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RESEARCH ARTICLE

EFFECT OF DIFFERENT SALIVA DECONTAMINATION PROCEDURES ON THE SHEAR BOND STRENGTH OF A UNIVERSAL EIGHTH GENERATION ADHESIVE SYSTEM TO DENTIN AFTER POLYMERIZATION – AN *IN VITRO* STUDY

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Abstract

Aim: To evaluate and compare the effect of saliva decontamination procedure on the shear bond strength of a Universal eighth generation adhesive system to dentin after polymerization.

Materials & Method: Forty extracted non-carious premolars were mounted on a acrylic block and their occlusal surfaces were grounded flat to exposed the middle depth dentin. The teeth were then randomly divided into five Groups: Group A(control), Group B(Bonding, Saliva, Air-dried), Group C(Bonding, Saliva, Air-dried, Rinse), Group D(Bonding, Saliva, Air-dried, Re-bonding) & Group E (Bonding, Saliva, Air-dried, Rinse, Re-bonding). A teflon tube of 2x3 mm dimension was used to pack composite on the treated dentin surface and light cured for 40s. Shear Bond strength was then measured using instron testing machine at a crosshead speed of 1mm/min. Data were statistically analysed using ONE WAY-ANOVA and TUKEY'S test.

Result: It was observed that, the highest mean shear bond strength was found in Group D(Bonding, Saliva, Air-dried, Re-bonding) & Group E(Bonding, Saliva, Air-dried, Rinse, Re-bonding) with a mean value of 23.87 each. The least mean value was seen in Group C(Bonding, Saliva, Air-dried, Rinse), 13.25. When an intergroup comparison was made, statistically significant difference was seen between the various groups except Group A(control)-Group D(Bonding, Saliva, Air-dried, Re-bonding)-Group E(Bonding, Saliva, Air-dried, Rinse, Re-bonding) and Group B(Bonding, Saliva, Air-dried)-Group C(Bonding, Saliva, Air-dried, Rinse).

Conclusion: Salivary contamination significantly decreases the bond strength of the adhesive to dentin after polymerization. Rinsing and Air-drying of the cured contaminated dentin surface was not sufficient to restore the shear bond strength. However reapplication of adhesive system after rinsing and air-drying of the cured contaminated dentin surface fully restored the bond strength.

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Introduction:-

Composites and adhesive systems are one of the most widely used and versatile dental material available to the dental professional because of their esthetics and easy direct filling capabilities. However dental adhesives and composites are highly undefended against contamination. Clinically there are many factors that hinders adhesion and retention of resin-containing restorative materials. Moisture such as gingival fluid, blood, hand piece oil and, in particularly saliva, can affect the quality of the bond. This may result in loss of restoration, recurrent caries, postoperative sensitivity and discoloration. Therefore the bonding procedures require proper isolation and prevention of contamination.

Rubber dam isolation is usually the standard method as it facilitates visualization and makes it possible for the dentist to control contamination. On the other hand, it can be difficult to place a rubber dam on a severely fractured tooth, in patients with restricted mouth opening, on a newly erupted molar, or in a mouth breathing patient. For these reasons, it is not always feasible to use a rubber dam in all clinical cases. Instead cotton rolls are used during procedures and some kind of contamination still might occur.

Saliva contamination can occur anytime during a restorative procedure. It has been shown that the protein content of saliva is responsible for the decrease in adhesive bond strength when contamination with saliva has occurred^{1,2}. And also, dentin adhesion is a complex process when compared to enamel bonding because it is a heterogeneous substrate, with much higher organic and water content than enamel. Therefore the result of many studies related to the bonding efficacy of saliva contaminated dentin bonding agents has varied. Some studies demonstrated that saliva contamination reduces the bond strength of dental adhesive to dentin, while other reported conflicting results.

