"Comparative Evaluation of Influence of Cavity Disinfectants on Immediate and Delayed Shear Bond Strength of Composite Resin to Dentin Using Total Etch And Self Etch Dentin Bonding Systems." An In-Vitro Study

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ABSTRACT

Restoring posterior teeth with resin-based composite material continues to gain popularity among clinicians, and the demand for such aesthetic restoration is increasing.

In this study the evaluation of the influence of different cavity disinfectants was done on immediate and delayed shear bond strength of composite to dentin using total-etch and self-etch dentin bonding systems.

Intact, caries, restoration and crack free, 120 permanent molars, was collected and horizontal sectioning of all the samples was done to expose mid coronal dentin. Samples were divided into three main groups, on the basis of use of disinfecting agents **(**Control group, Treatment with 2% Chlorhexidine digluconate, Treatment with 3% Sodium hypochlorite),and each main group was subdivided into two subgroups on the basis of type of adhesive systems (Total-etch and Self-etch dentin bonding system). After the application of dentin bonding systems, the composite cylinder was formed on treated dentin surfaces. After that half samples were stored for Immediate shear bond strength (SBS) (n=10) and other half for

delayed (6 months) (n=10) SBS testing under Universal testing machine. Significant difference in the immediate and delayed shear bond strength was observed among experimental groups for both self etch and total etch dentin bonding system.($P < 0.01$).

Keywords: Shear bond strength, Chlorhexidine digluconate, Sodium hypochlorite, Self etch, Total etch, Matrix metalloproteinases (MMPs).

INTRODUCTION

Magnificent asthetic, and improved physical and mechanical properties of newly developed and recently advanced composites has lead to increased demand and popularity of tooth coloured restoration in all permanent teeth.[1] A stable bond formation between restorative material and dentin is necessary for long term success of the restoration.[2] The bond strength of enamel is usually stable with restorative materials but in case of dentin, it is recorded to be decreased by '30-40% after 6 months and 60-70% after 1 year'.[3] After the introduction of acid-etch technique by **Buonocore**, the reliable execution of dentin bonding systems has increased in day-to-day practice.[4] Currently total etch and self-etch technique adhesives are often used .[5] Both the adhesive systems hampers the bond strength in different ways. However, maintaining the adequate bond strength for longer duration is still a challenging procedure.[4]

Many studies showed that the degradation of collagen bundles and hydrolysis of bonding resin is responsible for bond strength degradation over time.[1,3] This degradation is the result of growth of micro-organisms under the restoration, which causes tooth sensitivity, bond strength deterioration, microleakage, pulpitis and degenerative changes in pulp.[6] Some other studies showed that proteolytic action of different MMPs are responsible for decrease in bond strength.[7]

These MMPs are pH dependant and get activated in the low pH created by acid etching process in restorative procedure. They bind to zinc and calcium ions and cause degradation of the bond formed between restorative material and dental tissue.[7] To overcome this drawback antibacterial agents and Matrix metalloproteinase (MMP) inhibitors are used in prepared cavity before restoration.

MMP inhibitors are present in many cavity disinfecting agents likes Chlorhexidine digluconate (CHX), minocycline (MI), Sodium hypochlorite, Glutaraldehyde, Aloe barbadensis miller (Aloe vera) etc. Among them, CHX is well known large-spectrum MMP-inhibitor.[5] **Sinha D J et al** [7] in their in vitro study stated that application of chlorhexidine has profound MMP-inhibitory effect. Even **Rayar S et al** [5], in their study stated that the CHX gave improved bond strength with both self-etch and total-etch adhesive systems.

Another cavity disinfectant, Sodium hypochlorite (NaOCL) is also well known antimicrobial agent and have tissue hydrolysing property, hence when use in prepared cavity, it had potential effects on bond strength preservation [1]. **Hussan AM et al** [1] stated that application of sodium hypochlorite increases the bond strength of self-etch adhesive, and increases the longevity of restorations.

In most studies, bond strength of dentin with composite restorative material has been probed in shorter periods, such as after 24 hours, [6] which missed the durability of resin to dentine. Several studies have investigated the stability of resin and dentin bond after long-term storage in water, which resulted in a reduction in bond strength after 6 months immersion in water. [1-4] The long term storage in water gives the investigators benefit of knowing the long term success rate and effect of bond strength of composite resin and disinfecting agents.

