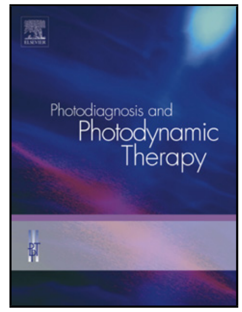
Accepted Manuscript



Title: Effect of Er,Cr:YSGG on bond strength and microleakage of dentin bonded to resin composite with different distance and irradiation time

Authors: Saud Abdulaziz Almojaly, Rana Sulaiman Al-Hamdan, Ali Alrahlah, Osama A. Qutub, Suleman Najashi, Fahim Vohra, Tariq Abduljabbar

PII: S1572-1000(18)30327-2
DOI: https://doi.org/10.1016/j.pdpdt.2018.10.017
Reference: PDPDT 1275

To appear in: *Photodiagnosis and Photodynamic Therapy*

Received date: 27-9-2018
Revised date: 21-10-2018
Accepted date: 25-10-2018

Please cite this article as: Almojaly SA, Sulaiman Al-Hamdan R, Alrahlah A, Qutub OA, Najashi S, Vohra F, Abduljabbar T, Effect of Er,Cr:YSGG on bond strength and microleakage of dentin bonded to resin composite with different distance and irradiation time, *Photodiagnosis and Photodynamic Therapy* (2018), https://doi.org/10.1016/j.pdpdt.2018.10.017

Title page

Effect of Er,Cr:YSGG on bond strength and microleakage of dentin bonded to resin composite with different distance and irradiation time

Running title: Effect of phototherapy on bond strength.

Saud Abdulaziz Almojaly,[a] Rana Sulaiman Al-Hamdan,[b] Ali Alrahlah,[c] Osama A. Qutub,[d] Suleman Najashi,[e] Fahim Vohra,[f] Tariq Abduljabbar[f]

Authors' affiliations:

[a] King Faisal Specialist Hospital and Research Center, Department of Dentistry.

[b] Department of Restorative Dental Science, Operative Division, College of Dentistry, King Saud University, Riyadh, Saudi Arabia.

[c] Department of Restorative Dental Sciences, College of Dentistry, King Saud University. Eng. Abdullah Bugshan research chair for Dental and Oral Rehabilitation. Riyadh, Saudi Arabia.

[d] Oral and Maxillofacial Prosthodontics, Faculty of Dentistry, King Abdulaziz University.

[e] Intern, College of Dentistry, King Saud University.
[f] Department of Prosthetic Dental Sciences, College of Dentistry, King Saud University. Eng. Abdullah Bugshan research chair for Dental and Oral Rehabilitation. Riyadh, Saudi Arabia

Corresponding author:

Dr Tariq Abduljabbar

Department of Prosthetic Dental Sciences, College of Dentistry, King Saud University, PO-Box-60169, Riyadh 11545, Saudi Arabia. E mail. academicksa1@gmail.com

Highlights

- Surface modification of dentin using phototherapy was evaluated.
- Bond strength and microleakage at two different distances and times of irradiation were explored.
- Ninety diseased-free third molars were collected for the experiment.
- Distance of laser and irradiation time has no significant effect on bond strength and microleakage

Abstract

Background: To explore the surface modification of dentin using Er,Cr:YSGG phototherapy and bonding system on the shear bond strength and microleakage scores at two different distances and times of laser irradiation.

Materials and Methods: Ninety disease-free third molars were collected for the experiment. The four laser groups were divided on the basis of non-contact mode distance and time of irradiation: Group-I: Distance of 1mm with duration of 60s; Group-II: Distance of 1mm with duration of 120s; Group-III: Distance of 2mm with duration of 60s and; Group-IV: Distance of 2mm with duration of 120s. The surface for control group (Group-EB: Etch & bond group) was treated with etch and rinse bonding regime. Specimens from all the groups were assessed for shear bond strength and microleakage scores. Samples from all groups were immersed in 2% methylene blue for 24 hours and assessed under a digital microscope for microleakage.

