ORIGINAL ARTICLE

# THREE YEARS CLINICAL ASSESSMENT OF LOW CONCENTRATION DIMETHYL SULFOXIDE PRIMER IN NON CARIOUS CERVICAL LESIONS: A RANDOMIZED CONTROLLED TRIAL

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## ABSTRACT

*Objectives*

to evaluate the clinical effectiveness of 1% dimethyl-sulfoxide (DMSO/$H_2O$) dentin pretreatment on the clinical performance of 2-step etch-rinse adhesive after 36-months of a follow-up.

*Methods*

Twenty-nine patients with 82 non carious-cervical lesions NCCLs were eligible for the study. NCCLs were randomly distributed into 2 equal groups. Both groups were acid etched then bonded with (Single-bond2, 3M-ESPE) and restored with a nanohybrid composite (Z350XT, 3M-ESPE) under rubber-dam isolation. Only for the intervention group 1% DMSO/$H_2O$ was applied for 60s then blot dried after etching and before bonding. The restorations were evaluated at baseline, 12-, 24-, and 36-months using FDI criteria for evaluation of the restoration. For the statistical analysis, intergroup comparison between interventions was performed using Chi-Square-test ($P \leq .05$), intragroup comparison within each intervention was performed using the Cochran's Q-test ($P \leq .0083$).

*Results*

In the current study, intergroup comparison between both groups at baseline and after 12, 24 and 36 months showed no statistically significant differences for all tested outcomes ($P > .05$), except for marginal discoloration at 36 months, where there was statistically significant difference ($P < .05$) favoring DSMO. Intragroup comparison within control revealed statistically significant deterioration through time ($P < .001$) for all tested parameters, also within DSMO there was statistically significant change through time ($P < .0083$).

*Conclusion*

The clinical performance of a 2-step etch-rinse adhesive in combination with 1% DMSO pretreatment was better than the control without pretreatment. Longer

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## KEYWORDS

*Dimethyl sulfoxide, DMSO primer, Adhesive dentistry, Bond pretreatment, Hybrid layer, Clinical trial*

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follow-up and higher concentrations of DMSO pretreatment should be evaluated in the future.

*Clinical Significance*
Several laboratory studies showed that DMSO pretreatment improves the hybrid layer quality and integrity. However, this clinical trial gave the proof of the clinical effectiveness of this protocol after 36-months follow-up.

## INTRODUCTION

Recent advancements in adhesive dentistry over the past decades have empowered dentists to employ minimal invasive nonretentive cavities for restoring tooth defects.[1] However, enhancing the durability of the current adhesive systems remains imperative, particularly to bolster resistance against hydrolytic degradation and host-derived enzymatic deterioration.[2] Despite the appeal of simplicity, studies indicate that bond strength achieved with 1-bottle universal adhesive systems with self-etch techniques still falls short of that attained with the etch and rinse method,[3,4] with self-etch adhesives also exhibiting more enamel marginal defects,[5] albeit with reduced technique sensitivity and lower activation of proteinase enzymes.[6,7]

Presently, there is no resin-dentin bonding methodology capable of producing adequately hybridized dentin interfaces within a clinically viable timeframe.[2,8] The longevity of hybrid layers depends entirely on the preservation of the demineralized collagen network over time, protected by a well-constructed polymer-network.[9]

In recent years, several studies have shown promising results with the use of dimethyl sulfoxide (DMSO) as a pretreatment before applying etch-and-rinse or self-etch adhesives.[10:13] This effect was explained with DMSO's potential in preventing hybrid layer degradation, enhancing dentin wettability, widening collagen interfibrillar spacing, and facilitating monomer infiltration into demineralized dentin.[12,9] This could be explained by the ability of DMSO to modify highly crosslinked collagen to split into a sparse fibril network by interfering with the ability of collagen fibrils to make hydrogen bonds with one another and the self-assembly of water molecules within dentine.[12,14] Collagen near the base of the hybrid layer is exposed by larger pores in the collagen meshwork, which also enable exposed collagen fibrils to impregnate the resin. DMSO's capacity to create hydrogen bonds with oxygen to support proteins and prevent the breakdown of collagen matrix.[10] Moreover, in vitro studies have indicated DMSO's ability to inhibit matrix metalloproteinase enzymes as well as its bacteriostatic effects.[12,15,16]

However, the beneficial effects of DMSO seems to be dose dependent. A previous study[17] showed that high concentrations of DMSO in dental adhesives, resulted in increased water sorption, solubility, and compromised mechanical properties, whereas low concentrations did not significantly affect bond properties. Furthermore, Aaqel and colleagues[18] investigated the cytotoxicity of DMSO concentrations up to 10% and found no adverse effects on biocompatibility when incorporated into hydrophobic resin. Hence, lower concentrations of DMSO were found to be safer, even as a pretreatment, to avoid any potential adverse effects if residual DMSO remains mixed with the subsequently applied adhesive.

Despite DMSO has got the FDA approval as a pharmaceutical solvent decades ago, there is a lack of clinical trials supporting long–term bonding efficacy of DMSO dentin pretreatment before using 2-step etch-and-rinse adhesives. Therefore, the objective of this randomized clinical trial was to evaluate the effect of 1% DMSO pretreatment on the clinical performance of a 2-step etch-and-rinse adhesive system placed in noncarious cervical lesions (NCCL). The null hypothesis of this study was that DMSO dentin pretreatment will not influence clinical performance of a 2-step etch and rinse adhesive system after a 36-month follow up period.

## MATERIALS AND METHODS

### Ethics Approval

The ethical requirements of this study's procedures involving human subjects were accepted and controlled by the Research Ethics Committee of Faculty of Dentistry (CREC), (Ref. 34/7/20). This randomized controlled clinical study was held in the clinics of the Faculty of Dentistry, Cairo University.

### Trial Registration

The trial protocol got the approval from the Evidence Based Dentistry Committee (EBD) of the Conservative Dentistry Department, Faculty of Dentistry, Cairo University. It was registered in (www.clinicaltrials.gov) database on 22 July 2020, with unique identification number NCT04492306.

### Trial Design and Setting

The study is a double-blind, randomized clinical trial with 2 parallel arms that is being conducted at Cairo University's Faculty of Dentistry's Conservative Dentistry Department clinic, from November 2020 to November 2023. Participant recruitment took place between September and November 2020.