Thats why in present study we examined the influence of 2% Chlorhexidinedigluconate (CHX) and 3% Sodium hypochlorite (NaOCL) at adhesive-dentine interfaces by using

two different adhesive techniques that is total etch technique and self etch technique, at two different time intervals, that is within 24 hrs of sample prepration and after 6 months of storage.

MATERIAL AND METHOD

This study was carried out in the Department of Conservative Dentistry and Endodontics Surendera Dental College and Research Institute, Sri Ganganagar, Rajasthan and Central Institute of Plastics Engineering & Technology, Jaipur, India.

Ethical Aspects

This research was approved by Research Ethical Committee of the institution (under no-SDCRI/IEC/2020/009)

Experimental design:

This in-vitro experiment evaluated the SBS (shear bond strength) of composite resin to dentine, before and after the treatment with disinfectants (2% Chlorhexidine digluconate (Prevest Chlor X) and 3% Sodium hypochlorite (Pyrax) and with two bonding systems (Total etch and self etch).

Teeth selection

Intact, decay, restoration and free of crack, 120 human permanent molars, free of debris, blood and calculus with sufficiently wide occlusal surface were collected for the study. All specimens were stored at 100% humidity during the experimental period.

Sample Characterization

The samples were debrided and polished with a rubber cup along with pumice stone under water at slow speed (10000 – 15000rpm) then washed well with distilled water. Then horizontal sectioning of all the samples was done at the mid coronal portion with the help of

double sided diamond cutting disk **(Denstply Maillefer, Ballaigues, Switzerland)** with a straight handpiece (**Marathon, Triodent Meditech, India**) using a slow-speed double sided cutting disc under water coolant spray to reduce the heat production during sectioning.

Mid-coronal dentin was exposed and the dentin surface was smoothened with 1000-grit silicon carbide paper (**3M Wetordry 1000 grit)** and the prepared samples were embedded in acrylic resin mold, which kept the flat dentin surface completely in horizontal position**.**

Now the samples were ready for surface treatment.

Grouping of samples:

All one hundred and twenty permanent molars were randomly divided into three groups which included one control group (n-40) and two experimental groups (n-80) such that experimental groups had 40 samples each.

Group 1(n=40) Control group (Fig 1)

Group 2(n=40) Treatment with 2% Chlorhexidine digluconate (fig 2)

Group 3(n=40) Treatment with 3% Sodium hypochlorite (fig 3)

Except control group the disinfectant in other two groups was applied with the micro-brush applicator (**Oro dental, Pune, India**) and, left undisturbed for 20 seconds, then it will be rinsed with water for 10 seconds, and dried with absorbent paper. (In total etch group the disinfectant was applied after acid etching process.)

Each main group was subdivided into two subgroups on the basis of type of adhesive systems. All the subgroups contained (n=20) samples each.

Subgroup a (n=20) Total-etch dentin bonding system

Subgroup b (n=20) Self-etch dentin bonding system

In subgroup (a) Total-etch dentin bonding system (Ivoclar Vivodent Te-Econom Bond Total Etch) Acid etching was done, with 37% phosphoric acid over the demarcated area of mounted samples for 15 sec and then rinsed with sterile water for 5 sec and excess of water was removed with absorbant paper. After that total etch adhesive was applied (**Ivoclar Vivodent Te-Econom Bond Total Etch**) and then light cured for 20 sec with LED light curing unit (**Woodpecker LED curing light**). A piece of polyvinyl tube (hollow cylinder shaped) with dimensions of (3mm×3mm) was positioned at the centre of flattened dentin surface of all the samples. With the help this tube, 2 increments of macrofill composite restorative material(IvoclarVivodentTe-Econom Plus Composite), of 1.5mm diameter was placed and light cured for 40 sec separately. A resin cylinder of 3 mm in diameter and 3 mm in depth was obtained. The polyvinyl tube was then cut and removed. (fig 4)

In subgroup (b) Self-etch dentin bonding system (Ivoclar Vivodent Tetric N Bond Universal)