Results: The lowest bond strength and microleakage was observed in the control group EB. The maximum bond strength score was observed in Group-IV with mean scores 23.41 ± 1.83, while the maximum microleakage scores were observed in Group-II and Group-IV with mean scores 1.0 ± 0.36 and 1.0 ± 0.11, respectively. The lowest bond strength and microleakage among laser groups was observed in Group-II and Group-III, respectively. However, specimens in laser groups showed comparable bond strength and microleakge scores ($p>0.05$). For bond strength and microleakage values, analysis of variance showed significant difference among the study groups (p<0.001). Using Tukey post hoc test, bond strength and microleakage of laser groups showed statistically significant values than EB group.

Conclusion: Use of phototherapy using Er,Cr:YSGG for dentin surface treatment has the potential for clinical application in comparison to conventional conditioning technique. Increasing the distance of laser application and time of irradiation has significant effect on the shear bond strength and microleakage scores of dentin bonded to resin composite. However, further in-vitro analysis should be undertaken in order to prove such findings.

Keywords: Er,Cr:YSGG laser; microleakage; bond strength; dentin; resin

1. Introduction

There is constant research in the field of conservative dentistry on how much should caries be removed from the infected tooth tissue. Conventional high speed hand piece is generally used in cavity preparation as they are cost effective, easy to prepare, and less time consuming [1]. However there are few unintended limitations such as pulpal hyperthermia, extreme sensitivity of the tooth, increase damage to the tooth structure, cross contamination and even fear among patients [2]. On the other hand, phototherapy have additional merits as they are proved to be more reliable, easy to handle, high patient acceptance, safe, with less invasive with no requirement of anesthesia [3]. In view of these benefits of phototherapy, its use in dentistry has noticeably increased in recent years.

The clinical application of phototherapy using Er,Cr:YSGG (Erbium Chromium-yttrium-scandium-gallium-garnet) lasers has developed much interest for both researchers and clinical practitioners [4]. Er,Cr:YSGG lasers operate at wavelength of 2780nm have been shown to be effective in cutting hard tissues including enamel, dentin and bone. Such phototherapy system works on the principal of micro explosion during tissue ablation resulting in micro and macroscopic surface irregularities without significant collateral damage to tooth structure, thermal effects or patients discomfort [5]. In addition, the purpose of Er,Cr:YSGG laser includes removal of smear layer and surface roughening of tooth mimicking acid etch technique [6]. Several studies have compared the efficacy of tooth surface conditioning using laser phototherapy and conventional acid etch technique [7,8]. The results of these studies indicate that bond strength of laser prepared surfaces are low or comparable to conventional acid etch technique [7,8].

Removal of smear layer remains a prerequisite in order to attain high marginal seal and bond strength [9]. There are various bonding systems which facilitates modification or removal of smear layer [10]. On the one hand, there is conventional total etch which is regarded as ‘etch and rinse bonding system’ which is highly technique sensitive and difficult to use and entirely eliminates the smear layer [11]. On the other hand, ‘self-etch bonding systems’ are associated with the use of non-rinse acidic monomer which rapidly conditions and prime dentin. Unlike etch and rinse bonding systems, self-etch bonding systems are considerably less technique sensitive and easy to use as it comprises of only single step technique and is generally referred to as “all in one” adhesive systems [11].

Research indicates that microleakage scores and bond strength of dentin treated with Er,Cr:YSGG phototherapy and conventional burs have shown significant convincing outcomes [12,13]. However, bonding regimes with their roles with regards to curing distance and time warrants further investigation. The purpose of this *in-vitro* study was to explore the surface modification of dentin using Er,Cr:YSGG phototherapy using different bonding systems on the shear bond strength and microleakage scores at two different distances and times of irradiation.

2. Materials and methods

This research follows the general guidelines as described in the CRIS (checklist for Reporting In-vitro Studies). Sample size was calculated using means from previous published studies set at 80% power.

2.1. Preparation of teeth samples

Teeth samples were prepared as explained in the previous study [4]. Ninety disease-free third molars were collected for the experiment. Hard deposits, stains or tissue remnants were removed with the help of a periodontal scaler (Sonic flex 2000, Biberach, Germany). Extracted teeth were stored for 5 days in the container containing disinfectant Chloramine T hydrate 95% (Sigma-Aldrich) and later shifted to distilled water at 4°C until use. The teeth were mounted perpendicularly in acrylic resin within the segments of polyvinyl chloride pipes with 10mm diameter. The roots were fixed in acrylic resin at the level of cemento-enamel junction (CEJ) revealing only the coronal part of tooth structure. The buccal surface of all molar teeth was inserted to a depth of 1mm. The occlusal surface was flattened by removing 1.5 mm of tooth structure form the deepest fissure using a grit machine (Isomet saw, Buehler, Illinois, USA) followed by polishing with 400 to 600 grit carbide paper on a rotatory polishing machine at 250 rpm under water irrigation for 10 seconds.