Table 1. Patients' inclusion and exclusion criteria.

| Inclusion criteria | Exclusion criteria |
|---|---|
| • Patients with noncarious cervical lesions (NCCL)<br>• 16-55 years<br>• Males or Females<br>• Patients who are cooperative and agree to take part in the study<br>• Pulp isn't inflamed and vital<br>• Good occlusion and normal contact with the neighboring teeth | • Xerostomia<br>• Facets of the posterior teeth that show signs of apparent wear and bruxism<br>• Known inability to attend recall appointments<br>• Any pathology exhibiting symptoms in teeth or supporting structures<br>• Fractured or visibly cracked candidate tooth<br>• Allergies to components of resin-based restorations<br>• Current desensitizing therapy, including desensitizing dentifrices<br>• long-term usage of painkillers, narcotics, or psychoactive substances<br>• Pregnancy or breastfeeding<br>• Therapy using an orthodontic appliance during the last 3 months<br>• Abutment teeth for fixed or removable prostheses<br>• Existing periodontal disease or periodontal surgery within the previous 3 months |

## Sample Size Calculation

The sample size was calculated based on a previous study,[19] in which retention rate of NCCLs restorations using total etch adhesive system was 96.7% after 36 months. By implementing a 2 tailed Z test for difference between 2 independent proportions with an alpha level of 5% and a power of 80%. The minimum sample size needed was 33 per group in order to detect a difference of 25%. Sample size was increased by 25% to compensate for possible dropouts to reach 41 restorations per group. Sample size was performed using G*Power version 3.1.9.2 for windows.

## Selection of the Participants and the Teeth

Out of fifty examined patients, twenty-nine patients were deemed eligible to participate in the study resulting in 82 eligible NCCL. The patient flow chart, adhering to CONSORT flow diagram, is presented in Figure 1. The inclusion and exclusion criteria utilized for this examination are presented in Table 1. Simplified scoring criteria for TWI (Tooth Wear Index) Table 2[20] was used for evaluation of severity of NCCL for patient enrolled in this study. Only score 1 "Dentin just visible (including cupping) or dentin exposed for less than 1/3 of surface" was included. Score 0 "No wear into dentin" was excluded as this study investigate the effect of DMSO pretreatment on dentin not enamel. Score 2 "Dentin exposure greater than 1/3 of surface" was excluded as the size of the cavity was to be very large that may act as extra variable in the current study. Score 3 "Exposure of pulp or secondary dentin" was excluded as pulp involvement or loss of vitality was among our exclusions criteria.

Table 2. Simplified scoring criteria for TWI tooth wear index.[20,19]

| Score | criteria |
|---|---|
| 0 | No wear into dentin. |
| 1 | Dentin just visible (include cupping) or dentin exposed for less than 1/3 of surface. |
| 2 | Dentin exposure greater than 1/3 of surface. |
| 3 | Exposure of pulp or secondary dentin. |

## Recruitment

Patients were recruited from the outpatient clinic of Faculty of Dentistry, Cairo University. Careful assessment of each patient, medical and dental history were registered in their charts. Facial and dental examinations were filled in the diagnostic chart. All participants were informed about the study's purpose, methods, safety measures, advantages, and anticipated period of participation. Participants were required to sign an informed consent that was supplied by the Research Ethics Committee (REC) covering all of the trial's ethical considerations. All participants were motivated, and oral hygiene practises were encouraged. Scaling and polishing done as a preventative measure.

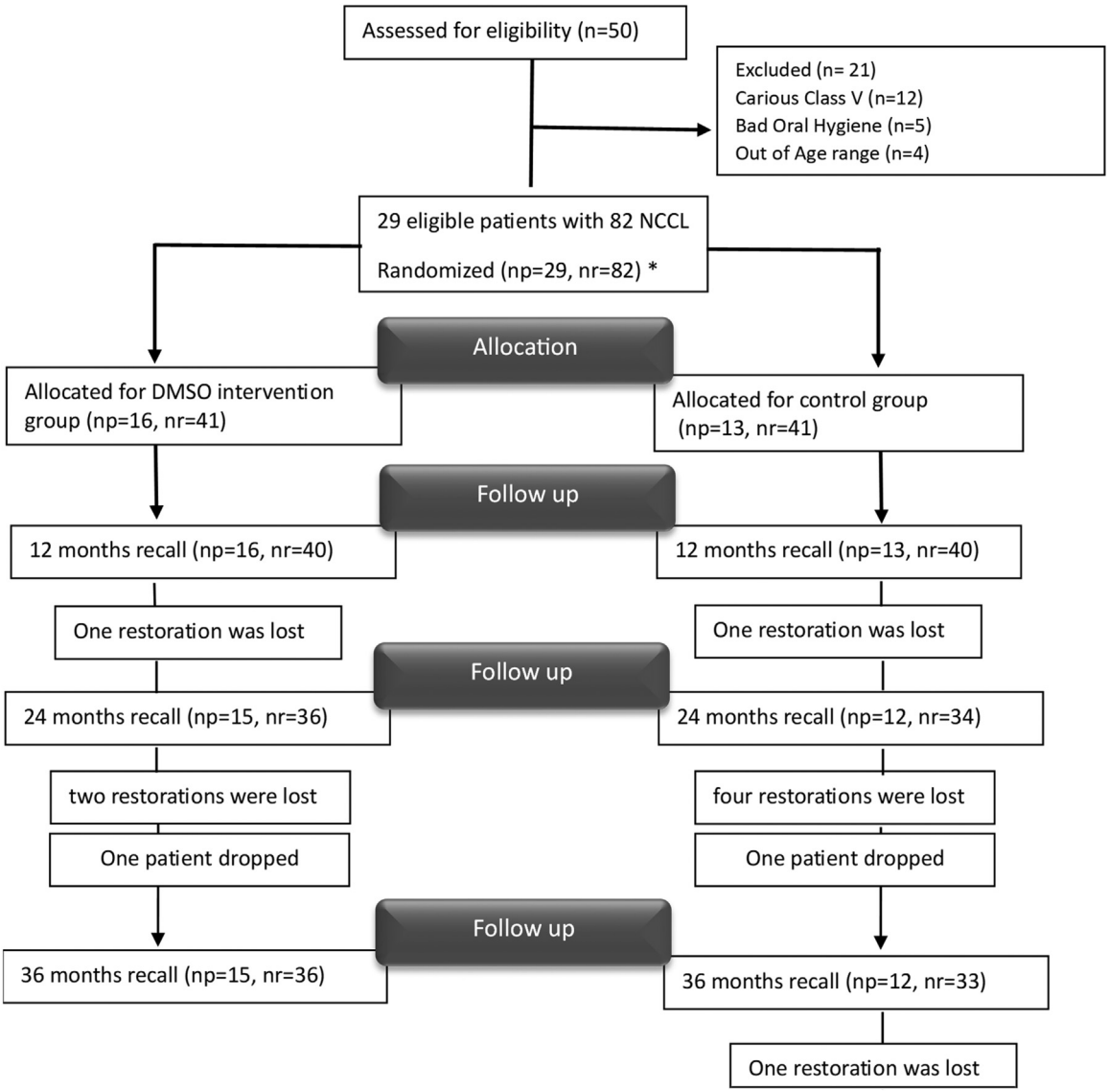
## Allocation and Sequence Generation

Using the Random Sequence Generator (https://www.random.org/), simple randomization was carried out by generating numbers starting from 1 to 82. Each randomly generated number represented the assignment of NCCL either as an intervention or a comparator with a 1:1 allocation ratio.

