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Research Article

EVALUATION OF EFFECT OF CHLORHEXIDINE ON MICROLEAKAGE OF CLASS-V COMPOSITE RESTORATIONS WITH DENTIN AND ENAMEL MARGINS USING TWO-STAGE SELF-ETCH ADHESIVE AFTER KEEPING THEM IN WATER FOR SIX MONTHS

Armaghan Alikhani and *Shima Heidari
Dental Faculty, Ahvaz Jundishapur University of Medical Sciences
**Author for Correspondence*

ABSTRACT
Use of materials with anti-bacterial effects including Chlorhexidine after tooth carving and before placing restorative materials has been prevailed; nonetheless, effect of this solution on amount of microleakage after restoration has been mentioned as one of the challenges to use this solution. With regard to few studies at the area of long term effects of Chlorhexidine on microleakage, the present research was conducted aiming at evaluating effect of Chlorhexidine on microleakage of Class-V composite restorations with dentin and enamel margins using two-stage self-etch adhesive after keeping them in water for six months. The present research has been a quasi-experimental and in vitro study conducted on 102 premolar teeth in Department of Restorative Dentistry- Ahvaz Jundishapur University of Medical Sciences. After creating class V cavities (2.3 mm × 1.5 mm) on the buccal surface of each tooth, teeth were divided into two groups of control(n=51) and treatment(n=51) groups in random. In treatment group, before use of bonding, cavities have been washed with Chlorhexidine and then restorated and the samples have been subjected to thermal cycling at the third month and kept in distilled water for 6 months and observed under Stereomicroscope magnified up to 20 times after being placed in 0.2% Methylene Blue Stock Solution and their buccolingual cut, whereby the results have been recorded based on amount of microleakage by means of dye penetration in gingival and occlusal margins. Findings of this study indicated that there is no significant difference on amount of dentin microleakage in control and treatment groups after six months ($P>0.05$). Yet, there is a significant difference on amount of enamel microleakage in control and treatment groups after six months ($P<0.05$). Results of this study indicated that use of Chlorhexidine in Class-V composite restorations before using bonding and after 6 months does not raise microleakage at dentin margin, yet it seems that use of Chlorhexidine in Class-V composite restorations has a negative effect on amount of microleakage at enamel margin. With regard to extensive use of Chlorhexidine in restorative dentistry, further studies on probable effects of this solution on microleakage after restoration are suggested.

Keywords: *Microleakage, Chlorhexidine, Class-V Composite Restorations, Two-Stage Self-Etch Adhesive*

INTRODUCTION
Nowadays, in restorative dentistry, maintaining the structure and function of the teeth is not sufficient, that attention to beauty of restored teeth is of great importance. Hence, with regard to increasing needs for beauty and concern about toxic effects of mercury amalgam as well as less invasive cavity preparation in tooth-colored restorations, use of tooth-colored restorations has increased (Singla *et al.,* 2011). Composites have been regarded as the best tooth-colored restorative materials which have enabled to provide this beauty as well as possible (Samimi, 2002; Asefzadeh *et al.,* 2010).
Nonetheless, polymerization shrinkage of dental composite resins has been mentioned as the major problem in use of these restorative materials that can result in rise of a gap between tooth and composite ending in microleakage, secondary caries and bond failure (Asefzadeh *et al.,* 2010; Shafiei *et al.,* 2010). Microleakage and leakage of bacteria and toxins have been provided through edges of teeth which were restored (Bergenholtz *et al.,* 1982). Continuity of microleakage at the edges of teeth which were restored can result in tooth sensitivity, color change and pulp irritation (Shafiei *et al.,* 2010). The factors pertaining to microleakage at the edges of teeth include physical characteristics of composite, hybrid layer

