

Effect of mechanical polishing and 35% hydrogen peroxide application on DIAGNOdent measurements in stained occlusal fissures: a randomized split-mouth clinical study

Anıl Loça  · Özlem Erçin  · Dilan Kopuz  · Özlem Kanar  · Bora Korkut  ·
Dilek Tağtekin  · Funda Yanıkoğlu 

Received: 9 September 2026 / Accepted: 14 October 2026
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Abstract

Background DIAGNOdent laser fluorescence measurements may be confounded by extrinsic staining in occlusal fissures, and the effect of common surface-preparation protocols on these readings remains unclear. The aim of this study was to compare the effects of mechanical polishing and 35% hydrogen peroxide application on DIAGNOdent Pen measurements in stained occlusal fissures.

Methods In this randomized split-mouth clinical study, 126 posterior teeth from 24 participants were evaluated. Opposing jaws received either mechanical polishing with a fluoride-free prophylaxis paste for 30 s or 35% hydrogen peroxide gel for 15 min. DIAGNOdent scores were recorded at baseline, after air–water spray cleaning and drying, and after the assigned procedure. Digital bite-wing radiographs obtained at baseline as a complementary diagnostic assessment. Data were analyzed using linear mixed-effects models and Wilcoxon signed-rank tests.

Results Air–water spray cleaning and drying did not significantly change DIAGNOdent values in either group (bleaching: $p=0.355$; polishing: $p=0.516$). Post-procedure scores increased significantly after bleaching ($p=0.023$) and polishing ($p<0.001$). The mean score change was significantly greater after polishing than bleaching (18.4 ± 26.0 vs. 4.6 ± 19.3 ; $p<0.001$).

Conclusions Surface-preparation protocols significantly influence DIAGNOdent readings in stained occlusal fissures. Post-polishing readings should be interpreted with caution due to potential paste retention and measurement artifacts, whereas 35% hydrogen peroxide application induces a more limited change without mechanical surface alteration. Standardized surface-preparation protocols and interpretation alongside clinical and radiographic findings are recommended.

Trial registration ClinicalTrials.gov NCT07763470. Registered 07/08/2026 (first submitted date) – Retrospectively registered, <https://clinicaltrials.gov/study/NCT07763470>.

Keywords Fluorescence · Dental caries · Hydrogen peroxide · Dental prophylaxis · Tooth bleaching

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Background

Dental caries is a complex, multifactorial oral disease resulting from the dynamic interaction between host factors, cariogenic microflora, and fermentable carbohydrates over time [1, 2].

In the initial stage of dental caries, a localized chalky-white lesion may form on the enamel surface [3, 4]. These lesions may become more evident after drying and are commonly described as non-cavitated enamel caries. If caries is not detected at an early stage, lesion progression may reduce the likelihood of successful remineralization [5, 6]. Timely and accurate diagnosis is therefore important for remineralization strategies and minimally invasive dentistry, helping to reduce unnecessary tissue loss and treatment burden [5, 7]. However, the detection of occlusal caries remains challenging [8]. Clinicians may have difficulty identifying dentin lesions beneath clinically intact enamel through visual examination alone. Bite-wing radiographs may provide additional diagnostic information, although their performance in detecting early occlusal lesions is limited [9, 10]. One clinical study reported that only one-third of occlusal dentin lesions were clinically detected, whereas the remaining lesions were identified radiographically [11]. Such lesions are commonly described as hidden caries [12, 13]. Detecting lesions before cavitation is important for preserving tooth structure and avoiding unnecessary invasive procedures [14, 15].

To support conventional methods, various adjunctive diagnostic techniques based on optical principles have been developed. These include fiber-optic transillumination (FOTI), digital imaging fiber-optic transillumination (DIFOTI), and near-infrared light transillumination (NILT; e.g., DIAGNOcam), which evaluate lesions based on differences in light transmission and scattering between sound and demineralized dental tissues [16, 17].

Studies have reported that the DIAGNOdent Pen may provide reproducible measurements for the detection of occlusal and approximal caries. However, plaque, calculus, hypomineralized areas, and staining may contribute to false-positive readings, representing an important limitation of the device [18].