2.2. Study groups

The teeth were divided into 5 groups. Eighteen teeth were treated with conventional flat wheel diamond bur and the remaining 72 teeth were treated with Er,Cr:YSGG laser. For 4 laser treated groups, all specimens were exposed to phototherapy using Biolase, Waterlase I-Plus laser (Biolase, Irvine, CA, USA) (tip MZ8) with frequency 50 Hz and power of 4.5 Watts (W). The four laser groups were divided on the basis of non-contact mode distance and time of irradiation:

Group-I: Distance of 1mm with duration of 60 s.

Group-II: Distance of 1mm with duration of 120 s

Group-III: Distance of 2mm with duration of 60 s

Group-IV: Distance of 2mm with duration of 120 s

Similarly, 18 teeth were treated with flat wheel diamond bur (Shenzhen Dian Fong Abrasives Co. China) for 1 minute at 1500 to 2500 rpm. The control group was denoted by Group-EB (etch & bond group) and the surface was treated with etch and rinse bonding regime. The samples in the EB group were etched using 37% phosphoric acid (Bisco; Schaumburg, IL, USA) for 30 seconds. The samples from all the groups were adhesively bonded using Harvard Bond TE Mono (Harvard Dental International, GmbH, Hoppegarten, Germany). The agent was applied to moist dentin for 30s and air-dried for 15s to form a uniform layer (as per the manufacturer's instructions). It was light cured for 20s with an intensity of 650 mWcm. (Bluephase. C8, Ivoclar Vivadent, Schaan, Liechenstein).

2.3. Bond strength testing

Resin composite build-ups of 3x3mm were made using standardized split moulds in cylindrical shape using Multi Core Flow (MC) (Ivoclar Vivadent Schaan Liechtenstein) composite resin. Excess material from the build-ups was excavated using hand instrument. Following removal of excess material, the build-ups were light cured for 40s. After bonding, all specimens were stored in distilled water (at room temperature) for 24 hours. Specimens underwent thermocycling for 3000 cycles, from 5ºC to 55ºC for 30s. Shear bond strength testing was performed by a single operator using a universal testing machine (Model 4411; Instron Corp, Canton, Mass) with a parallel knife-edge blade just touching the interface of dentin and composite resin build-up. A shear load was applied until failure at a crosshead speed of 1 mm/min. The mode of failure for all specimens was determined using a 3D digital microscope (Hirox-KH7700, 100 Commerce Way, Hackensack, USA).

2.4. Microleakage assessment

Microleakage was evaluated using samples that were immersed in methylene blue dye for 24 hours and then sectioned with Isomet disc after mounted in acrylic resin to prevent dislodgement of the composite. Specimens were cleaned with distilled water and air dried for examination under digital microscope. Scores were assigned using the following criteria:

0 No dye penetration

1 Dye penetration less than one-third of the restoration.

2 Dye penetration less than two-thirds of the restoration.

3 Dye penetration more than two-thirds of the restoration.

4 Dye penetration to the full extent of the restoration.

2.5. Statistical analysis

All quantitative outcomes of SBS and microleakage among the different study groups was tabulated using a specialized SPSS software. The statistical significance was set at

$p<0.05$. Means and standard deviations of SBS and microleakage was compared among the study groups using one-way analysis of variance (ANOVA) and *post hoc* Tukey for multiple comparisons.

3. Results

3.1. Shear bond strength

The lowest bond strength was achieved in the control group EB with mean value 11.73 ± 2.04. The maximum bond strength score was observed in Group-IV with mean scores 23.41 ± 1.83. The lowest bond strength among laser groups was observed in Group-II with mean values 13.52 ± 1.81. However, specimens in laser groups showed comparable bond strength scores ($p>0.05$). For bond strength values, analysis of variance showed significant difference among the study groups ($p<0.001$). Using Tukey post hoc test, bond strength of laser groups showed statistically significant values than EB group (Table 1).