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failure by small dirt suspended in the etch and disinfectants (Owens *et al.*, 2003). Hence, since providing resin bondings, an attempt has been constantly made to reduce microleakage problems. In this regards, there are different materials and methods to reduce microleakage of composite restorations, including use of layer method and use of different bondings under composite and etc. yet, these attempts have not come to an end in a desired way, and this problem has existed as a disturbing clinical agent (Salari *et al.*, 2014). Further, the concerns about working with composite can be time consuming stages of preparation of enamel and dentin surface. There is the possibility for saliva contamination and resin micromorphological adaptation to cavity wall during these stages, that the contamination has had a negative effect on longevity of restoration, resulting in repetition of all preparation stages (Santini *et al.*, 2004; Brackett *et al.*, 2006). Importance of this issue is doubled in importance in class-V composite restorations which are in proximity of edge of gum and gingival crevicular fluid, because insulation is much more difficult at these areas. Hence, during different generations of bonding, an attempt has been made to reduce the number of stages so as to reduce the contamination probability and failure in composite restoration including color change in edge of teeth, secondary caries and separation of restoration from teeth through reduction of preparation time at dentin and enamel margins (Asefzadeh *et al.*, 2010; Ateyah and Elhejazi, 2004). For this, new composite materials are increasingly supplied to market targeted in improving bonding and reducing the number of tooth preparation stages, because dentists prefer to use the materials that working with them is much easier (Roberson *et al.*, 2006). The newest invention at the area of simplification of bonding system can be production of self-bonding composites that aim to reduce the number of stages for preparation of tooth's surface and ease of use for dentist (Asefzadeh *et al.*, 2010). These new bonding systems do not require washing stage, including one-stage and two-stage self-etch bonding, welcomed by people due to ease of function. Yet, as there is no washing stage in self-etch bonding and Smear layer is not thoroughly removed, the need to disinfection of cavity increases before their use (Retief, 1994). In this regards, use of disinfectant solutions before restoration has been transformed to a common protocol in restorative dentistry aiming at disinfecting cavity, because with regard to the evidences, use of an anti- bacterial cleaning solution after providing cavity can help for removing potential hazards due to bacteria activity (Brannstrom, 1986). For this purpose, Chlorhexidine as an antiseptic for dentin margin and water-soluble chitosan on representative dental pathogens Streptococcus mutans is used at the root surfases with carries (Pashley *et al.*, 2004). Recent studies have shown that use of Chlorhexidine before bonding can remove the bacteria which have remained in smear layer and can suppress host matrix metalloproteinases (MMPs) that these enzymes can reduce durability of bond strength (Gendron *et al.*, 1999; Meiers and Kresin, 1996). Hence, use of Chlorhexidine with inhibitory effect on host bacteria helps for bond strength (Fure and Emilson, 1990) and with inhibitory effect on MMP in long term causes durability of bond (Ersin *et al.*, 2008; Hebling *et al.*, 2005). Despite these factors, probable effect of Chlorhexidine on amount of microleakage is one of the potential challenges in use of Chlorhexidine before restoration, for which different studies have been conducted. Some studies have shown that Chlorhexidine has no effect on amount of microleakage after restoration (Meiers and Kresin, 1996; AlDeeb, 2010; Geraldo-Martins *et al.*, 2007; Derhami *et al.*, 2005) and some studies have shown that this disinfectant can raise negative effects on microleakage (Tulunoglu *et al.*, 1998; Hiraishi *et al.*, 2009; Türkün *et al.*, 2005).

Hence, with regard to different results from studies conducted at the area of effect of Chlorhexidine on amount of microleakage after restoration and the limited studies conducted at the area of long-term effects of Chlorhexidine on amount of microleakage, the present research was conducted aiming at evaluating effect of Chlorhexidine on microleakage of Class-V composite restorations with dentin and enamel margins using two-stage self-etch adhesive after keeping them in water for six months.

MATERIALS AND METHODS

The present research has been a quasi-experimental and in vitro study conducted on 102 premolar teeth in Department of Restorative Dentistry- Ahvaz Jundishapur University of Medical Sciences. After creating class V cavities (2.3 mm × 1.5 mm) on the buccal surface of each tooth, teeth were divided into two

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groups of control(n=51) and treatment(n=51) groups in random. In treatment group, before use of bonding, cavities have been washed with Chlorhexidine and then restorated and the samples have been subjected to thermal cycling at the third month and kept in distilled water for 6 months and observed under Stereomicroscope magnified up to 20 times after being placed in 0.2% Methylene Blue Stock Solution and their buccolingual cut, whereby the results have been recorded based on amount of microleakage by means of dye penetration in gingival and occlusal margins. Then, class V cavities on buccal surface in each tooth at 3mm dimension at Mesiodistal dimension and depth of 1.5 mm were carved by a dentist via fissure bur and approved by two members of professors and residents in department of restorative dentistry. All the cavities have been in a way setting 1 mm under and above CEJ at the area of cavity carving. Fissure burs have been being replaced after each 10 carves. The carved teeth were divided into two groups including control (n=51) and treatment (n=51) groups in random. In the present research, bonding "CLEARFIL SE BOND " made by company KURARAY MEDICAL INC - Japan and composite " ESPE Filtek Z250 3M " made by company Dental products-America were used.