Tooth staining may result from organic debris, metal ions, proteolysis-related compounds, or food-derived pigments. Black staining has been associated with ferric sulfide formation through the interaction of iron in saliva or gingival crevicular fluid with hydrogen sulfide produced by bacteria [19]. Because pigmentation may interfere with DIAGNOdent measurements, the tooth surface should be cleaned before assessment. Mechanical cleaning with a rotary brush and prophylaxis paste is commonly used to remove plaque and extrinsic stains. However, the complex anatomy of deep fissures may limit the effectiveness of mechanical instrumentation. Hydrogen peroxide application may therefore be considered as an alternative approach for reducing fissure pigmentation through oxidative reactions [20].

Hydrogen peroxide exerts oxidative effects that may modify biofilm and surface characteristics. Experimental studies have reported antimicrobial effects of hydrogen peroxide (H_2O_2) photolysis against *Streptococcus mutans* and reductions in biofilm acidogenicity [21]. However, these findings were obtained using a photolysis-based protocol and should not be directly extrapolated to the 35% in-office bleaching protocol evaluated in the present study.

The color change induced by hydrogen peroxide is related to oxidative reactions that alter chromophore molecules and their optical properties. Because stain type and depth may influence the response to bleaching agents, changes in fissure pigmentation may vary between teeth [22]. These optical changes may also influence laser fluorescence measurements.

Previous studies have shown that professional cleaning and prophylaxis materials may influence laser fluorescence and DIAGNOdent readings [23–25]. However, clinical evidence regarding the effect of hydrogen peroxide on DIAGNOdent measurements remains limited. Studies evaluating hydrogen peroxide bleaching have reported changes in DIAGNOdent measurements following treatment, including protocols using 35% hydrogen peroxide [26, 27]. These findings suggest that hydrogen peroxide-induced changes in enamel mineralization and surface characteristics may influence laser fluorescence measurements. Therefore, further clinical investigation is

warranted to determine whether the application of 35% hydrogen peroxide affects DIAGNOdent measurements in stained occlusal fissures under clinical conditions.

Based on this gap, the aim of the present study was to compare the effects of mechanical polishing and 35% hydrogen peroxide application on DIAGNOdent Pen measurements in posterior teeth with stained occlusal fissures.

H1: Mechanical polishing does not significantly affect DIAGNOdent measurements in stained occlusal fissures.

H2: There is no significant difference between mechanical polishing and 35% hydrogen peroxide application in their effects on DIAGNOdent measurements.

Methods

This clinical study was conducted at Istanbul Kent University, Faculty of Dentistry, Department of Restorative Dentistry following approval by the Marmara University Faculty of Medicine Ethics Committee (approval no. 09.2024.248) and in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrollment in the study. The study was registered at ClinicalTrials.gov (NCT07763470) on August 12, 2026 and was retrospectively registered. The trial was conducted, analyzed, and reported in accordance with the CONSORT 2010 guidelines for randomized clinical trials.

An a priori power analysis was performed using G*Power software (Heinrich-Heine University, Düsseldorf, Germany). A medium effect size ($f=0.25$) was assumed. Using a significance level of 0.05 and a statistical power of 80%, the minimum required sample size was calculated as 34 observations. To account for potential variations and the clustered split-mouth design, 126 posterior teeth from 24 adult participants were included.

A total of 25 healthy adult volunteers who presented to the clinic and met the inclusion criteria were initially enrolled. The inclusion criteria were being between 18 and 65 years of age, having clinically visible staining in the fissures of at least two permanent posterior teeth, and the absence of cavitation, restoration, or sensitivity in these teeth. Individuals with restorations; evident cusp loss, wear, fracture, or cracks on the occlusal surface; enamel cracks; or hypocalcification lesions were excluded from the study. One participant was excluded before the final analysis because excessive debris accumulation prevented stable and reproducible baseline laser fluorescence measurements. The final analysis included 24 participants and 126 teeth. Participant recruitment, exclusion, allocation, and inclusion in the final analysis are presented in the CONSORT flow diagram (Fig. 1). Because multiple teeth were obtained from the same participants, the clustered data structure was accounted for using a linear mixed-effects model with patient as a random effect.

The study was designed as a randomized, open-label, split-mouth clinical trial. The assessor who evaluated the radiographs was blinded to the allocated surface procedure. For each participant, the two surface procedures were randomly assigned to opposing jaws using a computer-generated randomization sequence. (Fig. 2A). Both surface procedures were performed during the same clinical session after the baseline measurements.