## Blinding

In the present clinical trial, double blinding was adopted to reduce performance and ascertainment bias as well as skewed estimations of treatment effects. Participants and both assessors were blinded to the pretreatment. The different application protocols for the adhesive system either to

Figure 1. CONSORT flow diagram of the study.
*np, number of patients.
*nr, number of restorations.




use DMSO pretreatment or not, lead to forbidding blinding of the operator, the operator was not blinded to the material assignment.

## Outcome Data Collection

Two independent examiners were responsible for the assessment of the restorations at baseline, 12 months, 24 months and 36 months with the aid of explorer, mirror and assessment chart following FDI Criteria for direct and indirect restorations Table 3. In order to establish inter-examiner reliability, the examiners underwent a rigorous assessment training program at the start of the study, undertaking repeated assessments of 20 cervical restorations utilizing FDI criteria. Any conflict in the assessment between the 2 examiners was solved by the main supervisor and she took the final decision in order to obtain only 1 score for evaluation of each restoration.

*Primary outcome:* Biological properties of the restorations of the 2 groups were examined using FDI Criteria for direct and indirect restorations.

Table 3. FDI criteria used for clinical evaluation.[40,31]

| | Esthetic property | Functional properties | | Biological properties | |
|---|---|---|---|---|---|
| | 1. Marginal staining | 2. Fracture and retention | 3. Marginal adaptation | 4. Postoperative sensitivity | 5. Caries adjacent to the restoration |
| Clinically excellent/ very good | 1.1 No marginal staining | 2.1 Restoration retained, no fractures/ Cracks | 3.1 Harmonious outline, no gaps, no discoloration | 4.1 No hypersensitivity | 5.1 No secondary or primary caries |
| Clinically good | 1.2 Minor marginal staining, easily removable by polishing | 2.2 Small hairline crack | 3.2.1 Marginal gap (50 lm)<br>3.2.2 Small marginal fracture removable by polishing | 4.2 Low hypersensitivity for a limited period of time | 5.2 Very small and localized demineralization. No operative treatment required |
| Clinically sufficient/ satisfactory | 1.3 Moderate marginal staining, not esthetically unacceptable | 2.3 Two or more or larger hairline cracks and/or chipping (not affecting the marginal integrity) | 3.3.1 Gap .150 lm not removable<br>3.3.2 Several small enamel or dentin fractures | 4.3.1 Premature/ slightly more intense<br>4.3.2 Delayed/ Weak sensitivity; no subjective complaints, no treatment needed | 5.3 Larger areas of demineralization, but only preventive measures necessary (dentin not exposed) |
| Clinically unsatisfactory | 1.4 Pronounced marginal staining; major intervention necessary for improvement | 2.4 Chipping fractures that damage marginal quality; bulk fractures with or without partial loss (less than half of the restoration | 3.4.1 Gap .250 lm or dentin/base exposed<br>3.4.2 Chip fracture damaging margins<br>3.4.3 Notable enamel or dentin wall fracture | 4.4.1 Premature/ very intense<br>4.4.2 Extremely delayed/weak with subjective complaints<br>4.4.3 Negative sensitivity; intervention necessary but not replacement | 5.4 Caries with cavitation (localized and accessible and can be repaired) |
| Clinically poor | 1.5 Deep marginal staining not accessible for intervention | 2.5 Partial or complete loss of restoration | 3.5 Filling is loose but in situ | 4.5 Very intense, acute pulpitis or nonvital Endodontic treatment is necessary, and restoration has to be replaced | 5.5 Deep secondary caries or exposed dentin that is not accessible for repair of restoration |

*Secondary outcomes:* Functional properties of the restorations of the 2 groups were examined using FDI Criteria for direct and indirect restorations.

*Tertiary outcome:* Esthetic properties of the restorations of the 2 groups were examined using FDI Criteria for direct and indirect restorations.

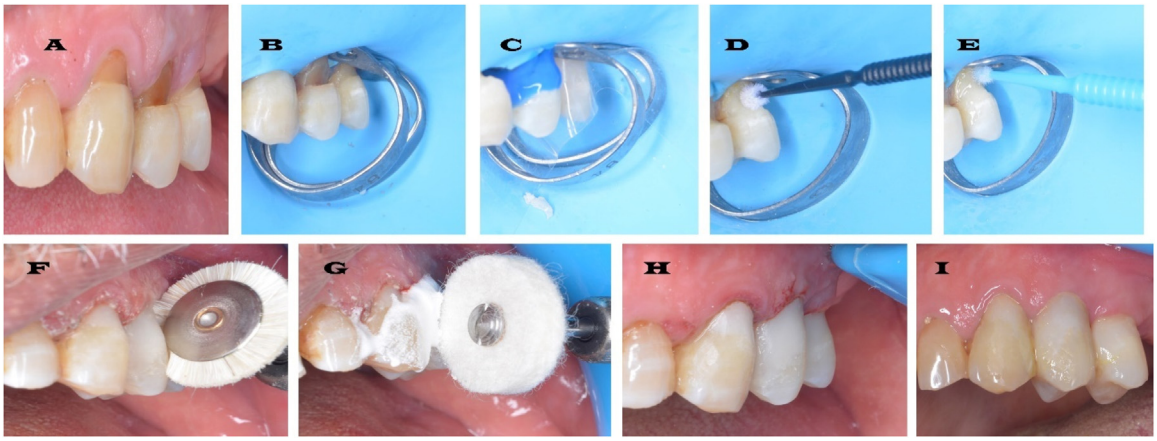
## Patient Retention

The patient's phone number recorded in the medical chart. The patient received a phone call to remind him/her of the time of his/her appointment. A follow-up appointment was set for the following week if the patient did not respond for whatever reason.

