International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Evaluation of the Effect of Diode Laser and Desensitising Agent on Dentinal Tubules in Fluorosed and Non-Fluorosed Teeth using SEM Analysis- An in Vitro Study

Roopalakshmi N.[1], Prabhuji MLV[2], Karthikeyan BV[3]

Abstract: ***Aim**: The aim of the present study was to evaluate the effect of a combination of diode lasers and desensitising agent on the dentinal tubules and to compare these effects in fluorosed and non fluorosed teeth. **Materials And Methods**: 72 fully erupted teeth were extracted and scaling and root planing were done. The sample was divided into two groups namely fluorosed (F) group, n= 36 and non fluorosed (NF) group, n= 36. The proximal region of each sample was taken and sectioned into 3 parts using diamond discs. The samples were treated according to the treatment protocol of the subgroups they were allotted to: Subgroup a, 5% Potassium Nitrate desensitising agent; Subgroup b, 810 nm Diode laser; Subgroup c, Combination of desensitising agent and diode laser. The samples were analysed using scanning electron microscopy. **Results**: The subgroup treated with a combination of diode laser and desensitising tooth paste showed maximum number of closed dentinal tubules and also the maximum amount of closure, followed by laser subgroup and least closure seen with desensitising agent subgroup. **Conclusion**: Thus the results of the present study suggest that, for both fluorosed and non fluorosed teeth, combination therapy with initial application of desensitising agent followed by irradiation with diode laser have a therapeutic benefit over the control groups, and this is a useful treatment for dentinal hypersensitivity.*

1. Introduction

Dentinal hypersensitivity is one of the most common and most troublesome complaints presented by dental patients. It can be described clinically as an exaggerated response on application of a stimulus onto a tooth with exposed dentine, regardless of its location.[1,2] Dentists are onto the mission of devising a permanent solution to this problem and the search is on for decades now.

Dental fluorosis leads to gradual increase in hypo-mineralisation of the teeth involved. The pits and discolouration presented on the surface of a tooth are not due to the fluoride directly but are actually a result of the hypo-mineralised and porous enamel, which eventually wear away and get damaged.[3] This finally leads to the exposure of the underlying dentin and in turn causes increased hypersensitivity to the patients.

In a study to evaluate the relationship between the dentin mechanical and structural properties, it is shown that, fluorosed dentin correlates positively with dentin tubule size and negatively with ultrasound velocity.[4] Thus, it can be affirmed that patients with increased fluorosis are at increased risk of dentinal hypersensitivity (DH).

Due to the reduced thickness of enamel on the cervical regions of the teeth,[5] the cervical areas are the most affected areas, which are seen in the form of cervical abrasions.

Various treatment modalities used for the treatment of hypersensitivity most commonly include, the occlusion of the exposed tubules by the use of topical agents such as fluorides, strontium acetate, calcium sodium phosphosilicate paste, calcium hydroxide paste, potassium oxalate, silver nitrate, fluoride iontophoresis, varnishes, dentin adhesives, etc. Though these can be used by the patient upon being instructed, the efficacy has been shown to be short lived. Other treatment modalities that have been tried include, placement of restorations, desensitisation of the pulpal sensory nerves using potassium nitrate toothpastes. But again, restorations if not maintained could lead to demineralisation under the restorations at the borders, leading to return of the symptoms. More recently, for the past 2 decades, lasers are coming into the fore front in various realms of dental and periodontal treatment, due to its decided advantages. It has been shown in various studies that lasers can be used in the effective management of DH. The proposed mechanism by which they alleviate the symptoms are by the occlusion of the dentinal tubules,[6] or by affecting the neural transmission in the dentinal tubules.[6,7] It has also been proposed that lasers coagulate the proteins inside the dentinal tubules and block the movement of fluid.[8]Compared to all the lasers used for the purpose, diode lasers are shown to have least effect on the pulp in terms of temperature rise, and also on the dentin surface in terms of damage to the dentin surface.[9,10]

Among the desensitising agents available for the treatment of DH, the oldest, proven and the most commonly used one, is potassium nitrate.[11] Therefore based on the evidence from previous literature, it can be hypothesised that, a combination of diode laser and potassium nitrate desensitising agent will have a better therapeutic benefit for the treatment of dentinal hypersensitivity.