In control group, self-etching primer and bonding system called CLEARFIL SE BOND was subjected to the cavity for 20 seconds through a microbrush and dried with puar air for 5 seconds and then the adhesive called CLEARFIL SE was subjected to cavity through another microbrush and cured for 10 seconds, and then composite Z250 was set in mesial and distal of cavity and cured for 20 seconds; ultimately final curing was considered for 40 seconds. Dental light cure (VIP Junior, Bisco, Schaumburg, IL, USA) used in this study has been regarded with intensity of 600 MW/CM2. In treatment group, before use of bonding, cavities were washed with Chlorhexidine (Consepsis 2%) made by company ULTRADENT for 60 seconds and then cavities were subjected to bonding (CLEARFIL SE BOND) and composite(ESPE Filtek Z250 3M) and restoration of teeth were fulfilled likewise that of in control group. After these stages, teeth restored at both groups were kept in distilled water at 37 ° C in incubator and distilled water has been being replaced every day to avoid from growth of fungi. Further, at the third month, the samples were set in Multifunctional Thermocycle LTC100 under 1000 thermal cycle (5-55 ° C) at transmission time (30 seconds).

After six months, the apex of the root of a tooth was covered with sticky wax and then all parts of tooth were covered via two layers of nail polish to 1 mm restoration margin so as to avoid leakage interference. Both control and treatment groups were subjected to 2% methylene blue dye aqueous solution and remained at room temperature for 24 hours. After passage of time, teeth were carved at the midline of teeth via carving machine (Struers, Denmark) and the samples were examined under Stereo Microscopes (Carl Zeiss Inc, Oberkochen, Germany) magnified up to 20 times by two persons and the results were recorded based on amount of microleakage by means of dye penetration in gingival and occlusal margins. How the rate of dye penetration was scored, has been represented as follow:

0: not-observed dye penetration
1: dye penetration under 1.2 distance to wall
2: dye penetration above 1.2 distance to the extended axial wall
3: dye penetration to the extended axial wall

Characteristics of the materials used in the study

Material	**Batch#**	**Manufacturer**	**Composition**
Clearfil SE Bond	41502	Kuraray Medical Inc, Okayama, Japan	Primer: MDP, HEMA, hydrophilic dimethacrylate, photoinitiator, water bond: 10-MDP, Bis-GMA, HEMA, hydrophilic dimethacrylate, microfiller, photoinitiator

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RESULTS AND DISCUSSION

Findings

In this research, 102 premolar teeth have been examined in vitro study in Department of Restorative Dentistry- Ahvaz Jundishapur University of Medical Sciences. Results from overview of amount of microleakage of Class-V composite restorations with dentin and enamel margins using two-stage self-etch adhesive after keeping them in water for six months in control group indicated no dye penetration in 70.6% of cases and extension of dye penetration to axial wall in 7.8% of cases in sake of amount of microleakage of Class-V composite restorations in control group in which Chlorhexidine was not used before bonding after six months. This overview under dentin separation has been in this way: no dye penetration was observed in 52.9% of cases and extension of dye penetration to axial wall was observed in 25.5% of cases (table 1).

Table 1: Overview of amount of microleakage of Class-V composite restorations in control group under separation of dentin and enamel margins

Amount of microleakage	Enamel Frequency(%)	Dentin Frequency(%)	Sum Frequency(%)
no dye penetration	36 (70/6)	27 (52/9)	63 (61/76)
dye penetration under 1.2 distance to wall	8 (15/7)	6 (11/8)	14 (13/73)
dye penetration above 1.2 distance to the extended axial wall	3 (5/9)	5 (9/8)	8 (7/84)
dye penetration to the extended axial wall	4 (7/8)	13 (25/5)	17 (16/67)
Sum	51 (100)	51 (100)	102 (100)

Overview of amount of microleakage of Class-V composite restorations with dentin and enamel margins using two-stage self-etch adhesive after keeping them in water for six months in treatment group has been a factor which undergone evaluation in this study. Findings from overview of amount of microleakage of Class-V composite restorations with dentin and enamel margins using two-stage self-etch adhesive after keeping them in water for six months in treatment group indicated no dye penetration in 27.5% of cases and extension of dye penetration to axial wall in 15.7% of cases in sake of amount of microleakage of Class-V composite restorations in treatment group in which Chlorhexidine was not used before bonding after six months. This overview under dentin separation has been in this way: no dye penetration was observed in 31.4% of cases and extension of dye penetration to axial wall was observed in 31.4% of cases (table 2).