## Data Management

The operator entered the data, and the co-supervisor reviewed it. Using a password, all computer data was encrypted. This was done to facilitate accurate data entry, allow for extensive revision, and guard against improper use of data. Data also backed up on a google drive to avoid losing it. Main operator informed the participants about any possible harm such as recurrent caries, postoperative hypersensitivity, fracture of the restoration or the tooth and complete loss of the restoration. If present, participants were in-

Figure 2. Showed restorative procedures of 23 and 24, (A) NCCL (Non carious cervical lesions), (B) Cavities isolation by rubber dam, (C) Cavities etching with 37% phosphoric acid (selective etching), (D) Application of 1% DMSO/H2O, (E) Application of Single Bond2, (F and G) Finishing and polishing, (H) Immediate restorations, (I) Restorations follow up at baseline (after 1 week).




structed to contact the operator immediately by a phone call.

## Data Monitoring

The main supervisor monitored this study and monitored any risk of bias from participants, operator or assessors, monitored the blinding of the assessors, patient safety.

## Restorative Procedure

The labial surfaces of the selected teeth were cleaned with polishing paste and brush. Local anesthesia was administered (Mepecaine–L Cartridges, Alexandria Co. for pharmaceuticals and chemical industries) as required. Rubber dam isolation (Dental Dam, Henry Schein, Germany) and subgingival clamps (KSK, DENTECH Corporation, Japan) were used to isolate the teeth. Surface roughening was carried out using a diamond point BR-31 (Mani Japan) Figure 2.

The details of the materials, compositions, and manufacturer information are listed at Table 4. Both enamel and dentin received etching treatments for 30- and 10-seconds respectively. Following etching, the cavities were rinsed with water for 15 seconds and blot dried leaving a slightly moist dentin surface. In the intervention group, etched dentin was actively treated with 1% DMSO/$H_2O$ solutions for 60 seconds, followed by blot drying. Two different color brushes were used to avoid unnecessarily mixing of the bond with the pretreatment Figure 2. Subsequently, 2 consecutive coats of the adhesive were applied for 15 seconds (Adper™ Single Bond 2, 3M ESPE, St Paul, MN, USA) followed by gentle air blowing for 5 seconds. The adhesive was light cured. Composite build-ups (Filtek Z350 XT, 3M ESPE, St Paul, MN, USA) were completed incrementally and each was individually light-cured for 20 seconds. Light curing of all resin materials in both groups was performed using a LED device (Bluephase 20i, Ivoclare Vivadent, Schaan, Liechtenstein) delivering 1100 mW/cm2 Figure. The power of light cure was regularly checked with Radiometer (LM-1, DTE) Figure 2(A:E).

## Finishing and Polishing

Finishing of the restorations was completed using a fine tapered diamond from Mani, Japan to give it the desired smooth texture, followed by polishing the surface with felt wheel and goat hair from ENA, Italy respectively Figure 2(F and G).

## Interventions-Adherence

Participants were encouraged to adhere to oral hygiene instructions both before and after intervention. Any additional complaint of patient was addressed promptly to ensure patient satisfaction. Moreover, regular phone calls were conducted with patient as effective means of maintaining a positive operator-patient relationship and to ensure patients engagement and cooperation throughout the study.

## Clinical Evaluation Using FDI Criteria for Evaluation of Restorations

Clinical evaluation using FDI Criteria for restoration assessment was conducted by 2 blinded assessors at baseline, and subsequently at 12 months, 24 months, and 36 months post-intervention. Each restoration, was evaluated using an assessment chart completed by the evaluators. In case of discrepancies in scores, resolution was achieved by the main

Table 4. Materials' composition and manufacturer.

| Materials | Composition | Lot number | Manufacturer |
|---|---|---|---|
| Scotchbond Universal etchant (3M) | 37% phosphoric acid, fumared silica (pH 0.6) | N7523 | 3M ESPE, St Paul, MN, USA |
| 3M Adper Single Bond2 | Ethyl alcohol; bis-GMA; silane-treated silica; HEMA; water (<10%); copolymer of acrylic and itaconic acid; UDMA | NA82584 | 3M ESPE, USA |
| Filtek Z350 XT | *The resin* contains bis-GMA, UDMA, TEGDMA, PEGDMA and bis-EMA resins. *The fillers* are a combination of nonagglomerated/nonaggregated 20 nm silica filler, nonagglomerated/nonaggregated 4 to 11 nm zirconia filler, and aggregated zirconia/silica cluster filler The inorganic filler loading is about 72.5% by weight (55.6% by volume) | NA81717 | 3M ESPE, USA |
| 1% DMSO/H2O (Dimethyl sulfoxide) | DMSO [$(CH_3)_2SO$] was diluted to 1% DMSO/H2O by volume using pipette. | RNBJ9944 | Sigma Aldrich, USA |

*Abbreviations:* bis-EMA, bisphenol A ethoxylated dimethacrylate; bis-GMA, bis-phenol diglycidylmethacrylate; HEMA, 2-hydroxyethyl methacrylate; PEGDMA, polyethylene glycol dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, diurethane dimethacrylate.

supervisor. All evaluations were performed under a dental operating light, utilizing flat-surfaced mouth mirrors and pointed dental explorers within a suitable, isolated field, in accordance with provided instructions.

## Statistical Methods

Data was analyzed using Medcalc software, version 22 for windows (MedCalc Software Ltd, Ostend, Belgium). Categorical data was described as frequency and percentage, intergroup comparisons between interventions was performed using Chi-Square test with statistical significance level set at ($P \leq .05$), intragroup comparison within each intervention was performed using the Cochran's Q test with statistical significance level set at ($P \leq .0083$) after Bonferroni correction followed by multiple comparisons. Intention to treat analysis was used, all lost restorations scoring 5 in fracture and retention was categorized as score 5 in other clinical parameters. Relative risk was used to assess the clinical significance. Survival rate was analyzed using Kaplan-meier and Log-rank test. Logistic regression was used to assess the effect of patients on success rate at each follow-up. The confidence limit was set at 95% with 80% power and all tests were 2 tailed.

# RESULTS

This clinical trial was conducted to evaluate the clinical performance of 2-step etch-and-rinse adhesive after DMSO dentin pretreatment in different time intervals (baseline, 12 months, 24 months and 36 months). Data was collected for each patient including survival of tooth restoration, primary outcome (Biological properties), secondary outcome (Functional properties) and tertiary outcome (Esthetic properties). Then, overall primary, secondary and tertiary outcome result scores were recorded.

