

patient's chair. In paediatric dentistry, these are desired benefits [6].

Presence of microorganism under the fissure sealant cause caries, because of that application of fissure sealant on disinfected area is so important. Some disinfection method was showed; one of them is ozone application. Lately, ozone has been employed for both remineralization of initial pit and fissure caries (non-invasive treatment) and surface pre-treatment prior to fissure sealants [7].

Some studies suggest that adjunct use of ozone is a useful prophylactic anti-microbial treatment before the etching and application of fissure sealants, that doesn't interact negatively with the physical properties of both: enamel and adhesive fillings [8]. The employment of ozone on teeth surfaces is a novel preventive and therapeutic process that is still being estimated in many dental parts involving: cardiology, oral surgery, endodontics, and periodontology [9].

The use of ozone has been shown to help tooth structures remineralization [10]. In addition to eliminating abiogenic bacteria by oxidizing biomolecules found in dental disease (e.g., cysteine, methionine, etc.) [11]. Ozone infusion into non-cariou dentin can help prevent the production of biofilms from *Streptococcus* mutants and *Lactobacillus acidophilus* for long periods of time [12]. Finally, there are human studies [13,14] clearly show that ozone has the potential to be effective in the treatment of minimum cavitated or incipient lesions in pits and fissures while causing the least amount of anxiety when compared to established methods. These promising scientific and clinical results point to the possibility of using ozone to disinfect pits and fissures before applying fissure sealants. It is well believed that ozone's powerful antibacterial action is due to its oxidant potential [15].

MATERIALS AND METHODS

This *in vitro* study was conducted with the approval of the Ethics Committee of the University of Baghdad 2021.

Teeth Preparation

The study comprised eighty human premolar teeth, with the number of teeth sampled determined by the G-power program. Soft tissue residues were removed from chosen teeth, and the pits and fissures were washed using a water-cooled hand piece and brush. The teeth were next inspected with the naked eye and under excellent lighting to rule out any cracks or defect. Teeth were kept in distilled water that was replaced once a week at room temperature.

Groups and used material

By using a random number generator the teeth were separated into five groups randomly according to the occlusal surfaces pre-treatment method before fissure sealant application as follows:

- Group 1: (Self-etching): Universal Bond Quick +Ozone application+Fissure sealant.
- Group 2: (Self-etching): Universal Bond Quick+Fissure sealant.
- Group 3: (Total-etching): Etch-rinse+Universal Bond Quick+Ozone application+Fissure sealant.
- Group 4: (Total-etching): Etch-rinse+Universal Bond Quick+Fissure sealant.
- Group 5: (control group): Phosphoric acid etching+Fissure sealant.

Ozonated water preparation

To produce Ozonated water, five mL of distilled water were spared with ozone gas from an ozone generating apparatus with a range of 300's (about 1000 mg/L) [16].

Application modalities

The application modalities were as follows: In ozone application including groups: before adhesive application, the pits and fissures were pre-treated with freshly prepared ozonated water for 80 second and rinsed off for 15 second. In acid pre-treated groups: The occlusal surfaces were etched for 30 seconds with 37 percent phosphoric acid gel, then rinsed for 15 seconds by water, and finally dried for 5 seconds with an air syringe.

In adhesives group with self-etching mode: After applying adhesive to the dried tooth surface, medium air pressure was applied for 5 seconds. Lastly, the adhesive was photo cured for 10 s. In adhesives group with total etch mode: prior to the self-etch steps, pits and fissures were etched for 10 seconds with 37 percent phosphoric acid gel, rinsed for 10 seconds with water, and dried for 5 seconds with an air syringe [17]. Following pre-treatment procedures of pit and fissures, sealants were applied regarding with the manufacturer's instructions.

Fifteen seconds per each application were waited for the penetration of sealants and then cured by light for 20 seconds. After sealant application, the specimens were stored in distilled water for 1 day at 37°C. To provide thermal stress the samples were then aged in a thermos cycling bath 500X at 5°C-55°C [18]. Then the root apices were sealed by wax, all teeth surface except 1 mm around the margins of the fissure sealant agents were covered with double layer nail varnish and immersed in Methylene blue dye for 24 h. Next the specimens were rinsed with water and sectioned in buccal-lingual direction with water coolant. Each specimen was cut into three sections (central, mesial, and distal), and all surfaces were digitally imaged at a magnification of 40X using a Stereo-Microscope. The photographs were saved as uncompressed TIFF files on an IBM-compatible computer. By using ImageJ software (V.1.42, National Institutes of Health, Bethesda, MD), the depth of dye penetration on the tooth-sealant contact was assessed in millimetre. In each section the micro leakage value was computed by multiplying the sum of buccal and lingual value of dye penetration by the total interface lengths of buccal and lingual enamel-sealant [19]. The

measurements were taken by a single calibrated operator who was blinded of the treatment groups. In each tooth the extent of micro leakage for each section was recorded and the mean value of the three sections for that specimen was obtained.

Statistical analysis

Data description, analysis and presentation were done by using: Statistical Package for social Science include:

- Shapiro Wilk test,
- Levene test,

One Way Analysis of Variance (ANOVA), General Linear model (Two Way Analysis of Variance, Bonferroni post hoc test. +Level of significance as: Not significant P>0.05, Significant P<0.05.