Table 2: Overview of amount of microleakage of Class-V composite restorations in treatment group under separation of dentin and enamel margins

Amount of microleakage	Enamel Frequency(%)	Dentin Frequency(%)	Sum Frequency(%)
no dye penetration	14 (27/5)	16 (31/4)	30 (49/41)
dye penetration under 1.2 distance to wall	16 (31/4)	15 (29/4)	31 (30/39)
dye penetration above 1.2 distance to the extended axial wall	13 (25/5)	4 (7/8)	17 (16/67)
dye penetration to the extended axial wall	8 (15/7)	16 (31/4)	24 (23/52)
Sum	51 (100)	51 (100)	102 (100)

Comparison of amount of microleakage of Class-V composite restorations with dentin margin in both control and treatment groups after six months via Wilcoxon signed-rank test and the statistics value equal to 1081 with significance level (0.12) indicates that there is no significant difference on amount of

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microleakage of Class-V composite restorations with dentin margin in both control and treatment groups after six months. Yet, comparison of amount of microleakage of Class-V composite restorations with enamel margin in both control and treatment groups after six months via Wilcoxon signed-rank test and the statistics value equal to 2051.5 with significance level (0.001) indicates that there is a significant difference on amount of microleakage of Class-V composite restorations with enamel margin in both control and treatment groups after six months, that such difference has been reported with more amount of microleakage of Class-V composite restorations with enamel margin in treatment group (table 3 & 4).

Table 3: Comparison of amount of microleakage of Class-V composite restorations with dentin margin in both control and treatment groups

Group	**Dentin microleakage**			
	Mean of ranks	**Sum of ranks**	**Test-value**	**P_Value**
Without Chlorhexidine	47/20	2407	1081	0/12
With Chlorhexidine	55/80	2846		

Table 4: Comparison of amount of microleakage of Class-V composite restorations with enamel margin in both control and treatment groups

Group	**Enamel microleakage**			
	Mean of ranks	**sum of ranks**	**Test-value**	**P_Value**
Without Chlorhexidine	40/23	2051/5	2051/5	0/001
With Chlorhexidine	62/77	3201/5		

Further, in this study, status of microleakage was examined after restoration in both control and treatment groups under separation of dentin and enamel margins via Wilcoxon signed-rank test. Findings of this study indicated that there is a significant difference on amount of microleakage of Class-V composite restorations with dentin and enamel margins in control group; In other words, amount of microleakage at dentin margin after six months has been more than the amount of microleakage at enamel margin, that such difference is significant statistically (p-value=0.021, Z=-2.31).
Yet, there has not been a significant difference on amount of microleakage of class-V composite restorations with dentin and enamel margins after six months (Z= -.687, P_value =0.49) (table 5&6, figure 1 & 2).

Table 5: Comparison of amount of microleakage of class-V composite restorations with dentin and enamel margins in control group

Group		**No**	**Mean of ranks**	**sum of ranks**	**z-value**	**P_value**
control	Negative Ranks	9	12/50	112/50	-2/312	0/021
	Positive Ranks	20	16/13	322/50		
	Ties	22				
	Total	51				