## Demographic Data

Twenty-nine patients with 82 non carious cervical lesions who matched the eligibility criteria were assigned to the intervention and control groups ($n = 41$ restorations). After 36 months 2 patients with 4 restorations were lost due to follow-up with 95% retention rate. Mean age of the participants in the current trial was 30 ± 7.6 years; mean age within DSMO group was 29.34 ± 7.5 years, while within the control group mean age was 30.65 ± 7.77 years, there was no statistically significant difference between both groups regarding age ($P = .438$). Gender distribution is shown in Table 5, there was no statistically significant difference between both groups regarding gender ($P = .5415$). Distribution of teeth is shown in Table 5, there was no statistically significant difference between both groups regarding teeth distribution ($P = .9195$).

## Fracture of Material and Retention

At baseline, both groups had excellent ratings for fracture and retention. At 12 months recall, both groups had a majority of teeth rated as excellent, with 1 tooth in each group rated as poor. At 24- and 36-months recall, both groups had a majority of teeth rated as excellent, with a higher percentage of teeth rated as poor in control group (5 and 6 teeth) respectively compared to intervention group where 3 teeth scored poor. In addition, at 24- and 36-months recall, 1 tooth in each group was rated as good Table 6 .

Table 5. Demographic data (gender and teeth distribution).

| Group | Gender | | Row total (RT) |
|---|---|---|---|
| | Male | Female | |
| Control | 8<br>50% CT<br>61.5% RT | 5<br>38.5% CT<br>38.5% RT | 13 (44.8%) |
| DSMO | 8<br>50% CT<br>50% RT | 8<br>61.5% CT<br>50% RT | 16 (55.2%) |
| Column total (CT) | 16<br>(55.2%) | 13<br>(44.8%) | 29 |
| *P*-value | | $P = .5415$ | |

| Group | Teeth | | Row total (RT) |
|---|---|---|---|
| | Control | DSMO | |
| Maxillary anterior teeth | 18<br>43.9% CT<br>47.7% RT | 20<br>48.7% CT<br>52.6% RT | 38<br>(46.3%) |
| Maxillary premolars | 8<br>19.5% CT<br>47.1% RT | 9<br>22% CT<br>52.9% RT | 17<br>(20.7%) |
| Mandibular anterior teeth | 5<br>12.2% CT<br>55.6% RT | 4<br>9.8% CT<br>44.4% RT | 9<br>(11%) |
| Mandibular premolars | 10<br>24.4% CT<br>55.6% RT | 8<br>19.5% CT<br>44.4% RT | 18<br>(22%) |
| Column total (CT) | 41<br>(50.0%) | 41<br>(50.0%) | 82 |
| *P*-value | | $P = .9195$ | |

Overall, it appeared that both intervention and control groups had good to excellent ratings for fracture and retention at all time points, the *P*-value for the median FDI score between both intervention and control groups is 0.756 at 24 months recall, the *P* value for both intervention and control groups was .567 at 36 months recall, which indicated that there was no significant difference between the 2 groups at this time point. $P < .05$ is statistically significant.

## Caries Adjacent to the Restoration

At baseline and after 12 months, the median FDI score for both intervention group and control group were 1, with a range of 1-1. The *P*-value for the median FDI score in both groups was 1.000 at each time point, which means that there was no significant difference between the 2 groups at any of the time points Table 6.

## Postoperative Hypersensitivity

Both groups had a majority of teeth rated as excellent for postoperative hypersensitivity, and there was no significant difference between the 2 groups Table 6.

## Marginal Adaptation

At baseline, both groups had excellent ratings for marginal adaptation. At 12 months recall, both groups had a majority of teeth rated as excellent, with a small percentage of teeth in each group rated as good. DMSO intervention group 36 months results showed there are 34 restorations scored "excellent" and 2 restorations scored "Satisfactory". While in

Table 6. Overall primary, secondary and tertiary outcome result scores at Baseline and after 12, 24, 36 months.

| Outcome | Follow-up | Control | | | | | | DSMO | | | | | | *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No | Success | | | Failure | | No | Success | | | Failure | | |
| | | | 1 | 2 | 3 | 4 | 5 | | 1 | 2 | 3 | 4 | 5 | |
| Fracture and retention | Baseline | 41 | 41 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 41 | 41 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 1.0000 |
| | 12 mo | 41 | 40 (97.6%) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (2.4%) | 41 | 40 (97.6%) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (2.4%) | 1.0000 |
| | 24 mo | 39 | 33 (84.6%) | 1 (2.6%) | 0 (0%) | 0 (0%) | 5 (12.8%) | 39 | 35 (89.7%) | 1 (2.6%) | 0 (0%) | 0 (0%) | 3 (7.7%) | .7562 |
| | 36 mo | 39 | 32 (82%) | 1 (2.6%) | 0 (0%) | 0 (0%) | 6 (15.4%) | 39 | 35 (89.7%) | 1 (2.6%) | 0 (0%) | 0 (0%) | 3 (7.7%) | .5671 |
| | *P*-value | $P < .001$[a] | | | | | | $P = .004$[a] | | | | | | |
| Marginal adaptation | Baseline | 41 | 41 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 41 | 41 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 1.0000 |
| | 12 mo | 40 | 38 (95%) | 2 (5%) | 0 (0%) | 0 (0%) | 0 (0%) | 40 | 37 (92.5%) | 3 (7.5%) | 0 (0%) | 0 (0%) | 0 (0%) | .6462 |
| | 24 mo | 34 | 31 (91.2%) | 1 (2.9%) | 2 (5.9%) | 0 (0%) | 0 (0%) | 36 | 34 (94.4%) | 0 (0%) | 2 (5.6%) | 0 (0%) | 0 (0%) | .5821 |
| | 36 mo | 33 | 30 (91%) | 1 (3%) | 2 (6%) | 0 (0%) | 0 (0%) | 36 | 34 (94.4%) | 0 (0%) | 2 (5.6%) | 0 (0%) | 0 (0%) | .5707 |
| | *P*-value | $P < .001$[a] | | | | | | $P = .004$[a] | | | | | | |
| Marginal staining | Baseline | 41 | 41 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 41 | 41 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 1.0000 |
| | 12 mo | 40 | 38 (95%) | 2 (5%) | 0 (0%) | 0 (0%) | 0 (0%) | 40 | 40 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | .1547 |
| | 24 mo | 34 | 28 (82.4%) | 0 (0%) | 5 (14.7%) | 1 (2.9%) | 0 (0%) | 36 | 34 (94.4%) | 2 (5.6%) | 0 (0%) | 0 (0%) | 0 (0%) | .0301* |
| | 36 mo | 33 | 22 (66.7%) | 0 (0%) | 4 (12.1%) | 6 (18.2%) | 1 (3%) | 36 | 31 (86.2%) | 2 (5.5%) | 2 (5.5%) | 1 (2.8%) | 0 (0%) | .0126* |
| | *P*-value | $P < .001$[a] | | | | | | $P = .003$[a] | | | | | | |