RESULTS

Findings in Table 1 showed that the micro leakage was the highest value in the control (G5) followed by G2 (Self etched without Ozone) and G4 (Total etched without Ozone) respectively while lower among groups G1 (Self-etched with ozone) and G3 (Total etched with ozone) respectively with significant difference when comparing

each group with the control group. Furthermore, using multiple comparisons between each group with control (Table 2), results appeared that only G1 (self-etched with ozone) and G3 (Total etched with ozone) had a significant difference with control group while G2 (Self etched without Ozone) and G4 (Total etched without Ozone) were not significant statistically (Figure 1).

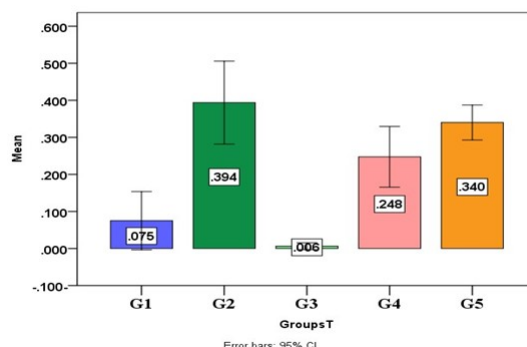


Figure 1: Micro leakage among the different groups.

Table 1: Descriptive and statistical test of micro leakage among groups.

Groups	Minimum	Maximum	Mean	± SD	± SE	F	P value
G1	0	0.45	0.075	0.148	0.037	22.833	0.000 Sig.
G2	0.16	0.75	0.394	0.21	0.053		
G3	0	0.05	0.006	0.017	0.004		
G4	0.09	0.59	0.248	0.154	0.039		
Control	0.21	0.58	0.34	0.089	0.022		

Levene test=2.243, p value=0.093 NS

Table 2: Statistical test of micro leakage of each group with control.

Multiple Comparisons of micro leakage between each group with control using Dunnett t (2-sided)			
(I) Groups	(J) Groups	Mean Difference (I-J)	P value
G1	Control	-0.265	0.000*
G2	Control	0.054	0.651
G3	Control	-0.334	0.000*
G4	Control	-0.093	0.197

*=significant at p<0.05.

DISCUSSION

The most frequent method for assessing micro leakage is measuring dye penetration on tooth sections [20]. To improve the accuracy of measurements, three sections were cut into each specimen in this investigation. Instead of relying on subjective assessment, this technique was integrated with image analysis to get quantifiable findings. When compared to traditional subjective scoring methods, this objective technique has the advantage of not requiring separate evaluators,

consensus scoring in questionable circumstances, or statistical processes to determine inter-examiner reliability [21].

Clinical recommendations for long-term retention and efficient placement of (resin-based sealants) typically involve: cleaning pits and fissures, acid-etching of the surfaces in appropriate manner, and keeping a dry field uncontaminated by saliva till placing and curing of the sealant to decrease micro leakage and sealant sensitivity to moisture contamination. Dentin bonding agents have

been proposed as an intermediary layer between the enamel and the fissure sealer. [22]. For paediatric patients, the self-etch adhesives have the ease of use, requires less technical sensitivity and acid etching is not needed. However, etch and rinse adhesive systems provide better entrance in to enamel rods compared to self-etch adhesive systems, this may result in a better bond strength. To overcome the weakness of previous methods including single step and self-etch adhesives, universal adhesives were introduced since 2011 into daily use. These systems combine the use of the simplest option for both one-steps Self-Etch (SE) and two-steps Etch and Rinse (ER). They are able to adhere different types of hard tissues (*i.e.*, sound, carious, sclerotic dentin, as well as enamel). Furthermore, they can be used to bond to different surfaces, such as glass ceramics, zirconia, and alloys [23]. Universal-bonding agents are composed of Nano-fillers, which provide stress absorption and increased bond strength. Combined use of them with acid etching has been suggested by many authors to achieve optimal bonding. In the study of Unverdi [24]. It was shown that fissure sealants applied following acid etching and ER or SE adhesive procedures lead to better retention compared to conventional sealant over a period of 24 months. These studies concluded that application of sealant along with universal bonding agents enhanced sealant agents' retention and when etch and rinse mode was applied, bond strength was superior compared to self-etch mode.

The current findings showed that in the absence of ozone pre-treatment, the extent of dye leakage was significant in the both totals etch and self-etch (G2,G4) greater than of the groups (G1,G3) that were pre-treated with Ozone. This positive outcome in the ozone pre-treated groups could be attributed to two factors: First, ozone causes the enamel to dehydrate in a reversible manner. As a result, removing remaining moisture from the enamel surface and fissures may considerably improve the penetration of the hydrophobic fissure sealer material. Second, as a disinfectant, ozone destroys minute residues of organic components in the fissure, such as bacteria and their products that are not completely removed by traditional fissure cleaning processes, resulting in a cleaner enamel surface and improved material adherence [25]. In study of Dukic removing such minute remnant from the fissure may allow the sealant material to penetrate deeper into the crack depth. This discovery is supported by the current.

Micro morphological studies, which show that ozone-pre-treated, fissure sealant groups adapt and penetrate better. The findings of this investigation revealed that ozone pre-treatment of fissures had no negative impact on the test materials' micro leakage.

CONCLUSION

According to the findings of this study's experimental circumstances, ozone can be applied as a preventive treatment before the application of the evaluated conventional and bonded fissure sealants, resulting in

better sealing qualities than their ozone-untreated counterparts.

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