The right and left posterior quadrants were isolated using a rubber dam. The anterior teeth were not exposed through punched openings in the rubber dam. The surface procedures were performed on the isolated posterior teeth to minimize contact of the prophylaxis paste or 35% hydrogen peroxide gel with adjacent oral tissues. Following the procedures, the treated surfaces were thoroughly rinsed with water and gently dried. Baseline images of the occlusal surfaces were recorded using an intraoral camera (Charming, Micro lens-1, China) according to a standardized protocol. The camera was positioned at an angle of approximately 90° to the occlusal surface and at a distance of 10–15 mm to optimize visualization of the fissures and obtain comparable images for all teeth.

Following digital imaging, laser fluorescence measurements were performed using the DIAGNOdent Pen device (KaVo, Biberach, Germany) operating with a red diode laser at a wavelength of $\lambda = 655$ nm and a peak power output of ≤ 1 mW, equipped with a dedicated cylindrical fissure probe (occlusal tip). Before each session,

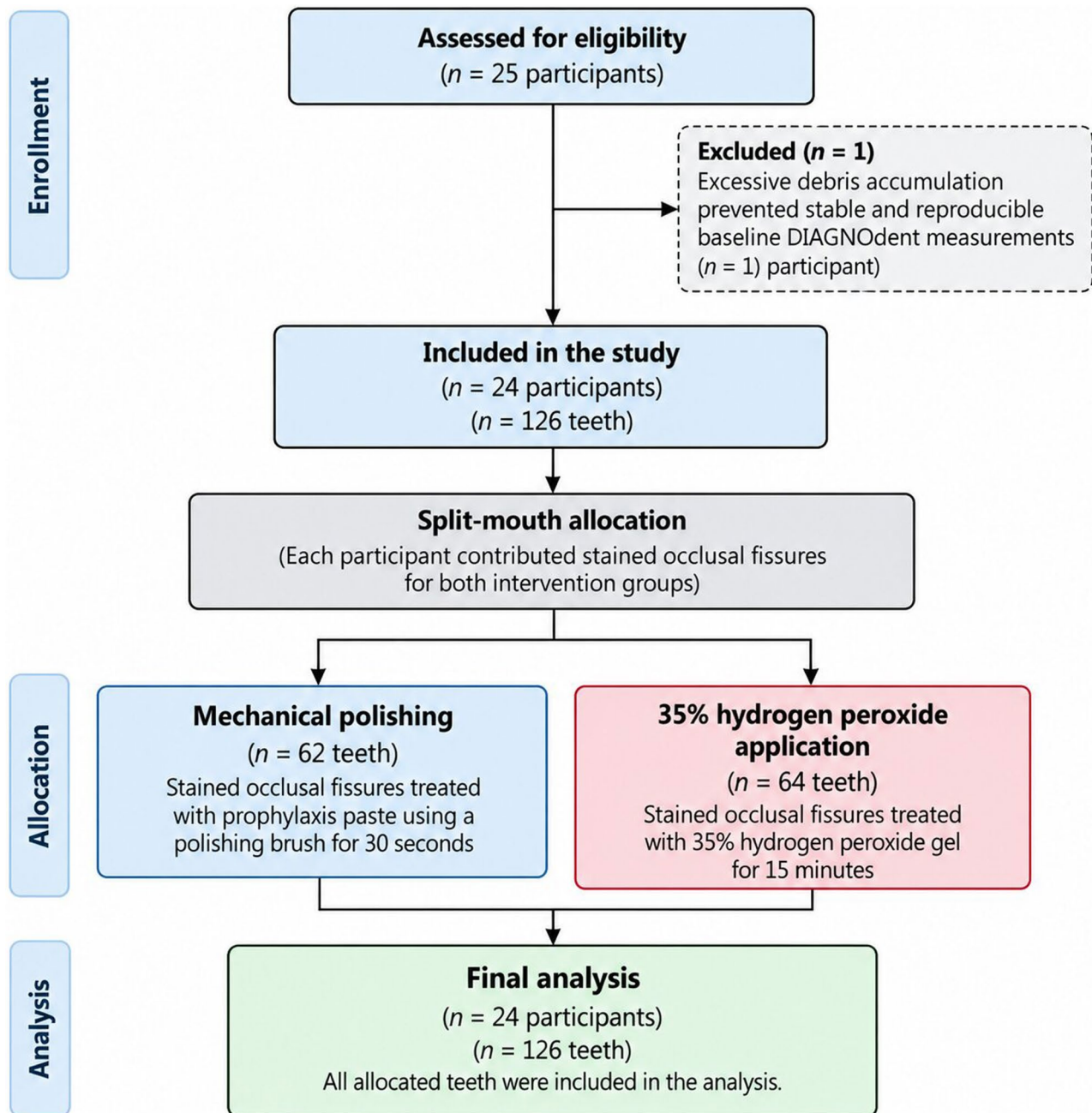


Fig. 1 CONSORT flow diagram

the device was calibrated against its ceramic standard according to the manufacturer's instructions. Baseline measurements were first obtained from the predetermined deepest points of the occlusal fissures. These measurement sites were identified during the baseline examination based on the clinical morphology of each fissure and were used for all subsequent measurements. After air–water spray cleaning and drying, measurements were repeated at the same predetermined sites. Following the assigned surface procedure, final DIAGNOdent measurements were again obtained from the same fissure sites. Each measurement was repeated three times, and the highest value was recorded. DIAGNOdent scores range from 0 to 99: 0–13 indicates a sound surface; 14–20 indicates a lesion in the outer half of the enamel; 21–29 indicates a lesion in the inner half of the enamel; and ≥ 30 indicates a dentin lesion.