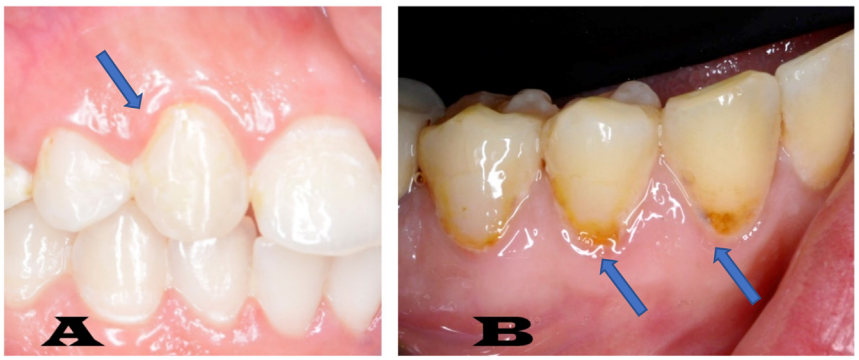
(*continued on next page*)

Table 6 (*continued*)

| Outcome | Follow-up | Control | | | | | | DSMO | | | | | | *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No | Success | | | Failure | | No | Success | | | Failure | | |
| | | | 1 | 2 | 3 | 4 | 5 | | 1 | 2 | 3 | 4 | 5 | |
| Postoperative hypersensitivity | Baseline | 41 | 39 (95.1%) | 2 (4.9%) | 0 (0%) | 0 (0%) | 0 (0%) | 41 | 38 (92.7%) | 3 (7.3%) | 0 (0%) | 0 (0%) | 0 (0%) | .6465 |
| | 12 mo | 40 | 39 (97.5%) | 0 (0%) | 1 (2.5%) | 0 (0%) | 0 (0%) | 40 | 40 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | .3173 |
| | 24 mo | 34 | 34 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 36 | 36 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | .8111 |
| | 36 mo | 33 | 33 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 36 | 36 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | .7180 |
| | *P*-value | $P < .001$[a] | | | | | | $P = .004$[a] | | | | | | |
| Caries adjacent to the restoration | Baseline | 41 | 41 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 41 | 41 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 1.0000 |
| | 12 mo | 40 | 40 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 40 | 40 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 1.0000 |
| | 24 mo | 34 | 34 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 36 | 36 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | .8111 |
| | 36 mo | 33 | 33 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 36 | 36 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | .7180 |
| | *P*-value | $P < .001$[a] | | | | | | $P = .004$[a] | | | | | | |

Frequencies and percentages not sharing the same letter throughout follow-up are considered statistically significant.
[a] Denotes statistically significant.

Figure 3. (. A) Showed a representative case of upper canine after 36 months follow-up recorded (excellent) at all FDI criteria. B: A representative case of restoration recorded (unsatisfactory) at marginal staining FDI criteria after 36 months.




the control group 36 months results showed there are 30 restorations scored "excellent", 1 restoration scored "good" and 2 restorations scored "Unsatisfactory". The difference between 2 groups is not significant ($P = .57$). Overall, there was no significant difference in the marginal adaptation between intervention and control groups at any of the time points measured Table 6.

## Marginal Staining

At baseline, both intervention and control groups had a median staining score of 1 with a range of 1-1, indicating no significant difference between the 2 groups at baseline. At 12 months recall, the median staining scores for both intervention and control groups were 1-2 and 1, respectively. Although the median score for control group was lower, the *P*-value of .155 indicated that the difference between the 2 groups was not statistically significant. At 24 months recall, after treatment, the median staining scores for intervention and control groups were comparable, ranging from 1 to 4. The *P*-values of .0301* suggested that there was statistically significant difference in staining between the 2 groups. DMSO intervention group 3 years results showed that there are 31 restorations scored "excellent", 2 restorations scored "good", 2 restorations scored "satisfactory" and 1 restoration scored "unsatisfactory". While in the control group 36 months results showed that there are 22 restorations scored "excellent", 4 restorations scored "satisfactory", 6 restorations scored "unsatisfactory" and 1 restoration scored "poor" Figure 3, and the *P*-value of .0126* suggested that there is a significant difference between the 2 groups. $P < .05$ is statistically significant. Overall, there was no significant difference in staining between both groups at all of the time points measured except after 24- and 36-months Table 6.

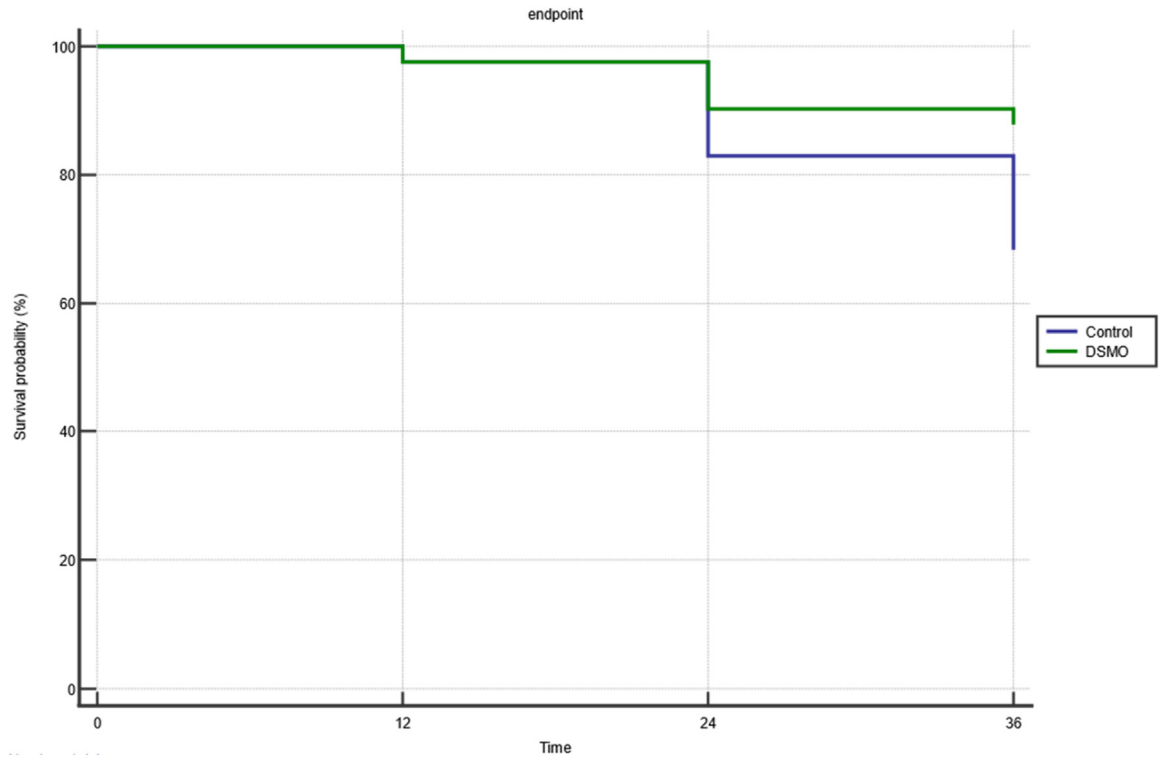
## Overall Primary, Secondary and Tertiary Outcome Results Scores