Keeping this in mind, for the first time, an initial attempt has been made in this in vitro study, to evaluate and compare the root surface changes on application of a combination therapy of diode lasers and potassium nitrate desensitising agent. The aim of the present study was firstly to evaluate and compare the effect of a combination therapy of diode lasers and potassium nitrate desensitising agent on the dentinal tubules; secondly, to compare the effect of treatment with lasers and desensitising agent when used alone, and thirdly to compare these effects in fluorosed and non fluorosed teeth.

2. Materials and Methods

Specimens

A total of 72 extracted teeth (36 fluorosed and 36 non fluorosed), with cervical non-carious lesions were included in this study. Freshly extracted teeth were obtained from the Department of Oral and Maxillofacial Surgery, Krishnadevaraya College of Dental Sciences, Bangalore, Karnataka, India. The teeth were used according to the protocol set forth by the Research Ethics Committee of Rajiv Gandhi University of Health Sciences, Karnataka, India.

The teeth specimens were selected according to the inclusion and exclusion criteria. Fully erupted teeth, extracted from systemically healthy patients, atraumatically extracted, preferably with non-carious cervical lesions were included in the study. For fluorosed teeth, the teeth were included based on the clinical examination and history of the patients from fluoride beds in and around the area. Any teeth with caries or restorations, or those that have been previously treated for periodontal reasons, impacted teeth or teeth with intrinsic stains due to other developmental anomalies were excluded.

After extraction, the teeth were stored in phosphate buffer saline (PBS) (pH= 7.4) at 37^{o}C until the preparation phase was begun.

Thorough scaling and root planing (SRP) was carried out using hand scalers and curettes in order to remove the over lying calculus and stains. The overlying layer of cementum was also removed using curettes. The specimens were stored in PBS (pH= 7.4) until the treatment phase was begun to prevent dehydration.

After the removal of calculus and the overlying cementum, the teeth were sectioned on the proximal surface using sterile diamond discs, under constant irrigation with saline.

For sectioning, first, a cut was made at the level of the cement enamel junction (CEJ), to separate out the crown and the roots. Following this, vertical cuts were made on the proximal surface of the roots. These vertical cuts were joined by a horizontal cut apically. Care was taken not to extend the sections into the apical third of the roots, as the apical third tends to have high frequency of anatomical and morphological variations.

Each specimen obtained was further sectioned into three parts and allotted to the three subgroups. The sections measured5 mm x 5 mm. Following this, 18% EDTA was used over the sections for 1min, to remove off the smear layer.

- Subgroup a- 1st part was treated with a combination of both diode laser and Potassium Nitrate paste
- Subgroup b- 2nd part was treated with 810nm diode laser
- Subgroup c – 3rd part was treated with 5% Potassium Nitrate desensitising paste

For the treatment with desensitising agent, a constant thickness of desensitising agent was applied over the teeth sections and allowed to stay for 1 minute.

Laser specifications

Diode laser irradiation was done using a laser system (Ga:Al:As) with a flash lamp-pumped laser operating at the wavelength of 810nm set at 1 Watt power and energy of 1mJ per second, for a time duration of 10 seconds per millimetre in a non-contact mode delivered by a hollow wave guide, with the tip placed perpendicular to the tooth surface as close to the tooth as possible.

Combination treatment was done by leaving the desensitising agent over the teeth sections for 1 minute, followed by laser irradiation for 10 seconds and then all the specimens were commonly washed off using isotonic saline solution.

SEM Analysis

For SEM analysis, the specimens were fixed with 2.5% gluteraldehyde in phosphate buffer saline (pH= 7.4) for 24 hours at 4^{o}C, and washed with a phosphate buffer (pH= 7.4) solution for 10 minutes each. The specimens were dried and desiccated in a desiccation jar and mounted with silver paint on SEM stubs. Gold and palladium sputter coating was done, and specimens were viewed under the scanning electron microscope. Photomicrographs of the samples under 2000x magnification were taken. Images of the root surfaces representing each group with its subgroups were obtained.