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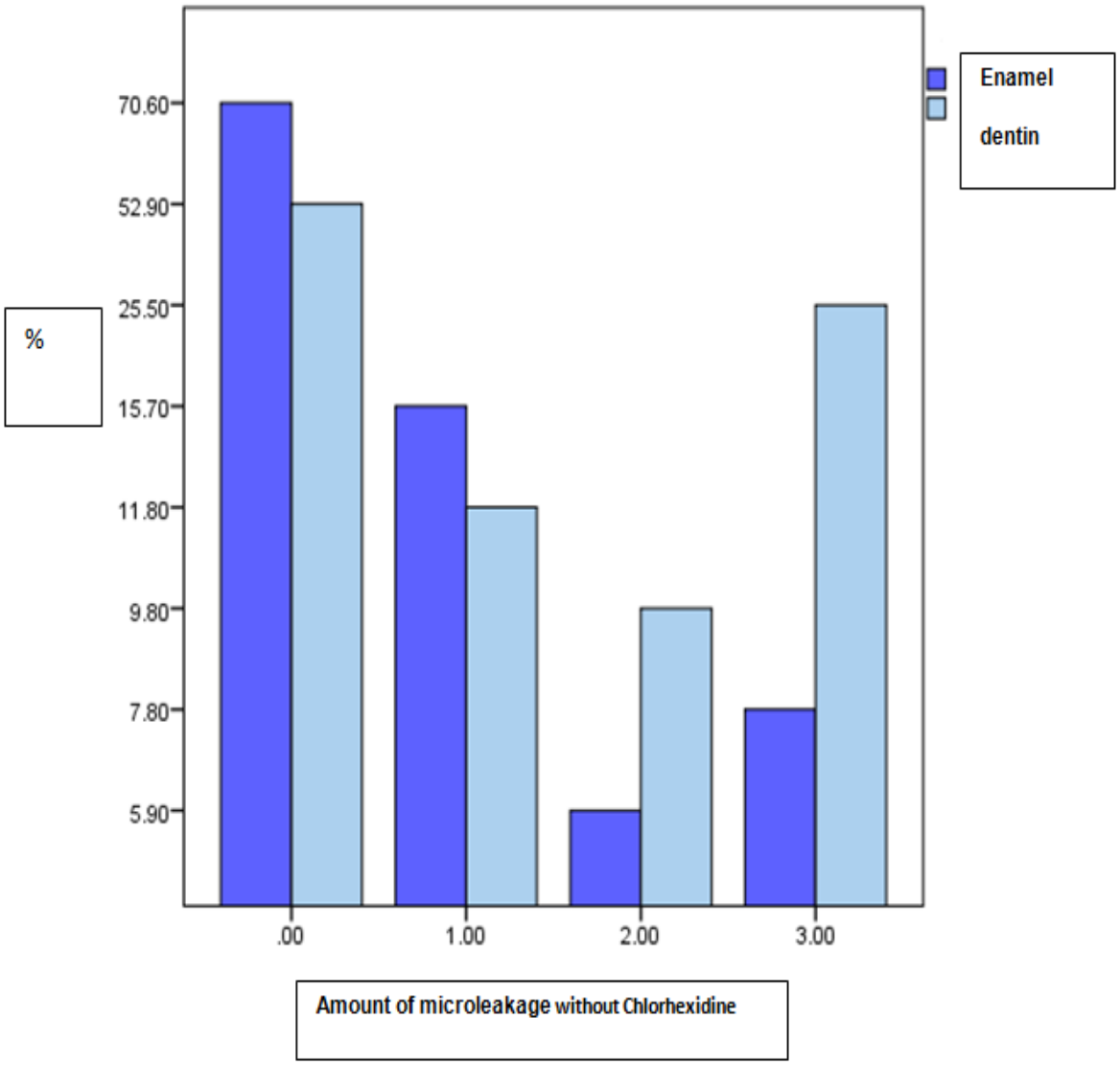

Figure 1: Comparison of amount of microleakage of class-V composite restorations with dentin and enamel margins in control group

Table 6: Comparison of amount of microleakage of class-V composite restorations with dentin and enamel margins in treatment group

Group		No	Mean of ranks	sum of ranks	z-value	P_value
Treatment	Negative Ranks	13	16/50	214/50	-0/687	0/492
	Positive Ranks	18	15/64	281/13		
	Ties	20				
	Total	51				

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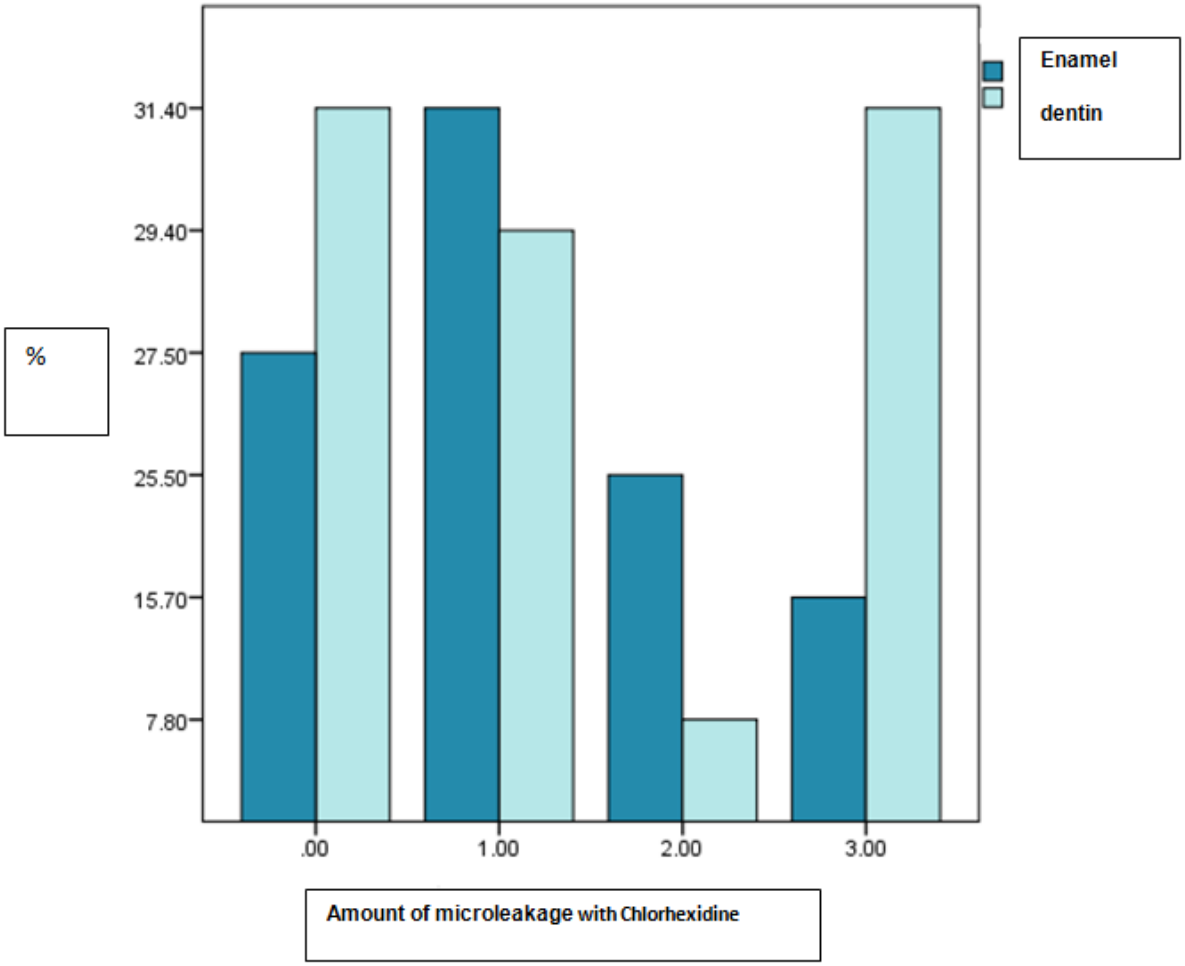