In the current study, intergroup comparison between both groups at baseline and after 12, 24, and 36 months showed no statistically significant differences for all tested outcomes ($P > .05$), except for marginal discoloration at 36 months, where there was statistically significant difference ($P = .0131$) favoring DSMO. Intragroup comparison within control revealed statistically significant deterioration through time ($P < .001$) for all tested parameters, also within DMSO there was statistically significant change through time ($P < .0083$) Table 6.

Logistic regression revealed statistically significant effect of only 2 patients (1 in each group) on success rate at 12 months ($P = .0123$), showing odds ratio of 0.013 (95% CI 0.0004-0.3900), as these 2 patients had 1 successful and 1 failing restoration after 12 months. After 24 and 36 months, logistic regression revealed no effect of patients on success rate ($P > .05$).

Overall survival of composite restorations was assessed after 36 months, 13 restorations in control group and 5 restorations in DSMO group failed after 36 months due to scoring 4 or 5 using FDI criteria. Kaplan-Meier analysis was used to obtain survival curves, comparison of survival curves was performed using Logrank test, there was statistically significant difference between both adhesives ($P = .0408$). Figure 4 The success rate of control was 68.29% after 36 months, while in the DSMO group the success rate was 87.8%. There was 61.5% less risk of failure when using DMSO when compared to the control for restoration of NCCLs with relative risk (RR) of 0.38 (95% CI 0.1508-0.9809 $P = 0.0454$).

Figure 4. Survival analysis of both adhesives for NCCLs restorations after 36 months.




## DISCUSSION

The available data concerning the use of dimethyl sulfoxide (DMSO) as pretreatment to dental adhesives are apparently limited and inconclusive in literature. All the data are in vitro studies, there isn't enough evidence to support the clinical performance of DMSO application in adhesive dentistry. The aim of this study is to evaluate the clinical effectiveness of 1% DMSO dentin pretreatment on the clinical performance of etch-and-rinse adhesive.

The etch-and-rinse strategy with phosphoric acid remains the gold standard for enamel. In comparison to etch-and-rinse systems, self-etching adhesives typically fail to generate a suitable and uniform hybrid layer, which results in lower bonding strengths.[21] On the other hand, phosphoric acid etches the dentin deeper than resin can infiltrate, leaving a gap of demineralized, unsupported collagen that significantly weakens the overlying hybrid layer. That is why self-etching adhesives have become the gold standard for dentin adhesion, despite yielding lower bond strength results.[2] DMSO pretreatment has emerged as a technique to minimize the sensitivity of etch-and-rinse technique to dentin and maintain the strength and resistance to degradation of the hybrid layer for a much longer time.[22] (Single bond2, 3M) was selected as a 2-step etch-and-rinse adhesive based on the availability of the laboratory studies supported the improvement of its performance after DMSO pretreatment[11,23,14] and the simplicity of the procedure comparing to 3-step etch-and-rinse adhesives.

DMSO was applied for 60 seconds based on the effectiveness of this time, rather than 20 seconds, in a previous study.[24] Tjäderhane et al.[25] found that 1% DMSO pretreatment improved the bond strength of both total etch and universal adhesives after aging for 30 months. The same concentration was used in this study.

Randomized control trials (RCTs) are considered the gold standard method for clinical assessment, owing to several key features that render them highly robust and reliable.[26] This robustness is achieved through the use of randomization, which in this study was facilitated by the website www.random.org, overseen by an individual not involved in the research. Another crucial aspect is allocation concealment, ensuring the integrity of the randomization process by employing sealed, opaque envelopes prepared by an independent party. Despite their numerous strengths, RCTs also come with certain limitations that must be acknowledged. Among the most significant is the substantial investment of

resources and time required. RCTs typically demand significant financial investment, meticulous planning, and the involvement of a substantial number of participants and study sites.[26]

This study focused on noncarious cervical lesions (NCCL) restorations due to their absence of macro retentive features, which makes them more susceptible to debonding compared to Class I occlusal lesions. Furthermore, NCCL restorations involve both enamel and dentin at margins, enabling assessment of adhesion to both substrates.[27] Although NCCL lesions display various cavity configurations that may induce interfacial stress, the mechanical properties of the composite used typically play a minor role.[28]

Parallel design and split-mouth design are 2 common approaches used in clinical trials to compare the effectiveness of interventions. Parallel design involves independent allocation of participants to different treatment groups, with each participant receiving only 1 of the interventions being compared. On the other hand, split-mouth design allocates different interventions to different sides (e.g., left and right) or different quadrants of the mouth of each participant. Split-mouth design is commonly used in dentistry.[29] Parallel design offers several advantages over split-mouth design in clinical trials.[30] It eliminates carryover effects, allows for a larger sample size and better statistical power, enhances blinding and reduces observer bias, offers greater flexibility and ensure the external validity in design and analysis, and simplifies the study protocol. That's why we chose parallel arm 1:1 design in this study.

One week was chosen as (the baseline), followed by 1, 2- and 3-years follow-up intervals. Hickel[31] recommended that baseline examination be performed roughly a week (or at the latest, a month) after the implantation of the restorations. Baseline testing 1 week later allowed for ample time for polymerization shrinkage strains to relax and for any sensitivity brought on by restorative operations like cavity preparation and rubber dam application to be resolved.