Tabulation

SAMPLE (Fluorosed)	Num of tubules seen on SEM	Num of tubules closed	%age of tubules closed	SAMPLE (Non Fluorosed)	Num of tubules seen on SEM	Num of tubules closed	%age of tubules closed
Sp a	37±	25±	70±	Sp a	56±	42±	75±
Sp b	60±	33±	55±	Sp b	65±	35±	52±
Sp c	25±	8±	30±	Sp c	7±	4±	50±

Quantitative Analysis

	Fluorosed Group					Non Fluorosed Group					
SAMPLE	100%	75%	50%	25%	<25%	SAMPLE	100%	75%	50%	25%	<25%
Sp 1a		✓				Sp 1a		✓			
Sp 1b			✓			Sp 1b			✓		
Sp 1c				✓		Sp 1c	-	-	-	-	-
Sp 2a	✓					Sp 2a	✓				
Sp 2b			✓			Sp 2b			✓		

Sp 2c		✓				Sp 2c	-	-	-	-	-
Sp 3a	✓					Sp 3a	✓				
Sp 3b		✓				Sp 3b			✓		
Sp 3c	-	-	-	-	-	Sp 3c	-	-	-	-	-

Qualitative Analysis

	Completely Blocked	Incompletely Blocked
Sp 1a	✓	
Sp 1b		✓
Sp 1c		✓
Sp 2a	✓	
Sp 2b	✓	
Sp 2c	✓	
Sp 3a	✓	
Sp 3b		✓
Sp 3c		✓

Fluorosed Group

	Completely Blocked	Incompletely Blocked
Sp 1a	✓	
Sp 1b		✓
Sp 1c		✓
Sp 2a	✓	
Sp 2b		✓
Sp 2c		✓
Sp 3a	✓	
Sp 3b		✓
Sp 3c		✓

Non- Fluorosed Group

Statistical Analysis

The inter group comparison with respect to the percentage of tubule closer was done using Mann- Whitney U Test. (Table 1,2) Intra group comparison in fluorosed group and non fluorosed group separately was done using Kruskal Wallis ANOVA. (Table 3) Comparison of the samples with the status of tubule blockage qualitatively in both the groups taken together were done using Chi square test, with $p < 0.05$. (Figure 3)

3. Results

The inter group comparison with respect to the percentage of tubule closer was done using Mann- Whitney U Test. The results showed that the difference between the two groups is not clinically significant. (Table 1)

Table 1: Comparison of Fluorosed and non- Fluorosed groups with respect to percentage of tubule closure by Mann-Whitney U test

Group	Mean	Sum of ranks	U-value	Z-value	p-value
Fluorosed group	58.67±25.30	85.00	40.00	-0.0442	0.9648
Non- Fluorosed group	59.56±23.77	86.00			

On comparing the percentage of tubule closure in fluorosed and non fluorosed teeth taking into consideration each subgroup separately (inter group comparison), also did not show any significant difference, suggesting that the treatment was equally effective in either of the groups. (Table 2)

Table 2: Intergroup comparison of degree of tubule closure in each subgroup using Mann-Whitney U Test

Subgroup	Group	Mean	SD	Sum of ranks	U-value	Z-value	p-value
Subgroup a	Fluorosed group	82.67	11.02	10.00	4.00	-0.2182	0.8273
	Non- Fluorosed group	84.33	13.65	11.00			
Subgroup b	Fluorosed group	54.33	6.03	9.00	3.00	-0.6547	0.5127
	Non- Fluorosed group	58.67	8.33	12.00			
Subgroup c	Fluorosed group	39.00	30.51	10.00	4.00	-0.2182	0.8273
	Non- Fluorosed group	35.67	15.04	11.00			

Intra group comparison in fluorosed group and non fluorosed group separately was done using Kruskal Wallis ANOVA. The analysis showed statistically significant results in the non fluorosed group, and the results were not statistically significant in the fluorosed group. (Table 3)

The graphical representation of the statistical analysis in the fluorosed teeth (Figure 1) and non fluorosed teeth (Figure 2) show clearly the better tubule closure with the subgroup a, though not statistically significant in fluorosed teeth.

Table 3: Intra Group comparison of tubule closure using Kruskal Wallis ANOVA and Post Hoc using Mann- Whitney U Test

	Non Fluorosed Teeth			Fluorosed Teeth		
Sub groups	Means	Std.Dev.	Sum of ranks	Means	Std.Dev.	Sum of ranks
Sub group a	84.33	13.65	24.00	82.67	11.02	23.00
Sub group b	58.67	8.33	15.00	54.33	6.03	12.00
Sub group c	35.67	15.04	6.00	39.00	30.51	10.00
H-value	7.2000			4.35550		
P-value	0.0273*			0.11330		
Pair wise comparison by Mann-Whitney U test						
SG a vs SG b	P=0.0495*					
SG a vs SG c	P=0.0495*					
SG b vs SG c	P=0.0495*					