3.2. Microleakage Outcomes

The lowest microleakage values were observed in the control group EB with mean value 0.3 ± 0.18. The maximum microleakage scores were observed in Group-II and followed by Group-I with mean scores 1.9 ± 0.36 and 1.5 ± 0.11. The lowest microleakage values among the laser groups were observed in Group-IV with mean values 0.5 ± 0.11. However, specimens in laser groups I, II and III showed comparable microleakage scores ($p>0.05$). For microleakage values, analysis of variance showed significant difference among the study groups ($p<0.001$). Using Tukey post hoc test, microleakage of laser groups showed statistically significant values than EB group (Table 2).

4. Discussion

To the authors knowledge, this is a maiden study to explore the surface modification of dentin using Er,Cr:YSGG phototherapy using different bonding systems on the shear bond strength and microleakage scores at two different distances and times of irradiation. The authors showed that distance of 2 mm for 120 sec has significant effect on the shear bond strength and microleakage scores of dentin bonded to resin composite.

There are considerable chances of tooth sensitivity with the use of high speed handpiece and dentinal burs for cavity preparation which may cause fear and anxiety among patients [12,14]. On the one hand, surface treatment is necessary for durable bond between composite resin and tooth substance. Smear layer is responsible for poor bonding between the two interfaces. To circumvent the problems related to bonding, surface treatment should ideally be performed to remove the smear layer entirely either with the help of total etch adhesive system or by incorporating the smear layer within the bonding layer with help of self-adhesive system [12,15,16]. On the other hand, phototherapy is found to be more comfortable to the patients, is less invasive and reduces the risk of sensitivity during tooth preparation [17].

The premise of erbium lasers including Er,Cr:YSGG and Er:YAG laser is generally well-absorbed by the mineralized tissues because both wavelengths show high absorption by water and hydroxyapatite. These lasers act at the dental hard tissue through explosive “thermo-mechanical” ablation. In this mechanism, the water molecules contained between the hydroxyapatite crystals absorbs the incident radiation and the water vaporization results in increased internal pressure and microexplosions leading to a substrate ejection in the form of inorganic particles and to a precise irradiated tissue removal [18]. Studies have evaluated the effect of erbium lasers in surface morphology of dentin, and they have shown an irregular appearance, without smear layer, with open dentinal tubules and prominent peritubular dentin, with a microretentive morphological pattern possibly favorable to bonding procedures and making it more resistance to demineralization at low pH [19-21]. Shear bond strength of the material was evaluated using universal testing machine to follow homogeneity and standardization. The present study is a laboratory based qualitative test used to provide data easily and quickly on specific parameters. It helps to measure specific variable while keeping other parameters constant such as distance and time [22]. A single type of bonding system was used as it was shown in the previous study that without etching removal of smear layer was not enough and bond strength was negligible [4]. The reason for treating one group with self-etch bonding system was based on the controversy within the available evidence that whether Er,Cr:YSGG laser is capable of complete removal of the smear layer [23]. Furthermore, there is no study reported previously showing self-etch group on laser surfaces. Microleakage scores were assessed by Dye Penetration Test (DPT) using 2% methylene blue to standardize and homogenize method reported in previous studies [24,25]. The dye penetration method is effective and gives a balance and a fair comparison between different restorative materials [24].

In the current study, samples were fired laser using Er,Cr:YSGG and non-laser sample bonded showed comparable bond strength values. This finding was concurrent with the study done by Hadley et al.[26] and Verma et al.[12]. However, using Er,Cr:YSGG from a distance of 2mm at 120 sec produced greater shear bond strength. A possible explanation of this result with total etch bonding system causing complete removal of the smear layer, opening of the tubular and peritubular dentinal surface which would reflect the unevenness of the surface with increasing time. It would be realistic to assume that this rough and uneven surface influences the bond strength of the adhesive agents [27]. In addition, the surface topography through SEM revealed that surface treated with Er,Cr:YSGG laser in the presence of etch and rinse bonding agents showed highly irregular, sharp jagged projections with evidence of open dentinal tubules.

Microleakage scores was found to be more in groups treated with laser in the presence of etch and rinse bonding agents. Multiple factors including polymerization shrinkage, coefficient of thermal expansion, bonding agent/liners, polymerization technique, quality of substrate (enamel or dentin), location of margins in the substrate and the surface topography are reported to influence marginal microleakage [28]. Recent published data used ageing method to challenge tooth resin interface [11,13]. In our study, thermocycling protocol was followed to make the results valid, comparable and standardized. From our understanding, high microleakage scores was found in laser treated groups due to heat damage, high power density and denaturation of dentin crystals. Further, experiments are warranted to investigate microleakage score at different power settings and number of applications.