The dentine surface of each sample was treated with self etch adhesive (**Ivoclar Vivodent Tetric N Bond Universal**). The adhesive agent was applied with the help of micro-brush applicator, left undisturbed for 5 seconds and then light cured for 20 sec with LED light curing unit (**Woodpecker LED curing light**). A piece of polyvinyl tube (hollow cylinder shaped) with dimensions of (3mm×3mm) was positioned at the centre of flattened dentin surface of all the samples. With the help this tube, 2 increments of macrofill composite restorative material(Ivoclar VivodentTe-Econom Plus Composite), of 1.5mm diameter was placed and light cured for 40 sec separately. A resin cylinder of 3 mm in diameter and 3 mm in depth was obtained. The polyvinyl tube was then cut and removed. (Fig 4)

After formation of composite cylinder **(Fig 4)** each subgroup was further subdivided into two subdivisions for Immediate shear bond strength (SBS) (n=10) and delayed (6 months) (n=10) SBS test.

Subdivision (I) Immediate shear bond strength (SBS) test was conducted without any waiting period, under UTM (Universal Testing Machine, **Shimadzu, Japan**) **(Fig 5).** A knife edge shearing rod with a crosshead speed of 0.5 mm/min was used. The force was applied perpendicular at the interface of enamel surface and the bonded composite cylinder. The load at failure was recorded by lab tech notebook software version 6.3. The shear bond strengths of the specimens were calculated and expressed in N.

Subdivision (D) For delayed shear bond strength (SBS), samples was stored in incubator water for 6 months The specimens were stored at room temperature and then tested for delayed SBS under Universal testing machine (**Shimadzu, Japan**).**(Fig 5)** The shear bond strength of all samples were recorded in MPa.

After that statistical analysis was performed using pair t test and Post Hoc Tucky test.

STATISTICAL ANALYSIS

The data obtained was statistically analyzed. Pair t test and Post-Hoc Tukey' tests were used for intragroup comparison for shear bond strength after different surface tretment. The data was analyzed using statistical package for social sciences (SPSS, version 10.5).

RESULT

Both the experimental groups **GpII (2% CHX) and GpIII (3% NaOCl)** showed increased immediate and delayed shear bond strength values as compared to the Control Group I (with no disinfectant treatment) regardless of the adhesive systems used.(Table 1)

There was significant difference ($p<0.01$) between the groups when tested using 'pair t test and Post-Hoc Tukey' tests.

In intergroup comparison statistically significant increase in shear bond strength was seen inch was tested immediately. This shows that both 2% CHX and 3% NaOCl acted as MMP inhibitors. But shear bond strength decreased in control group as no disinfectant was used.(Table 1)

In intergroup comparison, 2% CHX could reverse the delayed SBS values with total etch rather than self etch dentin bonding system. (Table 2) Whereas 3% NaOCl could reverse the delayed SBS values with both dentin bonding systems.(Table 3)

TABLES:-

Table1. Intragroup comparison of Immediate & Delayed mean and SD values of SBS (shear bond strength, MPa) for groups (disinfecting agents) divided according tothe type of adhesive systems (Total-etch and Self-etch) used, using paired t test.

Groups	Sub Groups	Time Interval	Mean	Std. Deviation	t test value	p value
Group I (Control group) (N=40)	**Sub Group Ia (Total etch) (N=20)**	**Immediate (N=10)**	7.69	1.08	1.4145	>0.01
		Delayed (N=10)	7.11	1.14		
	Sub Group Ib (Self etch) (N=20)	**Immediate (N=10)**	6.30	1.27	3.2282	0.01*
		Delayed (N=10)	4.81	0.93		
Group II (2%	**Sub Group IIa (Total etch)**	**Immediate (N=10)**	13.03	0.97	-8.0659	<0.01*

Chlorhexidine digluconate) (N=40)	**(N=20)**	**Delayed (N=10)**	18.82	1.97		
	Sub Group IIb (Self etch) (N=20)	**Immediate (N=10)**	10.46	0.74	6.3743	<0.01*
		Delayed (N=10)	7.62	1.47		
Group III (3%Sodium hypochlorite) (N=40)	**Sub Group IIIa (Total etch) (N=20)**	**Immediate (N=10)**	9.24	0.72	-6.2812	<0.01*
		Delayed (N=10)	14.89	2.73		
	Sub Group IIIb (Self etch) (N=20)	**Immediate (N=10)**	6.60	0.97	-7.0424	<0.01*
		Delayed (N=10)	11.43	1.76		

*Statistically significant

Table2. Pairwise intergroup comparison of mean values of immediate & delayedSBS (shear bond strength) of composite restorative material by using total etch dentin adhesive system, using Post Hoc Tukey test.