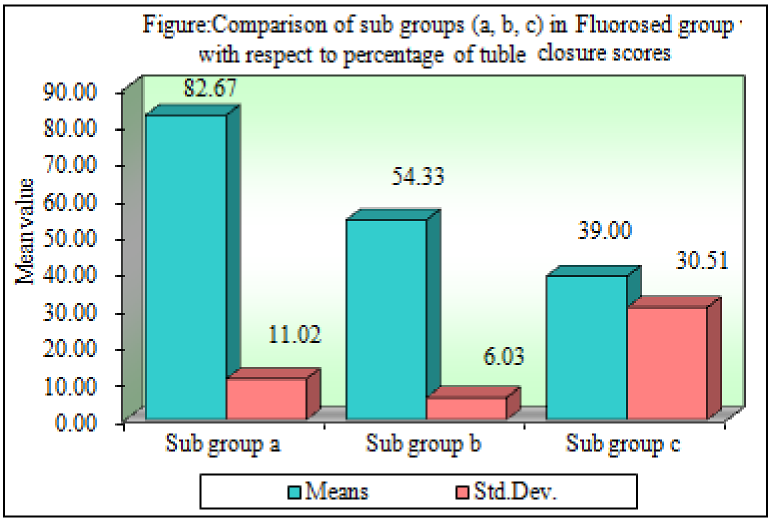

Figure 1: Comparison of sub groups (a, b, c) in Fluorosed group with respect to percentage of tubule closure scores using Kruskal Wallis ANOVA

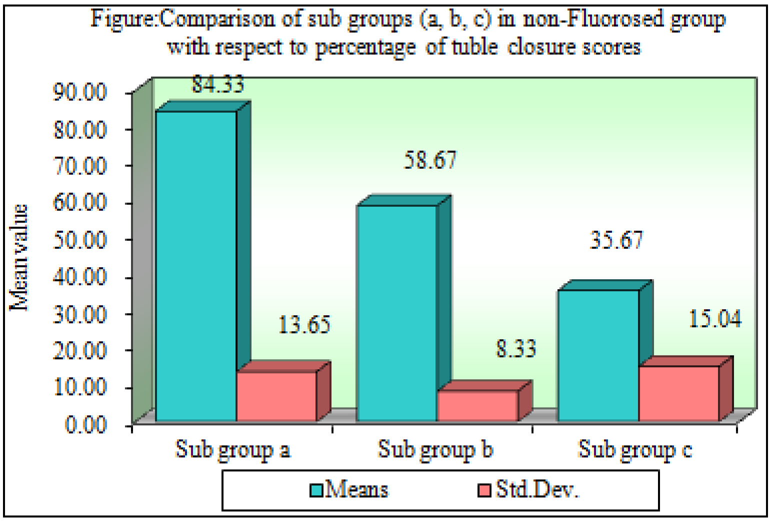

Figure 2: Comparison of sub groups (a, b, c) in non-Fluorosed group with respect to percentage of tubule closure scores using Kruskal Wallis ANOVA and Mann-Whitney U Test

Comparison of the samples with the status of tubule blockage qualitatively in both the groups taken together were done using Chi square test, with $p < 0.05$. The results showed statistical significance, favouring subgroup a suggesting that the treatment of the teeth samples with the combination of desensitising agent followed by diode laser blocked the tubules better than the other two control groups. (Figure 3)

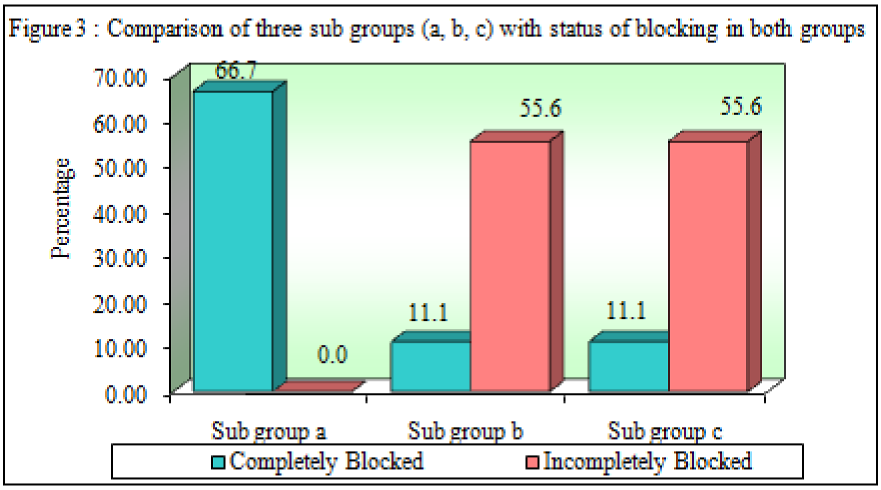

Figure 3: Comparison of the status of tubule blockage qualitatively in both the groups taken together using Chi square test