Some limitations should not be overruled. The results are pertinent only on Er,Cr:YSGG laser, type of bonding agent and materials used. In addition, the surface modification of dentin after laser application in comparison to bur treated specimens was not quantitatively evaluated. Furthermore, the dentin surface among different teeth is impossible to standardize, due to sclerotic dentin, ethnic variations, and density of dentinal tubules. Therefore, further studies are recommended to explore the influence of Er,Cr:YSGG on the surface roughness and strength of dentin in a standardized environment. On the basis of current findings, Er,Cr:YSGG laser treatment for tooth preparation and bonding of resin composite to dentin has shown considerable potential. However multiple factors including, laser parameters, dentin surface and type of bonding agent influence the bonding outcomes of specimens after laser application should further be investigated for enhanced clinical success.

Conclusion

Use of phototherapy using Er,Cr:YSGG for dentin surface treatment has the potential for clinical application in comparison to conventional conditioning technique. Increasing the distance of laser application and time of irradiation has significant effect on the shear bond strength and microleakage scores of dentin bonded to resin composite. However, further in-vitro analysis should be undertaken in order to prove such findings.

Conflict of interest statement

None declared

Acknowledgment

The authors are grateful to the Deanship of Scientific Research, King Saud University for funding through Vice Deanship of Scientific Research Chairs

References

1. Banerjee A, Watson TF, Kidd EA. Conservative dentistry: dentine caries excavation: a review of current clinical techniques. Br Dent J. 2000;188(9):476-482.

2. Hailu K, Lawoyin D, Glascoe A, Jackson A. Unexpected Hazards with Dental High Speed Drill. Dent J. 2017;5:10.

3. Sun G, Tunér J. Low-level laser therapy in dentistry. Dent Clin North Am. 2004 Oct;48(4):1061-76.

4. Vohra F, Alghamdi A, Aldakkan M, Alharthi S, Alturaigi O, Alrabiah M, Al-Aali KA, Alrahlah A, Naseem M, Abduljabbar T. Influence of Er: Cr: YSGG laser on adhesive strength and microleakage of dentin bonded to resin composite. In-vitro study. Photodiagnosis Photodyn Ther. 2018;23:342-346.

5. Walsh LJ. The current status of laser applications in dentistry. Aus Dent J. 2003;48(3):146-55.

6. Hossain M, Nakamura Y, Yamada Y, Kimura Y, Matsumoto N, Matsumoto K. Effects of Er, Cr: YSGG laser irradiation in human enamel and dentin: ablation and morphological studies. J Clin Laser Med Surg. 1999;17(4):155-9.

7. Üşümez S, Orhan M, Üşümez A. Laser etching of enamel for direct bonding with an Er, Cr: YSGG hydrokinetic laser system. Am J Orthod Dentofac Orthoped. 2002;122(6):649-56.

8. Ceballos L, Toledano M, Osorio R, Tay FR, Marshall GW. Bonding to Er-YAG-laser-treated dentin. J Dent Res. 2002;81(2):119-22.

9. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine AJ, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dent Mater. 2011;27(1):17-28.

10. Oliveira SS, Pugach MK, Hilton JF, Watanabe LG, Marshall SJ, Marshall Jr GW. The influence of the dentin smear layer on adhesion: a self-etching primer vs. a total-etch system. Dental Materials. 2003 Dec 1;19(8):758-67.

11. Deliperi S, Bardwell D, Wegley C. Restoration interface microleakage using one total-etch and three self-etch adhesives. Operative Dent. 2007;32(2):179-184.

12. Verma M, Kumari P, Gupta R, Gill S, Gupta A. Comparative evaluation of surface topography of tooth prepared using erbium, chromium: Yttrium, scandium, gallium, garnet laser and bur and its clinical implications. J Ind Prostho Soc. 2015;15:23.

13. España T. Microleakage in Class V cavities with self-etching adhesive system and conventional rotatory or laser Er, Cr: YSGG. Laser Ther. 2012;21:255-268.