According to the results obtained from the current study, after 3 years all restorations were evaluated except 4 dropouts, 2 from each group. Regarding the FDI criteria results, 5 items were analyzed; Caries adjacent to the restoration, Postoperative hypersensitivity, fracture and failure of material retention, marginal adaptation and marginal staining. The retention rate recorded for the study participants was 95.1%. Out of the 41 restorations assessed in both groups. There was no significant difference between the 2 groups after 3 years for all items except for the marginal staining, where more discoloration was observed in the control group than the intervention group ($P = .0126$*) which leads to the partial rejection of the null hypothesis.

The significant difference in marginal staining after 36 months can be attributed to 1% DMSO/$H_2O$ preventing the degradation of the hybrid layer, consistent with laboratory results.[11,25] These findings contrast with other reports,[32] where other pretreatments failed to reduce marginal staining, such as chlorohexidine in the first study after 36 months[33] or proanthocyanidins in the second study after 24 months.[32]

Marginal staining criteria play a crucial role in evaluating the quality and longevity of dental restorations.[31] The International Dental Federation (FDI) recognizes the importance of this criterion in assessing the success of dental restorations. Marginal staining refers to the presence of visible color changes at the interface between the restoration and the natural tooth structure.[34] Such staining often indicates the presence of micro gaps at the restoration-tooth interface, providing pathways for microleakage, bacterial accumulation and secondary caries development. Consequently, marginal staining can compromise the integrity of the restoration, increasing the risk of failure and the need for premature replacement or repair.

The enhancement of adhesive penetration and increased wettability of dentin surfaces attributed to DMSO pretreatment may be a result of its ability to disrupt collagen fibrils and water molecules within dentin. DMSO causes highly crosslinked collagen to separate into a sparser fibril network, leading to greater gaps in the collagen meshwork.[25] This could potentially result in less exposed collagen at the base of the hybrid layer and a more even encapsulation of exposed collagen fibrils in the adhesive resin.[25] Additionally, DMSO has the capacity to form hydrogen bonds with oxygen to backbone amide proteins, preventing the collagen matrix from collapsing.[10] One of the challenges in achieving optimal dental adhesion is controlling the moisture present on the tooth surface. Excessive moisture can impair the bonding process and compromise the longevity of the restoration.[1] DMSO has hygroscopic properties, meaning it can absorb and control moisture. By applying DMSO before the adhesive, it can help to displace and control moisture, creating a drier surface that promotes better adhesion.[22] DMSO features a high dielectric constant, low surface tension, and a great balance between polar and surface tension characteristics, making it an ideal wetting agent, particularly for porous surfaces.[35] The tooth surface often contains a thin layer of debris called the smear layer, which is created during dental procedures such as drilling or scaling. The smear layer can interfere with adhesion by acting as a barrier between the tooth structure and adhesive materials. DMSO has been found to have the ability to partially dissolve or degrade the smear layer, allowing better penetration of the adhesive into the tooth surface and enhancing the bond strength.[36]

Previous studies[15,16,37] have shown that DMSO pretreatment can suppress metalloproteinase activities within the hybrid

layer and exhibit anti-bacterial properties by obstructing the binding sites that allow MMPs to interact with dentin collagen. Unlike other polar solvents, DMSO can dissolve typical proteins quickly and interact dynamically with protein peptides. This provides reliable support to the collagen matrix for enzyme stripping, reducing the breakdown of collagen fibrils.[38]

Regarding the marginal adaptation of the restorations, there was no significant difference between the 2 groups at all follow ups. This difference in significance between marginal adaptation compared to staining may be because marginal staining reflects micro gaps, while marginal adaptation reflects macro gaps that require more time and further degradation of the bonding interface to form.[31] It is widely recognized that marginal staining criteria are more sensitive than marginal adaptation criteria in evaluating dental restorations. Several reasons contribute to this observation. Marginal staining can occur even without clinically significant defects in the restoration, due to factors like stains from oral fluids or cement remnants.[34] These factors may not necessarily compromise the function or longevity of the restoration but can still be detected by visual inspection. In contrast, marginal adaptation criteria are more focused on the fit and contour of the restoration. They assess the presence of gaps or discrepancies at the interface between the restoration and the tooth. While the presence of such gaps can indicate inadequate adaptation, it is important to note that slight discrepancies may not always impact the clinical performance or longevity of the restoration. This finding is in agreement with a previous study[39] that found that no significant different regarding marginal adaptation criteria after 2 years. However, this findings is in disagreement with another study[33] that found that marginal adaptation of intervention group CHX showed less scores than the control group 3M Adper Single Bond2 after aging.

Regarding the fracture and failure of material retention, the DMSO group achieved a retention rate of 92.3% after 3 years, while the control group using SB achieved an 84.6% retention rate after 3 years. The ADA requires a 90% retention rate after 2 years for the acceptance of the adhesive protocol. Despite higher retention rate in the DMSO group, there was no significant difference between 2 groups ($P = .567$). This lack of significance may be due to the need for longer follow-up to evaluate these criteria. Regarding caries adjacent to the restoration and postoperative sensitivity, both criteria scored "Excellent" at all recall follow ups for both groups. The good clinical performance of the control group regarding these criteria was somewhat expected, based upon the results from earlier clinical trials that tested Adper Single Bond2 3M and yielded similar scores.[33,21,39]

Furthermore, it is worth mentioning that the success rate of any dental intervention depends on several factors beyond the adhesive or intervention itself. Factors such as patient compliance, oral hygiene habits, and the skill of the clinician can impact the long-term outcomes. Therefore, it is crucial to consider these contributing factors when interpreting the success scores obtained in this study.

## CONCLUSIONS

Dimethyl Sulfoxide dentin pretreatment showed a comparable clinical performance to the control Adper Single bond2 in in restoring noncarious cervical lesions after 36 months follow up intervals. 1% DMSO/H2O pretreatment had delayed the marginal staining in NCCL after 24 and 36 months follow up.

## RECOMMENDATIONS

To endorse the current findings, extended follow-up clinical investigations are advised. Different concentrations of DMSO that pass the cytotoxicity tests should be considered in the future clinical trials. Adding extra bonding steps will not please dentists so future investigations could consider DMSO incorporation into the adhesive.

## ETHICAL POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT

Faculty of Oral and Dental Medicine, Cairo University, Ethical committee approved and gave the code: CU, REC,34,7,20.

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