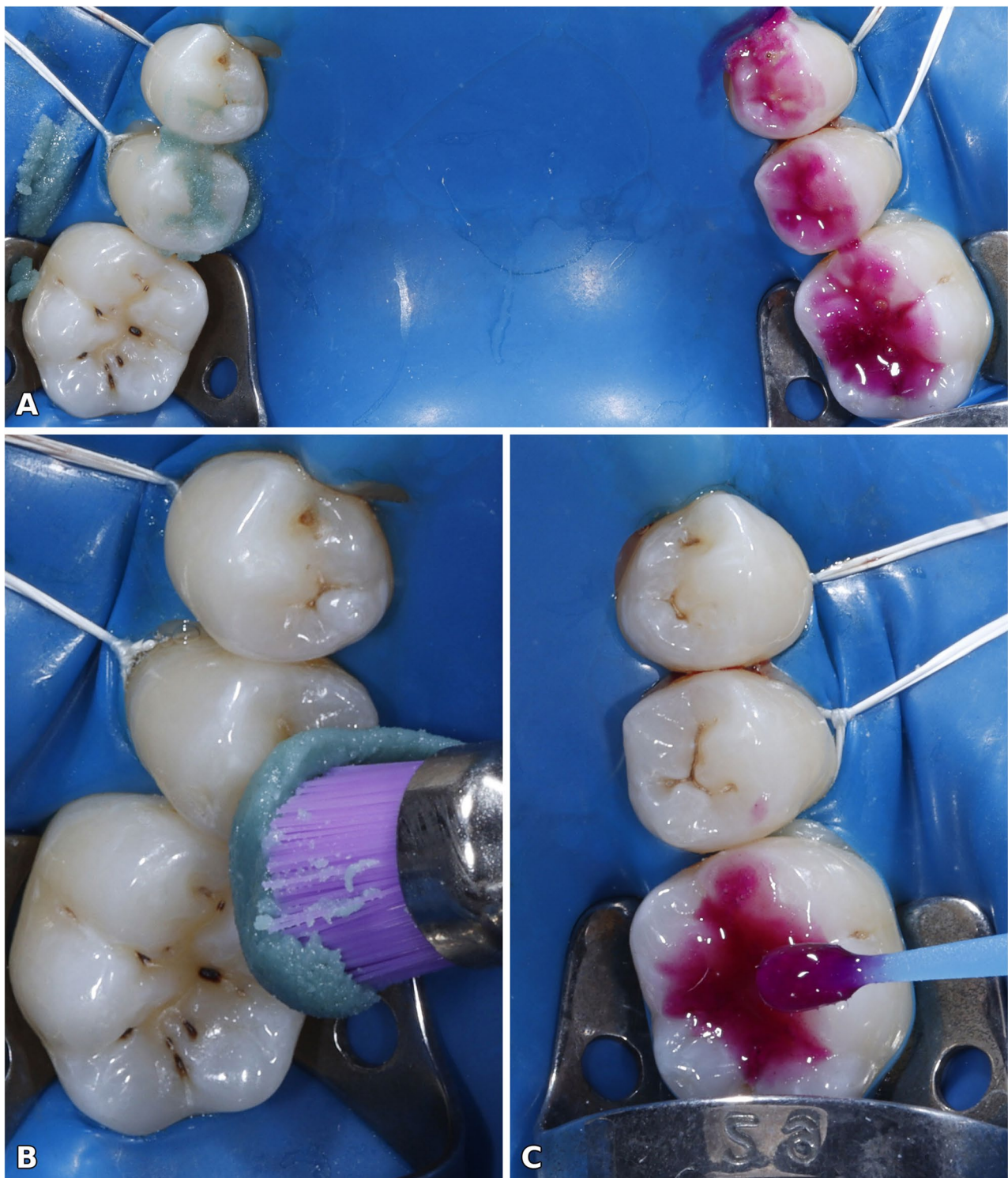


Fig. 2 Representative clinical images of the surface procedures applied under rubber dam isolation. **A** Representative split-mouth clinical view showing the two surface procedures applied to opposing jaws. **B** Mechanical polishing of stained occlusal fissures using a fluoride-free prophylaxis paste and a polishing brush. **C** Application of an in-office bleaching agent containing 35% hydrogen peroxide to stained occlusal fissures

Following baseline measurements, the assigned surface procedure was applied according to the split-mouth randomization protocol. In Group 1, a fluoride-free prophylaxis paste (Cleanic, Kerr, Salerno, Italy) was applied to the fissure area using a polishing brush attached to a micromotor operating at 10,000 rpm. In each tooth, the fissures were polished for 30 s with light pressure using circular movements (Fig. 2B). After the procedure, the tooth surface was rinsed with water spray and gently dried with air.

In Group 2, an in-office bleaching agent containing 35% hydrogen peroxide (Whiteness HP, FGM Dental, Brazil) was prepared according to the manufacturer's instructions (3 drops peroxide and 1 drop thickener) and applied to completely cover the stained fissure areas for 15 min (Fig. 2C). At the end of the time period, the gel was aspirated; the teeth were then rinsed thoroughly with water and gently dried with air.

Postoperative occlusal photographs were then obtained under the same standardized conditions as the baseline images. Final DIAGNOdent measurements were recorded from the same fissure sites. Accordingly, DIAGNOdent scores were obtained at three time points: baseline, after air–water spray cleaning and drying, and after the assigned surface procedure.