14. Firat D, Tunc E, Sar V. Dental anxiety among adults in Turkey. J Contemp Dent Pract. 2006;7:75-82.

15. Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems: I: Depth of penetration beyond dentin smear layers. Dent Mater. 2001;17:296-308.

16. Oliveira PHC, Cassoni A, Brugnera AJ, Tenorio IP, Rodrigues JA. Bond strength of abraded and non-abraded bleached enamel to resin after Er,Cr:YSGG laser irradiation. Photomed Laser Surg. 2017;35:530-536.

17. Yilmaz HG, Cengiz E, Kurtulmus-Yilmaz S, Leblebicioglu B. Effectiveness of Er,Cr:YSGG laser on dentin hypersensitivity: a controlled clinical trial. J Clin Periodontol. 2011;38:341-346.

18. Meister J, Franzen R, Forner K, Grebe H, Stanzel S, Lampert F, Apel C. Influence of the water content in dental enamel and dentin on ablation with erbium YAG and erbium YSGG lasers. J Biomed Opt.2006;11(3):34030

19. Aranha AC, De Paula Eduardo C, Gutknecht N, Marques MM, Ramalho KM, Apel C. Analysis of the interfacial micromorphology of adhesive systems in cavities prepared with Er, Cr:YSGG, Er:YAG laser and bur. Microsc Res Tech.2007;70:745–751.

20. Esteves-Oliveira M, Zezell DM, Apel C, Turbino ML, Aranha AC, Eduardo CP, Gutknecht N. Bond strength of self-etching primer to bur cut, Er, Cr:YSGG, and Er:YAG lased dental surfaces. Photomed Laser Surg.2007;25:373–380.

21. Malkoc MA, Taşdemir ST, Ozturk AN, Ozturk B, Berk G. Effects of laser and acid etching and air abrasion on mineral content of dentin. Lasers Med Sci.2011;26:21–27.

22. Sirisha K, Rambabu T, Ravishankar Y, Ravikumar P. Validity of bond strength tests: A critical review-Part II. J Conser Dent. 2014;17:420.

23. Lin S, Caputo AA, Eversole LR, Rizoiu I. Topographical characteristics and shear bond strength of tooth surfaces cut with a laser-powered hydrokinetic system. J Prosthet Dent. 1999;82:451-455.

24. Santini A, Ivanovic V, Ibbetson R, Milia E. Influence of Cavity Configuration on Microleakage around Class V Restorations Bonded with Seven Self-Etching Adhesives. J Esthet Rest Dent. 2004;16:128-135.

25. Silveira de Araújo C, Incerti da Silva T, Ogliari F, Meireles S, Piva E, Demarco F. Microleakage of seven adhesive systems in enamel and dentin. J Contemp Dent Pract. 2006;7(5):26-33.

26. Hadley J, Young DA, Eversole LR, Gornbein JA. A laser-powered hydrokinetic system: for caries removal and cavity preparation. J Am Dent Assoc. 2000;131:777-785.

27. Ayad MF, Johnston WM, Rosenstiel SF. Influence of dental rotary instruments on the roughness and wettability of human dentin surfaces. J Prosthet Dent. 2009;102:81-88.

28. Arias VG, Campos IT, Pimenta LAF. Microleakage study of three adhesive systems. Braz Dent J. 2004;15:194-198.

Table 1: Means (SD) for shear bond strength values among study groups using ANOVA and Tukey multiple comparisons test.

Study groups	Mean	SD	SE	Variance	P-value
Group-I	16.18[a]	2.12	0.88	5.99	<0.000
Group-II	13.52[a]	1.81	0.49	1.23	
Group-III	14.14[a]	3.01	0.12	2.89	
Group-IV	23.41[b]	1.83	0.53	2.73	
Group-EB	11.73[a]	2.04	0.45	1.38	

* Dissimilar letters denotes inter-group significant difference.

Table 2: Means (SD) for micro leakage values among study groups using ANOVA and Tukey multiple comparisons test.

Study groups	**Mean**	**SD**	**SE**	**Variance**	**P value**
Group-I	1.5[a]	0.51	0.155	0.32	<0.001
Group-II	1.9[a]	0.36	0.161	0.36	
Group-III	1.2[a]	0.14	0.183	0.19	
Group-IV	0.5[b]	0.11	0.291	0.41	
Group-EB	0.3[b]	0.18	0.148	0.22	

* Dissimilar letters denotes inter-group significant difference.