The current adhesive systems such as the universal adhesives has claimed to be resistant to salivary contamination. In the last decade, universal adhesive system has been developed, giving great versatility and freedom of choice for the clinician. Universal adhesives can be used according to self-etch, selective etch and total etch bonding. In these systems, functional monomer of 10-methacryloxydecyl Dihydrogen Phosphate (10-MDP) that allow chemical bonding to the calcium of hydroxyapatite are generally used, making it possible to create a stable adhesive interface over time³. These simplified systems allows an easier handling protocol, eliminating the rinsing and drying steps which simplifies the bonding procedure. In addition to this, the possibility of over wetting or over drying is reduced, which in turn helps in better adhesion. These dentin bonding agents have a reduced number of components and application steps and this reduces the risk of saliva contamination in the field of operation.

Hence the purpose of this study is to evaluate the effect of different salivary decontamination procedures on the shear bond strength of a single bottle universal eighth generation adhesive system to dentin when saliva contamination occurred after polymerization.

Materials And Method:-

The present in vitro study was carried out in the Department of Pediatric and Preventive Dentistry, D.J. College of Dental Sciences & Research, Modinagar in collaboration with Apex Assessment Laboratory, Mohan Nagar, Ghaziabad, Uttar Pradesh.

A total of forty human premolar teeth extracted for orthodontic or periodontal reasons fulfilling the inclusion criteria were taken as a sample for the study. The following inclusion criteria was used - teeth free from restoration and fluorosis, teeth without any caries, teeth with intact buccal/lingual surface and teeth without any developmental anomaly.

All the teeth were scaled with ultrasonic scaler to remove debris and calculus, autoclaved and stored in distilled water at room temperature until use for the experiment. The selected teeth was used within three months of collection as per recommendations of occupational safety and health administration guidelines(OSHA).

The collected forty samples were mounted in self cure acrylic resin and the occlusal surface of each sample was grounded on wet diamond disks to remove enamel and to expose area of middle depth dentin(Fig1 & Fig2). The exposed dentin surfaces were polished with 600-grit abrasive for 20-30s.



Fig1:- Acrylic mounting of tooth.



Fig2:- Preparation of occlusal surface.

The prepared samples were then randomly divided into the following five groups with eight samples in each group:

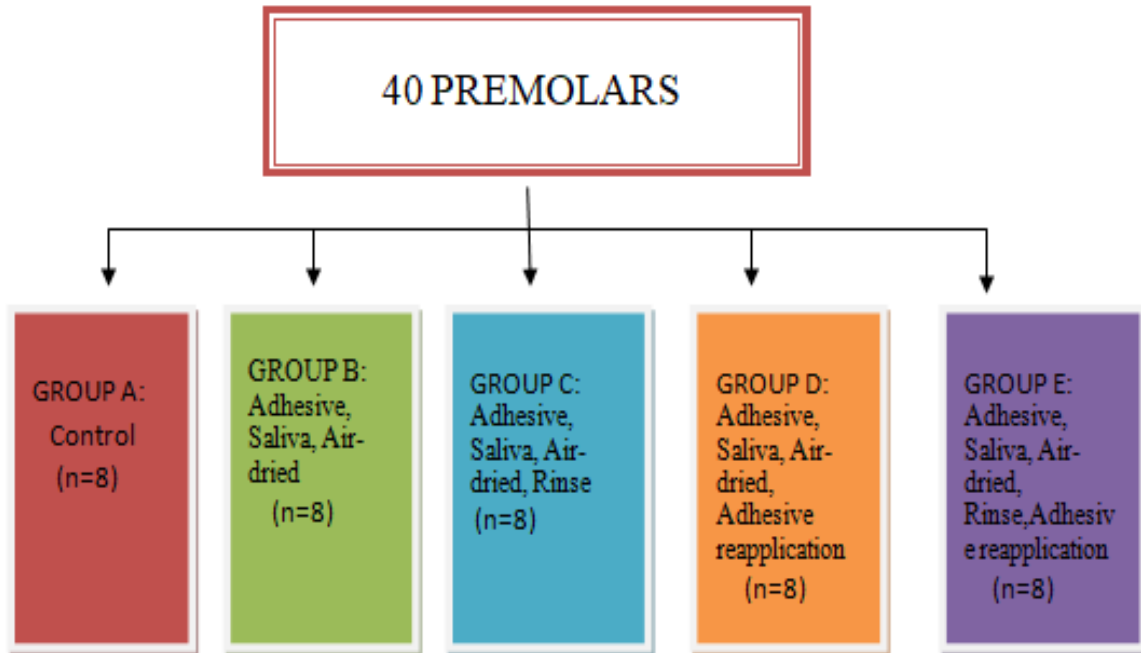
Group A: Control group. No saliva contamination. The single bottle 8th generation universal adhesive system (G-Premio bond) was applied to the dentin surface according to the manufacturer's instruction. The adhesive is then gently air dried for 5 seconds to evaporate the solvent and then light cured for 20 seconds.