All clinical procedures and laser fluorescence measurements were performed by a single investigator using a standardized measurement protocol. Digital bite-wing radiographs were obtained at baseline as a complementary diagnostic assessment during the initial clinical evaluation. They were used to document the baseline radiographic status of the posterior teeth and to support the clinical assessment and study eligibility. Radiographic findings were not defined as an outcome measure and were not included in the statistical analysis of DIAGNOdent measurements.

Statistical analysis

Statistical analyses were performed using SPSS software (version 29.0, IBM, Armonk, NY, USA). Data distribution was assessed before the analyses. Descriptive within-group comparisons of repeated measurements were performed using the Wilcoxon signed-rank test. Because multiple teeth were nested within participants and the study used a split-mouth design, the primary between-protocol analysis was performed using a linear mixed-effects model with patient as a random effect and surface procedure as a fixed effect. The change score was calculated as the post-surface procedure value minus the baseline value. The Mann–Whitney U test was used as a supplementary unadjusted analysis. A two-sided p -value of <0.05 was considered statistically significant.

Results

In both groups, DIAGNOdent scores obtained after air–water spray cleaning and drying did not differ significantly from baseline values (bleaching: $p=0.355$; polishing: $p=0.516$) (Table 1). In contrast, DIAGNOdent scores increased significantly after the assigned surface procedures. In the bleaching group, post-procedure values were

Table 1 Descriptive statistics of DIAGNOdent measurements and within-group comparisons according to the study groups

Group	Time/Stage	Mean $\pm$ SD	Median (Min–Max)	p^a	p^b	p^c
Bleaching ($n=64$)	1. Before air–water spray	15.4 $\pm$ 13.0	12.5 (2–87)	—	—	—
	2. After air–water spray	17.2 $\pm$ 17.2	12.0 (1–99)	0.355	—	—
	3. After surface procedure	20.0 $\pm$ 18.0	15.5 (0–99)	—	0.023	0.032
Polishing ($n=62$)	1. Before air–water spray	14.6 $\pm$ 11.0	11.0 (3–60)	—	—	—
	2. After air–water spray	14.2 $\pm$ 10.1	12.0 (3–59)	0.516	—	—
	3. After surface procedure	33.0 $\pm$ 28.1	23.0 (5–99)	—	<0.001	<0.001