SEM analysis of the teeth specimens demonstrated various degrees of tubule seal when treated with different modalities.

When lasers are used alone or in a combination with desensitising paste, the removal of organic debris is seen, enabling better visualisation of the tubules. Subgroup (c) showed a lot of organic debris left behind and was a hindrance for the assessment of the dentinal tubules.

Comparing qualitatively, the 3 subgroups w.r.t to the status of blocking of tubules in fluorosed and non fluorosed teeth, significant result was seen with combination therapy (Subgroup a), showing 75-100% closure of dentinal tubules, while the subgroups (b) and (c) showed comparatively lesser tubule closure.

Both fluorosed and non fluorosed teeth respond to laser therapy with and without desensitising agent, equally well. Fluorosed teeth showed good closure in all the specimens, showing increased sensitivity to lasers and toothpaste. Non fluorosed specimens showed significantly high closure of dentinal tubules in subgroup (c).

4. Discussion

Dentinal hypersensitivity is said to be one of the most troublesome problems faced by patients.

Conventional therapies for the treatment of DH like the topical use of desensitizing agents such as protein precipitants,[12,13] tubule-occluding agents,[14, 15] tubule sealants,[16] and, recently lasers,[17-23] have been tried to give a long term solution to the patient. But the longevity of these treatment methods have been questioned.

Several studies describe a synergistic action of lasers in association with desensitizing agents.[24,25] The authors have hypothesised that the laser system causes the permanence of the desensitising agent for longer time than when they are used alone. **If laser device is used in addition to a conventional desensitizing agent, the latter remains above the tooth surface for 60 seconds before the irradiation.**

Due to the increased diameter of dentinal tubules in fluorosed teeth, it was hypothesised that, the teeth would show higher frequency of hypersensitivity too. In agreement to this, studies comparing the frequency of hypersensitivity in non fluorosed teeth and fluorosed teeth, showed that fluorosed teeth may have higher incidence of hypersensitivity.[26,27]

The reduced thickness of enamel on the cervical regions of the teeth,[5] makes these the most affected areas, seen in the form of cervical abrasions. Therefore in this present study, we have used the cervical and proximal regions of extracted teeth, removing off the overlying cementum and exposing the underlying dentin, simulating the abraded tooth surface.

Many treatments have been tried in the treatment of the same, but none have compared the treatment methods under a scanning electron microscope and also none have compared the results in fluorosed and non fluorosed teeth. Thus, in the present study, an in vitro SEM analysis of teeth sections were carried out after using three different treatment options. By comparing the degree of dentinal tubule closure and also the number of tubules closed in the particular visualised field, it was seen that the subgroup (a), using a combination therapy showed best results compared to the other two subgroups.

Clinical studies comparing the combination therapy with laser and desensitising agent used alone, on a group of 10 patients have shown best results with the combination therapy. It is suggested that, the better tubule sealing with the combination treatment was due to the higher desensitising agent adhesion to the dentinal tubules when combined with laser energy.[28]

Studies comparing the effect of different lasers on the tooth surface have shown that, diode lasers are less harmful than Er:YAG lasers in causing cracks and tooth surface melts. Both the lasers didn’t show harmful rise in intrapulpal temperature and hence is shown to be safe for use in the treatment of hypersensitivity.[25]

The expected root surface changes on laser irradiation are, mild thermally induced change, primarily surface melting. Other surface alterations include surface etching, intermittent smear layer, exposure of collagen tufts. Except for melting of root surface, other undesirable morphological changes were found to be more common in non fluorosed than fluorosed root specimens.[29]

5. Conclusion

Thus, fluorosed and non fluorosed teeth, both show equally good dentinal tubule closure, and de-sensiting agent application prior to the irradiation with diode lasers, can be used to occlude the dentinal tubules. Diode lasers along with Pot. Nitrate paste provides better occlusion of dentinal tubules, than when the agents are used individually. It can be safely concluded that, for both fluorosed and non fluorosed teeth the treatment of dentinal hypersensitivity with a combination of diode lasers and desensing agent has an added benefit compared to them being used separately. Further studies need to be done to check the validity of the same in vivo and also to check the permanence of the treatment method.