Time Interval	Groups		Mean Difference	p value
Immediate	**Group I (control gp)**	**Group II (CHX gp)**	-5.33	<0.01*
		Group III (NaOCLgp)	-1.54	0.002*
	Group II (CHX gp)	**Group I (control gp)**	5.33	<0.01*
		Group III (NaOCLgp)	3.79	<0.01*
	Group III (NaOCLgp)	**Group I (control gp)**	1.54	0.002*
		Group II (CHX gp)	-3.79	<0.01*
Delayed	**Group I (control gp)**	**Group II (CHX gp)**	-11.70	<0.01*
		Group III (NaOCLgp)	-7.78	<0.01*
	Group II (CHX gp)	**Group I (control gp)**	11.70	<0.01*
		Group III (NaOCLgp)	3.92	<0.01*
	Group III (NaOCLgp)	**Group I (control gp)**	7.78	<0.01*
		Group II (CHX gp)	-3.92	<0.01*

*Statistically significant

Table3. Pairwise intergroup comparison of mean values of immediate & delayed SBS (shear bond strength) of composite restorative material by using self-etch dentin bonding system, using Post Hoc Tukey test.

Time Interval	Groups		Mean Difference	p value
Immediate	**Group I (control gp)**	**Group II (CHX gp)**	-4.16	<0.01*
		Group III (NaOCLgp)	-0.30	0.7885
	Group II (CHX gp)	**Group I (control gp)**	4.16	<0.01*
		Group III (NaOCLgp)	3.86	<0.01*
	Group III (NaOCLgp)	**Group I (control gp)**	0.30	0.7885
		Group II (CHX gp)	-3.86	<0.01*
Delayed	**Group I (control gp)**	**Group II (CHX gp)**	-2.80	<0.01*
		Group III (NaOCLgp)	-6.61	<0.01*
	Group II (CHX gp)	**Group I (control gp)**	2.80	<0.01*
		Group III (NaOCLgp)	-3.82	<0.01*
	Group III (NaOCLgp)	**Group I (control gp)**	6.61	<0.01*
		Group II (CHX gp)	3.82	<0.01*

*Statistically significant

FIGURES

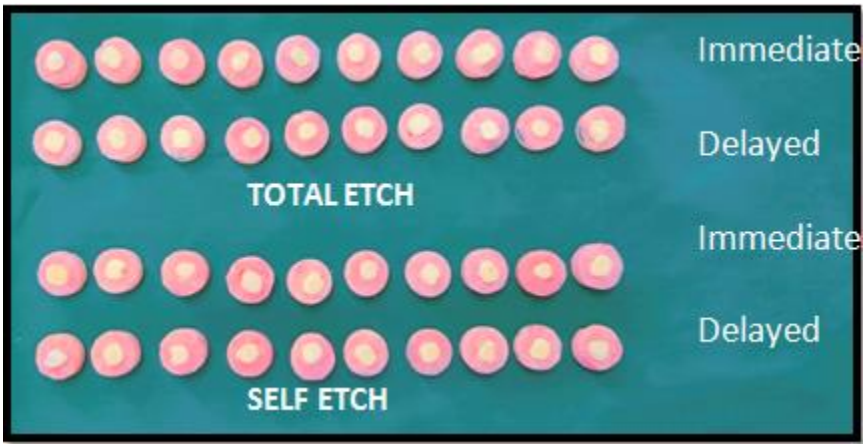

Fig 1 40 Samples prepared for control group (Group I)