Figure 2: Comparison of amount of microleakage of class-V composite restorations with dentin and enamel margins in treatment group

Discussion and Conclusion

Nowadays, use of materials with anti-bacterial effects including Chlorhexidine after tooth carving and before placing restorative materials has been prevailed; nonetheless, effect of this solution on amount of microleakage after restoration has been mentioned as one of the challenges to use this solution (Türkün *et al.*, 2004; Chandra *et al.*, 2013; Abed *et al.*, 2011). With regard to results from different studies at the area of effect of Chlorhexidine on amount of microleakage in Class-V composite restorations and overview of amount of microleakage after restoration after 24 hours, the present study has examined effect of Chlorhexidine on amount of microleakage after six months. Findings of this study indicated that there is no significant difference on amount of amount of microleakage of class-V composite restorations with dentin margin in both control and treatment groups after six months (p-value>0.05). Yet, comparison of microleakage of class-V composite restorations with enamel margin in both groups indicated a significant difference on amount of microleakage of class-V composite restorations with enamel margin in both groups after six months, tending to increasing amount of microleakage in treatment group (Chlorhexidine) (p-value<0.05). Further, overviews indicated that there is no significant difference on amount of microleakage of Class-V composite restorations with dentin and enamel margins in control group, indicating more amount of microleakage of Class-V composite restorations with dentin margin than enamel margin (p-value=0.021). Yet, there is no significant difference on amount of microleakage of Class-V composite restorations with dentin and enamel margins in treatment group (p-

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value=0.49). On one hand, higher amount of microleakage of Class-V composite restorations with dentin margin than enamel margin and insignificant difference of microleakage of Class-V composite restorations with dentin and enamel margins in treatment groups, and on the other hand higher amount of microleakage of Class-V composite restorations with enamel margin in treatment group rather than control group and insignificant difference in microleakage of Class-V composite restorations with dentin margin in both groups, indicate role of Chlorhexidine in increasing microleakage of Class-V composite restorations with dentin margin than enamel margin in treatment group; as a result increasing microleakage of Class-V composite restorations with dentin and enamel margins justifies insignificance of amount of microleakage of Class-V composite restorations with dentin and enamel margins in treatment group, yet lower microleakage of Class-V composite restorations with dentin margin has been indicated in control group. Chlorhexidine has been regarded as an antiseptic for dentin margin used for a long time in dentistry. This material due to a huge effect on a wide range of negative and positive bacteria has been recommended for washing carving cavity (AlDeeb, 2010; Chandra *et al.,* 2013). Nonetheless, effect of this solution on amount of microleakage after restoration has been mentioned as one of the challenges to use this solution. In this regards, according to the study by Meiers *et al.,* (1996), use of Chlorhexidine in adhesive and syntec has not had any effect on microleakage. Further results from the study by Abed *et al.,* (2009) examined effect of Cavity Disinfection with Chlorhexidine on Microleakage of Gingival Margin in Cl-V Composite Restorations Restored with One-step Self-etch Adhesive Resin in Vitro Study; further their study indicated that use of Chlorhexidine before carving and after restoration has not effect on microleakage of gingival margin in Cl-V Composite Restorations Restored with One-step Self-etch bonding. According to the study by AlDeeb (2010) who examined Effect of 2% Chlorhexidine Gluconate on Microleakage of Total-Etch, Self-Etch and Selective Etch Adhesives, it can perceive that Chlorhexidine before self-etch bonding has not had any effect on amount of microleakage. According to the study by Geraldo-Martins *et al.,* (2007) who examined two-step self-etch bonding, it can perceive that Chlorhexidine has not had any effect on amount of microleakage after restoration.