Group B: The single bottle adhesive system was applied as in the Group A, and then the dentin surface was subjected to contamination with the saliva for about 20 seconds using a micro brush. The surface was then air dried for 5 seconds using an air-water syringe.

Group C: The same procedure as in Group B, but after contamination, the surfaces were rinse with water for 10 seconds using an air-water syringe, followed by air drying for 5 seconds.

Group D: After the same procedure used in Group B, the adhesive resin was reapplied and light cured.

Group E: After the same procedure used in Group C, the adhesive resin was reapplied and light cured(Flowchart1).



Flowchart1: Division of samples

A teflon tube of inner diameter 2 x 3 mm dimension was then used for the application of composite on the treated dentin surfaces. The tube was filled with resin composite (FILTEK Z250) and light cured for 40s. The prepared specimen were stored in distilled water for 24 hours.

The shear bond strength was measured using Instron universal testing machine at a crosshead speed of 1mm/min. The force required to separate the composite material from the tooth surface was registered in newtons(N) and converted into megapascals(Mpa) as a ratio of newtons to surface area of the bonded surface.

The same procedure was followed for all the remaining samples.

Data was collected and tabulated and subjected for statistical analysis

Statistical Analysis

The statistical analysis was done using SPSS Version 15.0. The Analysis of Variance (ANOVA) and Post-Hoc Test (Tukey-HSD) were performed to know the effect of each variable and to reveal the statistical significance. The values were represented in number and mean \pm SD. For the purpose of statistical interpretation p value of 0.05 was considered statistically significant.

Results:-

The result showed that the highest mean shear bond strength was found in Group D(Bonding, Saliva, Air-dried, Re-bonding) & Group E(Bonding, Saliva, Air-dried, Rinse, Re-bonding) with a mean value of 23.87 each, followed by Group A(control), Group B(Bonding, Saliva, Air-dried) & Group C (Bonding, Saliva, Air-dried, Rinse) with a mean value of 23.50, 14.12 & 13.25 respectively. The least mean value was seen in Group C(Bonding, Saliva, Air-dried, Rinse), i.e. 13.25(Table1). When the post hoc Tukey analysis was done it was found that the intergroup comparison between the various groups showed statistically significant except Group A(control)-Group D(Bonding, Saliva, Air-dried, Re-bonding)-Group E(Bonding, Saliva, Air-dried, Rinse, Re-bonding) and Group B(Bonding, Saliva, Air-dried)-Group C(Bonding, Saliva, Air-dried, Rinse) (Table2).