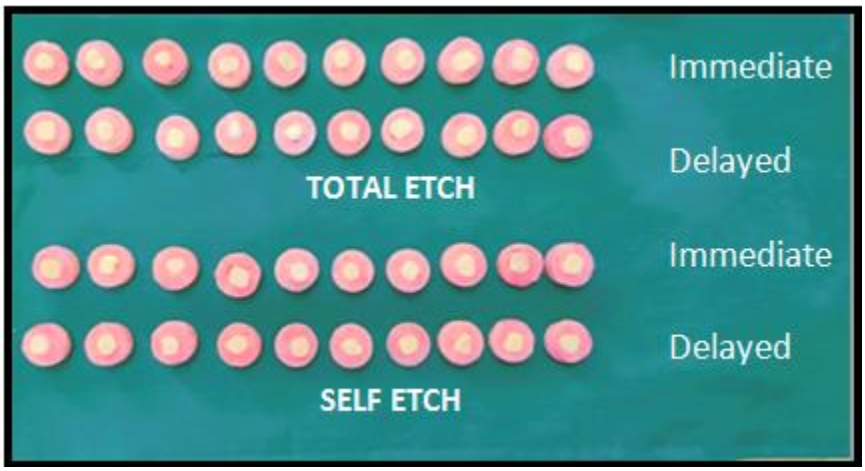

Fig:- 2- 40 Samples prepared for Chlorhexidine group (Group II)

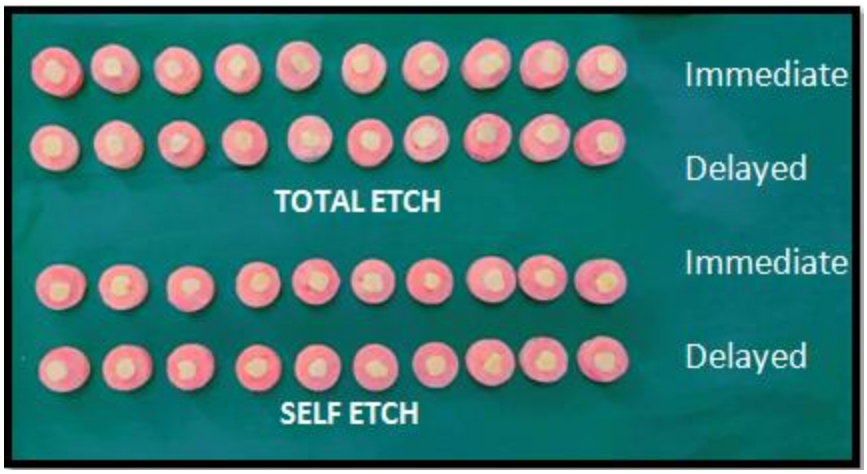

Fig:- 3 – 40 Samples prepared for Sodium hypochlorite group (Group III)

Fig 4 - Composite cylinder formation on flat dentinal surface

Fig:- 5 Universal Testing Machine procedure

DISCUSSION

There is a general thought of acceptance, that the bond formed by hydrophilic adhesive with dental hard tissue dissolved over time leading to decreased bond strength. The early loss of the bond stability or nanoleakage from the interface is a major problem that still affects stability of bonded restorations.[8]

Dentin matrix has shown to contain at least four MMPs: the stromelysin-1 (MMP-3) [15], the true collagenase (MMP-8) [14] and the gelatinases A and B, (MMP-2 and MMP-9 respectively) [9,13]. These host-derived proteases are thought to play an important role in numerous physiological and pathological processes occurring in dentin, including the degradation of collagen fibrils that are exposed by sub-optimally infiltrated dental adhesive systems after acid etching.[9]

A study by **Pashley DH et al in 2004[10]** previously suggested that high concentration of 35% H_3PO_4 (pH <1) have the ability to inactivate pro-MMPs present in the human dentin. However, in a recent work **Mutluay AT et al in 2013[11]** suggested that dentin treatment with 37% H_3PO_4 activate the MMPs trapped in the peripheral dentin instead of deactivating it.

Thus to inactivate these MMPs many potent MMP inhibitors were introduced and are evident in literature. Many new materials were also introduced in the literature like, **Davallo R at el in 2020[3]** used EDTA, gluma desensitizer and an innovative material containg 35% HEMA, 2% gutraldehyde and 2 % chlorhexidine (all dissolved in CHX**), Sinha DJ et al in 2018 [7]** introduced Aloe barbadensis miller, **Maper M et al in 2020 [12]** introduced collagen linked cross-linking agent (Quercus Extract) as a potent MMP inhibitor. Some of the authors used

LASERs (Er Cr YSGG laser), photodynamic therapy and ultraviolate- A (UVA activated Riboflavin) in addition to CHX. **[13,14]**

In this study 2 potent MMP inhibitors and cavity disinfectants, 2% Chlorhexidine digluconate and 3% Sodium hypochlorite are being used to examine its effect on bond stability.

