore, LASER activated SDF with Potassium iodide can be used as an aesthetic alternative to SDF as LASER activated SDF with Potassium iodide showed higher uptake of fluoride on the enamel surface without causing discoloration of tooth surface.

Limitations

Only two most commonly used topical fluoride agents were used in the present study, Also, other topical fluoride agents, with different concentrations of fluoride can be included to expand the scope of research. Single type of LASER (Diode LASER) with standard settings was used; a study with different types of LASERs with different power settings can be designed to evaluate the effect of different LASERs on enamel surface after application of topical fluoride to assess their effect on the surface enamel.

CONCLUSION

Laser activated SDF has shown to have better outcome in comparison with other groups followed by Laser Activated SDF with Potassium Iodide then SDF group and SDF with Potassium Iodide as the least. Remineralizing Agents Aided with Laser has shown and proven to be more superior and yield better results than conventional materials and has demonstrated the efficacy and ease of polarized light microscope in caries research studies.

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