According to the study by Shafiei *et al.,* (2010) who examined effect of the application of chlorhexidine 2% on the microleakage of composite restorations in class V using four adhesives, the results indicated that use of chlorhexidine after etching with 37% phosphoric acid and washing in two systems SBMP (Scotchbond Multi-Purpose) & Ex(Excite) and before use of acidic primer of CSEB (Clearfil SE Bond) and/or self-etch adhesive(i bond) has not had any effect on microleakage of Gingival Margin in class V (Shafiei *et al.,* 2010). Results from their study have been consistent with the results from the present reserath at the area of dentin margin, whereby no significant difference on amount of microleakage was observed in control and treatment groups. Nonetheless, results from some studies have indicated that use of chlorhexidine in some systems especially self-etch systems can result in increasing microleakage; further, in some studies, it has been announced that pre-treatment with 2% chlorhexidine has a positive effect on etch adhesives, resulting in increasing microleakage in self- etch adhesives (Singla *et al.,* 2011; Hiraishi *et al.,* 2009). Hiraishi *et al.,* (2009) have reported in a study that use of 2% chlorhexidine before self-etch adhesives results in increasing amount of microleakage, whereby this has been due to the lateral effects under use of chlorhexidine in a direct way on smear layer. Tulunoglu *et al.,* (1998) conducted a study entitled "effect of cavity disinfectants on microleakage in dentin bonding systems" and indicated that use of chlorhexidine has had a negative effect on microleakage of two adhesives including Syntac and Prime & bond and has had a negative effect on dentin bonding systems, resulting in increase of microleakage, that such difference can be due to the difference on structure of primary dentin than permanent dentin.

On the other hand, Clearfil SE Bond was used in this study, thus some differences in the results from this study and other studies can be attributed to the self-etch adhesive. Clearfil SE Bond encompasses functional monomer, that is, the MDP monomer (10-Methacryloyloxydecyl dihydrogen phosphate) having two hydroxyl groups (Kubo *et al.,* 2001). In addition, MDP-10 has caused dissolving the smear layer and smear plugs to a minimum level and opening dentinal tubules and reducing penetration of dentin (Dunn and Söderholm, 2001). Further, MDP-10 causes facilitating penetration, saturation,

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polymerization and involvement of monomer with demineralized dentin matrix (ADDM) so as to form a thick hybrid layer (Gordan *et al.,* 1998). Recently, Yoshida *et al.,* in a study "comparative study on adhesive performance of functional monomers" reported that MDP sticks in hydroxyapatite and its Calcium salt hardly dissolves in water. They have suggested that less dye penetration in the samples bonded with Clearfil SE Bond can be attributed to difference in chemical composition of self-etch bond (Yoshida *et al.,* 2004). Hence, it seems that effect of antiseptic solutions might be in line with their constituents and the type of bonding system (Shafiei *et al.,* 2010). In this regards, results from study by Siso *et al.,* (2009) indicated that use of 2% chlorhexidine as an antiseptic solution before use of Clearfil SE bonding influences this bonding system and increases amount of microleakage in chlorhexidine; yet, no significant difference was observed in Gingival Margin, yet a significant difference was observed in occlusal margin between chlorhexidine group and other groups, such that the amounts of microleakage has been higher in chlorhexidine group (Siso, 2009). According to the study by Tulunoglu, it was announced that increase of microleakage can be due to negative interactions between antiseptic solution and dentinal bonding factors (Tulunoglu *et al.,* 1998). In this regards, this discussion has continued that whether use of chlorhexidine before adhesive influences function of self-etch bonding and microleakage of Class-V composite restorations with dentin and enamel margins or not (Soares *et al.,* 2008). The aforementioned results have been consistent with the results of this study at the area of dentin margin in both control and treatment groups.