References

[1] Addy M. Tooth wear and sensitivity: Clinical advances in restorative dentistry. In: Addy M, Embery G, Edgar WM, Orchardson R, editors. Dentine hypersensitivity: Definition, prevalence distribution and aetiology. London: Martin Dunitz; 2000;239–248.
[2] Addy M. Etiology and clinical implications of dentine hypersensitivity. Dent Clin North Am. 1990 Jul; 34(3):503-14.
[3] Aoba T, Fejerskov O. Dental Fluorosis: Chemistry and Biology. CROBM 2002;13:155-170.
[4] Vieira APGF, Hancock R, Dumitriu M, Limeback H, Grynpas MD. Fluoride’s effect on human dentin ultrasound velocity (elastic modulus) and tubule size. Eur J Oral Sci 2006; 114: 83–88.
[5] Histology text book.

[6] Aoba T, Fejerskov O. Dental Fluorosis: Chemistry and Biology. Crit Rev Oral Biol Med 2002; 13: 155.
[7] Tonguca M. O, Ozata Y, Sertb T, Sonmezc Y, Kirziogluat F.Y. Tooth sensitivity in fluorotic teeth. Eur J Dent 2011;5:273-280.
[8] Hawker GA, Mian S, Kendzerska T, French M. Measures of Adult Pain. Arthritis Care & Research. 2011;63(S11): S240–S252.
[9] Theodoro LH, Haypek P, Bachmann L, Garcia VG, Sampaino JEC, Zezell DM et al. Effect of Er:YAG and Diode Laser irradiation on the root surface: Morphological and thermal analysis. J Periodontol 2003;74:838-843.
[10] Gojkov-Vukelic M, Hadzic S, Zukanovic A, Pasic E, Pavlic V. Application of Diode Laser in the Treatment of Dentine Hypersensitivity. Med Arch. 2016; 70(6): 466–469.
[11] Wang Y, Gao J, Jiang T, Liang S, Zhoua Y, Matis BA. Evaluation of the efficacy of potassium nitrate and sodium fluoride as desensitizing agents during tooth bleaching treatment—A systematic review and meta-analysis. J Dent. 2015; 43(8): 913-923.
[12] McCarthy D, Gillam DG, Parson DJ. *In vitro* effects of laser radiation on dentine surfaces. J Dent Res.1997;76:233.
[13] L. P. Gangarosa, "Current strategies for dentist-applied treatment in the management of hypersensitive dentine," *Archives of Oral Biology*, vol. 39, no. 1, pp. S101–S106, 1994.
[14] D. G. Kerns, M. J. Scheidt, D. H. Pashley, J. A. Horner, S. L. Strong, and T. E. Van Dyke, "Dentinal tubule occlusion and root hypersensitivity," *Journal of Periodontology*, vol. 62, no. 7, pp. 421–428, 1991.
[15] R. Ikemura, "Studies on new treatment agents for dentin hypersensitivity," *Japanese Journal of Conservative Dentistry*, vol. 36, pp. 1686–1698, 1993.
[16] T. G. Wichgers and R. L. Emert, "Dentin hypersensitivity," *General Dentistry*, vol. 44, no. 3, pp. 225–232, 1996.
[17] A. Moritz, U. Schoop, K. Goharkhay et al., "Long-term effects of CO2 laser irradiation on treatment of hypersensitive dental International Journal of Dentistry necks: results of an *in vivo* study," *J Clin Laser Med and Surg* 1998; 16: 211–215.
[18] Asnaashari M, Moeini M. Effectiveness of Lasers in the Treatment of Dentin Hypersensitivity. J Lasers Med Sci 2013; 4(1):1-7
[19] Hashim NT, Gasmalla BG, Sabahelkheir AH, Awooda AM. Effect of the clinical application of the diode laser (810 nm) in the treatment of dentine hypersensitivity. BMC Research Notes 2014 7:31.
[20] A. Moritz, N. Gutknecht, U. Schoop et al., "The advantage of CO2-treated dental necks, in comparison with a standard method: results of an *in vivo* study," *Journal of Clinical Laser Medicine and Surgery*, vol. 14, no. 1, pp. 27–32, 1996.
[21] C. Zhang, K. Matsumoto, Y. Kimura, T. Harashima, F. H. Takeda, and H. Zhou, "Effects of CO2 laser in treatment of cervical dentinal hypersensitivity," *Journal of Endodontics*, vol. 24, no. 9, pp. 595–597, 1998.
[22] F. Schwarz, N. Arweiler, T. Georg, and E. Reich, "Desensitizing effects of an Er:YAG laser on hypersensitive dentine: a controlled, prospective clinical study," *Journal of Clinical Periodontology*, vol. 29, no. 3, pp. 211–215, 2002.
[23] H. Watanabe, K. Kataoka, H. Iwami, T. Shinoki, Y. Okagami, and I. Ishikawa, "*In vitro* and *in vivo* studies on application of erbium:YAG laser for dentine hypersensitivity," *International Congress Series*, vol. 1248, pp. 455–457, 2003.
[24] S. Pesevska, M. Nakova, K. Ivanovski et al., "Dentinal hypersensitivity following scaling and root planing: comparison of low-level laser and topical fluoride treatment," *Lasers in Medical Science*, vol. 25, no. 5, pp. 647–650, 2010.
[25] U Romeo, Claudia R, Gaspare P, Gianluca T, Alessandro DV. Treatment of Dentine Hypersensitivity by Diode Laser: A Clinical Study. Int J Dent. 2012; Article ID 858950.
[26] 63. Corona SA, Nascimento TN, Catirse AB, Lizarelli RF, Dinelli W, Palma-Dibb RG. Clinical evaluation of low-level laser therapy and fluoride varnish for treating cervical dentinal hypersensitivity. J Oral Rehabil.2003;30:1183–9.
[27] Tonguca MO, Ozat Y, Sert T, Sonmez Y, Kirzioglu FY. Tooth Sensitivity in Fluorotic Teeth. Eur J Dent 2011;5:273-280.
[28] Zhang Y, Cheng R, Cheng G, Zhang X. Prevalence of dentine hypersensitivity in Chinese rural adults with dental fluorosis. Journal of Oral Rehabilitation 2014 41; 289—295.
[29] Dhingraa K, Vandana KL, Shah A, Cobb CM. Effect of Er:YAG Laser Irradiation on Fluorosed and Non fluorosed Root Surfaces: An In Vitro Study. J Oral Laser Applications 2010; 10: 87-97.