Groups	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
				Lower Bound	Upper Bound		
Group A	23.50	2.449	0.866	21.452	25.547	20.00	27.00
Group B	14.12	2.695	0.953	11.871	16.378	11.00	18.00
Group C	13.25	2.549	0.901	11.118	15.381	10.00	16.00
Group D	23.87	3.440	1.216	20.998	26.751	18.00	28.00
Group E	23.87	4.223	1.493	20.343	27.406	18.00	29.00

Table1: Mean values of shear bond strength in different groups

	<u>Mean</u>	<u>StdDev</u>	<u>Std Error</u>	<u>Mean Diff</u>	<u>P value</u>
Group A	23.50	2.449	0.866	9.37500*	0.001 (Sig)
Group B	14.12	2.695	0.953		
Group A	23.50	2.449	0.866	10.25000*	0.001 (Sig)
Group C	13.25	2.549	0.901		
Group A	23.50	2.449	0.866	-.37500	0.813 (Non-Sig)
Group D	23.87	3.440	1.216		
Group A	23.50	2.449	0.866	-.37500	0.813 (Non-Sig)
Group E	23.87	4.223	1.493		
Group B	14.12	2.695	0.953	.87500	0.581 (Non-Sig)
Group C	13.25	2.549	0.901		
Group B	14.12	2.695	0.953	-9.75000*	0.001 (Sig)
Group D	23.87	3.440	1.216		
Group B	14.12	2.695	0.953	-9.75000*	0.001 (Sig)
Group E	23.87	4.223	1.493		
Group C	13.25	2.549	0.901	-10.62500*	0.001 (Sig)
Group D	23.87	3.440	1.216		
Group C	13.25	2.549	0.901	-10.62500*	0.001 (Sig)
Group E	23.87	4.223	1.493		
Group D	23.87	3.440	1.216	.00000	1.000 (Non-Sig)
Group E	23.87	4.223	1.493		

Table2: Intergroup comparison of means of shear bond strength using Post hoc analysis

Discussion:-

Ever since the introduction of adhesive systems and their rapid advancement in dentistry, one of the most frequent concerns has been to obtain a completely dry field, so that the adhesive procedures are performed in an ideal condition because both the dental adhesives and composite are highly undefended against contamination. Therefore, in a standard dental operative treatment procedures, a rubber dam isolation is recommended. However, there are situations where rubber dam isolation is not possible or isolation protocol is violated, which leads to the operative site exposed to contamination. The most common and probable contaminant a clinician has to confront is the saliva.

Saliva is an extracellular fluid, which constitutes of 99.4% water and 0.6% solids. It contains aggregates of molecules like glycoproteins, sugar, proteins, amylase and inorganic components. The presence of water and glycoproteins of saliva on the surface may hamper with the proper infiltration of adhesives and subsequently hamper the micromechanical adhesion.

Thus this present study was done to evaluate the effect of salivary decontamination procedures on the shear bond strength of a newly introduced eighth generation universal adhesive material (G-Premio Bond, GC) to dentin after polymerization.

The result of the present study revealed that the highest mean shear bond strength was found in Group D (Bonding, Saliva, Air-dried, Re-bonding) & Group E (Bonding, Saliva, Air-dried, Rinse, Re-bonding) with a mean value of 23.87 each and the least mean value was seen in Group C (Bonding, Saliva, Air-dried, Rinse), i.e. 13.25. When the post hoc Tukey analysis was done it was found that the intergroup comparison between the various groups showed statistically significant except Group A (control)-Group D (Bonding, Saliva, Air-dried, Re-bonding)-Group E (Bonding, Saliva, Air-dried, Rinse, Re-bonding) and Group B (Bonding, Saliva, Air-dried)-Group C (Bonding, Saliva, Air-dried, Rinse).

This result showed, reapplication of the adhesive system after the salivary contamination on a cured bonding system improved the bond strength values. **Santschi et al (2015)**⁴ conducted a study on effect of salivary decontamination on bond strength of two one-step self etching adhesives to dentin and found reapplication of the adhesive restored the bond strength. A study done by **Darabi F et al (2012)**⁵ on Effect of different decontamination procedures from a saliva-contaminated cured bonding system (Single Bond) concluded that reapplication of the adhesive remove the adverse effect of saliva and reinstated the bond strength. Another study by **Sattabanasuk V et al (2006)**⁶ stated saliva contamination reduced the dentin bond strength and reapplication of the adhesive is an empirical recommendation for restoring bond strength. This can be attributed to the fact that reapplication of the adhesive increases the adhesive thickness and promotes a lower hydrolytic degradation potential and also the adhesive will probably provide a new layer of free bonding monomers with a capacity of chemical bonding to the above restorative resin, thus increasing the quality of the adhesion.