In point of view of some researchers, chlorhexidine at few concentrations can suppress dentin degradation activity (AlDeeb, 2010; Hebling *et al.,* 2005). Breschi *et al.,* (2010) in a study entitled "Chlorhexidine stabilizes the adhesive interface: A 2-year in vitro study" indicated that use of 2% Chlorhexidine for 30 seconds reduces dentin degradation activity which had been developed in an artificial way during 2 years. Some other researchers believe that use of Chlorhexidine after stage of etching acid in total etch bonding due to inhibition of matrix metalloproteinase has caused avoiding collagen breakdown and keeping hybrid layers and improving strength of bond (Abed *et al.,* 2011; Pappas *et al.,* 2005; Carrilho *et al.,* 2005). Further, Chlorhexidine has strong positive ion charge that allows connecting to phosphate group simply (Salari *et al.,* 2014; Meiers and Kresin, 1996). For this, Chlorhexidine has a high tendency to etch to the tooth's surface. This tendency increasing through etching tooth that causes proper Wettability at dentin surface, avoiding increase of amount of microleakage (Salari *et al.,* 2014). Further, some studies have shown that resin bond to dentin which is raised through Hydrophilic adhesive systems undergoes degradation during time (De Munk *et al.,* 2003; Tanaka *et al.,* 1999). Recent studies have shown that two main factors are involved in long-term destruction of bond: Presence of water in hydrophilic bonding systems and proteolytic enzymes derived from MMP (De Munk *et al.,* 2003; Tanaka *et al.,* 1999; Martin-De *et al.,* 2000). Recently, it has been indicated that bond reduces due to Hydrolytic degradation of composite resins and proteolysis of unprotected collagen fibrils inside the decalcified dentin (). Matrix metalloproteinases (MMPs) are targeted in degradation of hybrid layer and reduction of resin bond to dentin, that the compositions which inhibit MMPs can be effective in increasing bond (Carrilho *et al.,* 2007). In this regards, Chlorhexidine through inhibition of Matrix metalloproteinases (MMPs) in long term causes durability of bond in dentin (Ersin *et al.,* 2008; Hebling *et al.,* 2005). This is consistent with the results of this study at the area of dentin, yet Chlorhexidine has not had any effect on durability of bond in long term due to lack of lithic collagen at enamel margins. On the other hand, justifying an increase in microleakage in enamel margin in treatment group lies on this fact that Chlorhexidine is a solution in water hydrolyses in a better way in PH and avoids etching bacteria to the surface under the competition over trapping the calcium in the tooth's structure. With regard to PH close to 2, thorough demineralization of dentinal tubules does not occur and Chlorhexidine absorbed in smear layer enters into the hybrid layer, whereby thorough bonding will be avoided (Singla *et al.,* 2011). Further, Meiers and Kresin indicated that cavity disinfectants which are used on dentin surfaces are resistant to acidic conditioning (Meiers and Kresin, 1996). This resistant layer against acid might inhibit ability of hydrophilic resin to saturate dentin surface, whereby this can be a reason for increasing microleakage in use of 2% Chlorhexidine gluconate (Siso, 2009).

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The differences in results from different studies in relation to effect of Chlorhexidine on microleakage can be due to different composition of solution in used bonding, lack of washing disinfectants before bonding, use of different bonding substrate, different chemical composition in bonding, combining disinfectants with other washing materials and duration of intra-experimental investigations after use of this solution, that the duration has been mentioned 24 to 72 hours after restoration, yet this duration has been after passage of six months, where this can be a reason for different point in this study rather than other studies (Abed *et al.*, 2011).

Conclusion

Infection caused by bacteria attack whether existing in smear layer or emerges as the result of microleakage after restoration is one of the main factors stimulating the sensitivity of the pulp, under which some researchers suggest use of chemical solutions before restoration to prevent from accumulation of bacteria. These anti-bacterial solutions after preparation of cavity are prescribed (Singla *et al.*, 2011; AlDeeb, 2010; Türkün *et al.*, 2004; Soares *et al.*, 2008). Chlorhexidine has been regarded as an antiseptic for dentin margin.

With regard to results from different studies in relation to effects of Chlorhexidine on amount of microleakage after restoration and few studies at the area of long-term effects of Chlorhexidine, the present research has intended to investigate long-term effect of Chlorhexidine on amount of microleakage after restoration. Results from this study indicated that use of Chlorhexidine in microleakage of Class-V composite restorations using two-stage self-etch adhesive after keeping them in water for six months does not cause creation of microleakage at dentin surface, yet it causes increasing amount of microleakage at enamel surface. With regard to extensive use of Chlorhexidine in restorative dentistry, further long-term studies on probable effects of this solution on microleakage after restoration are suggested.

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