SD Standard deviation

p^a : Stage 2 vs. Stage 1; p^b : Stage 3 vs. Stage 1; p^c : Stage 3 vs. Stage 2

*Wilcoxon signed-rank test

Table 2 Comparison of the “post-surface procedure–baseline” difference score between groups (Mann–Whitney U test)

Change Parameter (Δ Score)	Group	Mean $\pm$ SD	Median	Mean Rank	Z	p^*
Post-surface procedure–Baseline	Bleaching	4.6 $\pm$ 19.3	3	52.30	-3.499	<0.001
	Polishing	18.4 $\pm$ 26.0	10	75.06		

SD Standard deviation

*Mann–Whitney U test

significantly higher than both baseline values ($p=0.023$) and post air–water spray values ($p=0.032$). In the polishing group, post-procedure values were significantly higher than both baseline and post air–water spray values ($p<0.001$ for both comparisons) (Table 1).

In the between-group comparison, the “post-surface procedure–baseline” difference score was significantly higher in the polishing group (18.4 $\pm$ 26.0; median=10) than in the bleaching group (4.6 $\pm$ 19.3; median=3) ($p<0.001$) (Table 2).

A linear mixed-effects model including patient as a random effect revealed a significant effect of the surface procedure on DIAGNOdent change scores ($F(1, 124)=13.247$, $p<0.001$). Compared with polishing, the bleaching protocol resulted in significantly lower change scores ($\beta=-16.0$, 95% CI: -24.8 to -7.3).

Discussion

The present study demonstrated that both mechanical polishing and 35% hydrogen peroxide application significantly affected DIAGNOdent measurements, although the magnitude of change differed between the two surface preparation protocols. Mechanical polishing produced a significantly greater increase in DIAGNOdent measurements than hydrogen peroxide application. Therefore, both research hypotheses were rejected. Because this in vivo design lacked a histological reference, these increases cannot be definitively attributed to true lesion progression versus a measurement artifact; this distinction is explored further below.

Contemporary caries management is based on early detection of lesions, careful assessment of activity and progression risk, and the implementation of individualized, risk-based preventive strategies accordingly. When necessary, the disease process is intended to be controlled using minimally invasive operative approaches that aim to preserve tooth structure at the highest possible level [28]. Although dark discolorations observed in occlusal fissures are a common clinical finding, it is accepted that these discolorations are not always indicative of active caries and may also be associated with extrinsic staining, organic debris, or color changes accompanying arrested lesions. Therefore, before deciding on an invasive intervention, the extent of the lesion and its potential activity should be evaluated objectively and supported with adjunctive diagnostic methods [29].

Laser fluorescence devices assist clinicians by converting fluorescence signals generated in dental hard tissues or lesion-associated areas into a numerical score; however, their diagnostic performance may vary depending on lesion characteristics, surface conditions, and the applied diagnostic protocol. Current consensus recommendations consider visual examination the first-choice method for caries detection on accessible surfaces, with bite-wing radiography and adjunctive non-ionizing diagnostic methods used when clinically indicated. Therefore, laser fluorescence should be considered an adjunctive diagnostic method rather than a stand-alone diagnostic tool [30]. Our findings reinforce this principle, as the surface-preparation protocol itself was shown to meaningfully alter the numerical score independent of any underlying lesion change.

In measurement standardization, the “clean and dry surface” condition is a critical step. A previous study reported that professional cleaning procedures may significantly affect laser fluorescence values, and that the cleaning step—particularly in areas with complex fissure morphology—can directly alter the interpretation of the measurement [23]. In the present study, the absence of a significant change in DIAGNOdent values after cleaning/drying with an air–water spray (Table 1) indicates that this step alone was not clinically sufficient to eliminate discoloration and organic deposits covering the fissure entrance.

Several studies have demonstrated that prophylaxis pastes and abrasive particles/residues remaining on the tooth surface may increase DIAGNOdent values. Therefore, adequate rinsing prior to measurement and standardization of measurement conditions for each patient/tooth have been recommended [24]. Moreover, since it has been reported that some abrasive polishing procedures can markedly increase DIAGNOdent scores and that this effect may not completely reverse in the short term, the possibility of a “measurement artifact” should be considered when interpreting post-polishing measurements clinically [25]. In the present study, the marked elevation in scores following polishing is consistent with paste entrapment and surface alteration rather than confirmed true lesion progression, although this could not be verified histologically in the present design. Therefore, post-polishing measurements should be interpreted cautiously and in conjunction with visual and radiographic findings.