The result of the present study is in contrary with the result of an earlier study done by **Fritz et al (1998)**⁷ using a one-step adhesive, which concluded that contamination of the already cured adhesive layer compromises the bond strength and reapplication of the adhesive system was not enough to restore the bond strength. The possible reasons for this might be the presence of 2-hydroxyethyl methacrylate (HEMA) in the experimental adhesive resin (ARX) used by him, as HEMA-containing adhesives are vulnerable to moisture in saliva, as their presence encourages absorption of water and will end up diluting the monomers to a degree that polymerization process is hindered⁸.

Group C (Bonding, Saliva, Air-dried, Rinse) showed the least shear bond strength. When intergroup mean shear bond strength comparison was made among the various groups, Group B (Bonding, Saliva, Air-dried) and Group C (Bonding, Saliva, Air-dried, Rinse) showed no significant difference. This showed salivary contamination drastically decreases the bond strength of the adhesive to dentin after polymerization, and decontaminant procedures such as air-drying and rinsing was not sufficient to restore the lost shear bond strength.

Several studies, such as **Kewlani M et al (2021)**⁹, **Nair P & Illie N (2020)**¹⁰, **Fujiwara S et al (2018)**¹¹, **Ulker E et al (2017)**¹², **Munaga S et al (2013)**¹³ stated that saliva contamination adversely affects the shear bond strength of dentin, which is in agreement with our present study. This can be explained by the theory that when the dentin surface is contaminated with saliva after polymerization process, absorption of salivary proteins to the polymerized surface takes place, which may prevent complete infiltration of the subsequent resin layer and prevent copolymerization, resulting in drastic reduction in the shear bond strength. A study by **Salz et al (2005)**¹⁴ evaluated

the hydrolytic stability of self etch adhesives and suggested that cured self-etch adhesives acted like a permeable membrane, thus allowing fluid to pass through the polymerized adhesive. **Patil SB(2014)**¹⁵ also stated glycoproteins from the saliva may adsorb into the polymerized surface and acts as a barrier, thereby decreasing the wettability of the composite resin and preventing adequate polymerization. Another study by **Pashley et al(1982)**¹⁶ showed that glycoproteins could leak into dentin tubules and form a barrier that prevented the polymerization of the composite resin layer. At the same time, it has also been reported that enzymes in saliva can break down bis-GMA in composite resin, and this hydrolytic activity may cause deterioration at the adhesive interface.

In the present study, rinsing and air-drying was not sufficient enough to restore the bond strength. This result of our study is in agreement with studies conducted by **Kim J et al(2015)**¹⁷, **Darabi F et al (2012)**¹⁸, **Ari H(2008)**¹⁹, who also found rinsing and air-drying not adequate to restore the bond strength to dentin after polymerization. It may be assumed simple steps like rinsing and air-drying was not able to completely remove the salivary glycoproteins that got adsorbed to the polymerized surface.

Therefore, in the present study it can be concluded that salivary contamination has an adverse effect on the shear bond strength of the eighth generative adhesive system(G-Premio Bond, GC)to dentin after polymerization. Decontamination procedures such as rinsing and air-drying was not adequate enough to restore the bond strength. However reapplication of the adhesive after the decontamination procedures fully recovered the bond strength.

Conclusion:-

Within the limitation of this in vitro study the following conclusions were drawn:-

1. Salivary contamination significantly decreased the bond strength of the adhesive to dentin after polymerization
2. Rinsing and Air-drying of the cured contaminated dentin surface was not sufficient to restore the shear bond strength.
3. Reapplication of adhesive system after simple rinsing and air-drying of the cured contaminated dentin surface fully restored the bond strength.

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