Tables and Figures

Table 1: Comparison of Fluorosed and non- Fluorosed groups with respect to percentage of tubule closure by Mann-Whitney U test

Group	Mean	Sum of ranks	U-value	Z-value	p-value
Fluorosed group	58.67±25.30	85.00	40.00	-0.0442	0.9648
Non- Fluorosed group	59.56±23.77	86.00			

Table 2: Intergroup comparison of degree of tubule closure in each subgroup using Mann-Whitney U Test

Subgroup	Group	Mean	SD	Sum of ranks	U-value	Z-value	p-value
Subgroup a	Fluorosed group	82.67	11.02	10.00	4.00	-0.2182	0.8273
	Non- Fluorosed group	84.33	13.65	11.00			
Subgroup b	Fluorosed group	54.33	6.03	9.00	3.00	-0.6547	0.5127
	Non- Fluorosed group	58.67	8.33	12.00			
Subgroup c	Fluorosed group	39.00	30.51	10.00	4.00	-0.2182	0.8273
	Non-Fluorosed	35.67	15.04	11.00			

Table 3: Intra Group comparison of tubule closure using Kruskal Wallis ANOVA and Post Hoc using Mann- Whitney U Test

	Non Fluorosed Teeth			Fluorosed Teeth		
Sub groups	Means	Std.Dev.	Sum of ranks	Means	Std.Dev.	Sum of ranks
Sub group a	84.33	13.65	24	82.67	11.02	23
Sub group b	58.67	8.33	15	54.33	6.03	12
Sub group c	35.67	15.04	6	39	30.51	10
H-value	7.2			4.3555		
P-value	0.0273*			0.1133		
Pair wise comparison by Mann-Whitney U test						
SG a vs SG b	P=0.0495*					
SG a vs SG c	P=0.0495*					
SG b vs SG c	P=0.0495*					