Chlorhexidine digluconate (Prevest Chlor X) is a broad based antimicrobial which is potently effective in reducing cariogenic bacteria**.[15-16]** Many authors had stated that the use of chlorehexidine to pre-etched dentin can prevent the collagen fibril degradation. Thus apart from being commonly known disinfectants ,CHX also function as a potent MMP inhibitor.[17] It inactivate the MMP enzyme by blocking its binding to metal ions like Zn and Ca.**[18]**

Sodium hypochlorite (Pyrax) is a potent antimicrobial and cleasing agent used routinely as irrigant in endodontic treatment. In water it dissociates into Na^+ and OCl^- ions. These ions are not stable and change its state if change in pH occurs. In the pH ranges between 4-7, chlorine ion exists as HClO i.e hypo-chlorous acid while, if pH increases to 9, only OCl dominates.**[19]** The $HClO^-$ is potent antibacterial agent compared to OCl^- due to its strong affinity towards oxidative phosphorylation distruption and other membrane-related actions.**[20]**

In this study there was decrease in shear bond strength when control group was undergone for delayed shear bond strength testing. Although a lot of factors are responsible for these reduction in bond strength with time in both the cases, but MMPs are responsible for bond hydrolysis over time.[1] Some aging phenomena like water absorption, leakage of unreacted monomers, plasticization of polymer-chains and hydrolysis of the bond, are also responsible for reduction in bond strength and can harmed the hybrid layer integrity and bond strength stability.**[26]**

The CHX group showed significant increased shear bond strength with total etch dentin bonding system ($p<0.01$). Which favors the study by **Davallo R et al in 2020 [3]**, they stated that a statistically significant increased in shear bond strength can be seen when CHX

pretreated dentin is tested for shear bond strength after 6 month storage. **Gendron et al [21] in 2002** stated that "CHX showed two mechanism of action as MMP inhibitor: 1. Binding to Zn and Ca (a chelating mechanism) for inhibition of MMP-2 and -9, and for MMP-8, it may react with the sulfhydryl groups and/or cystein present in its reactive site". CHX potently prevented the hydrolysis of incompletely infiltrated areas in hybrid layer by MMPs.[22]

But in case of CHX with self etch, there was decrease in shearbond strength, with p values <0.01, means highly significant. Which was in favour with the study by **Sharma V et al in 2011** [23] which showed that CHX solution deteriorate the bond stability with self etch dentin adhesive system, because of its increased wettability and greater penetration ability which block available space for adhesive systems present in dentinal tube, whereas no bond strength deterioration was seen with the etch-and-rinse adhesive as the separated etching process remove the excess material and also made more space in dentinal tubules for adhesive system penetration.

A study by **Deng D et al (2013)[4],** also mentioned that CHX's use with a self-etching adhesive did not work to secure the bond. According to their findings, the low shear bond strength of the resultant polymer is due to the large concentration of acidic monomers in one-step resin monomers.

Shear bond strength increased significantly when NaOCl was used as cavity disinfectant with both the adhesive systems, used in this study.

The use of NaOCl leads to the formation of an acid-base resistant zone beneath the hybrid layer; this reduce the occurrence of secondary caries below the restorations hence also prevent the bond strength deterioration with time.[24]

Numerous investigations have shown that single bond etchants more effectively remove the collagen meshwork and residual NaOCl from treated dentin because they remain in the

dentinal tubules for longer. When using NaOCl-treated dentin, the SBS with single-bond adhesives is higher than with total etch systems, according to this theory.[25-26]

It is also noted in the literature: that the SBS values increase after using cavity disinfectants if evaluated immediately.[1-5,7,15,17] and after six month storage, the shear bond strength either increased or reduces to some extent but the deterioration was reduced to much extent by CHX and NaOCl which was in favour of previous study done by **Davallo R et al in 2020**.[3]

CONCLUSION

- 2% CHX had given maximum SBS values with total-etch,in both immediate and delayed shear bond strength testing. whereas for selfetch adhesive system 2% CHX gave highest SBS in immediate testing but in delayed SBS testing 3% NaOCl gave highest SBS values.
- So for long term success 2% CHX could be used with total etch and 3% NaOCl with self etch dentin bonding system.

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