The more limited increase in DIAGNOdent values in the bleaching group compared with polishing (Table 2) may be related to the absence of a mechanical abrasive effect and a lower likelihood of residue-related measurement artifacts. Although clinical studies have investigated the effects of hydrogen peroxide-containing bleaching agents on cariogenic bacteria and plaque accumulation [31], the present study did not assess biofilm outcomes. Therefore, no clinical recommendation regarding the use of bleaching agents as a diagnostic pretreatment can be made based on the present findings. It should also be noted that the 15-minute in-office bleaching application used in this study is more time- and cost-intensive than routine 30-second mechanical polishing, which may limit its practical adoption solely as a diagnostic surface-preparation step.

When interpreting the relationship between bleaching and our findings, it should be noted that the primary mechanism of action of hydrogen peroxide (H_2O_2) is to reduce visible light absorption by breaking down organic chromophores through oxidative reactions [32]. Bleaching alters the optical properties of the tooth surface, and DIAGNOdent measurements may therefore be influenced not only by lesion presence but also by surface characteristics such as light transmission and absorption. Accordingly, changes in surface pigmentation may directly affect the measured values [15, 33]. In this context, a reduction in chromophore load due to bleaching may alter the amount of light reaching and returning from the fissure base, thereby leading to a measurable difference in laser fluorescence scores. Changes in fluorescence values after bleaching should not be interpreted solely as changes in lesion status [34]. On the other hand, it has been reported that high-concentration in-office H_2O_2 agents may cause morphological changes on the enamel surface and may lead to differences in parameters related to microhardness and mineralization level. Therefore, such multidimensional changes induced by bleaching at optical and structural levels should be considered when interpreting the outcomes of laser fluorescence-based diagnostic systems [35, 36]. In addition to these optical and structural effects, an increase in enamel permeability following 35% H_2O_2 application under in vivo conditions has also been reported. This finding indicates that high-concentration bleaching agents may induce not only optical but also microstructural changes in enamel [37]. Taken together, these optical, structural, and permeability-related changes indicate that bleaching affects DIAGNOdent readings through multiple, overlapping mechanisms rather than a single pathway, further underscoring that post-bleaching scores should not be interpreted as a direct proxy for lesion status.

Finally, these findings should be interpreted in light of their limited generalizability. The study was conducted at a single center using a single prophylaxis paste, a single bleaching product, and a single laser fluorescence device, so the results may not extend to other product or device combinations. In addition, because all procedures and measurements were performed by a single calibrated investigator who was also aware of the allocated treatment, the possibility of observer-related measurement bias cannot be entirely excluded.

This study has several limitations. First, because this was an in vivo clinical study, histological validation of dentin caries was not feasible. Second, discoloration changes were not quantified using an objective image-analysis method, and no microbiological analysis was performed. Finally, measurements were obtained immediately after the procedures; therefore, the findings do not reflect the long-term effects of polishing or bleaching on discoloration, biofilm dynamics, or laser fluorescence measurements. Future studies should include longer follow-up periods, standardized image analysis, microbiological assessments, and histological or advanced optical reference methods where feasible.

Conclusion

Within the limitations of this randomized split-mouth clinical study, both mechanical polishing with a fluoride-free prophylaxis paste and the application of 35% hydrogen peroxide significantly increased laser fluorescence scores, with a more pronounced increase observed following mechanical instrumentation. Because mechanical polishing resulted in significantly higher score increases, post-polishing DIAGNOdent readings should be interpreted with caution due to potential paste retention or surface artifacts, whereas 35% hydrogen peroxide application may represent a more stable surface-preparation approach that avoids mechanical surface distortion prior to laser fluorescence measurements.

Acknowledgements Not applicable.

Authors' contributions AL and ÖE conceived and designed the clinical trial. AL conducted the clinical procedures, radiographic evaluations, and laser fluorescence measurements. ÖE and DK performed data curation and formal statistical analysis. ÖK, BK, DT, and FY contributed to methodology validation, resources, and critical intellectual revision of the draft. AL and ÖE wrote the original draft. FY supervised the research. All authors read and approved the final manuscript.

Funding This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate The study protocol was approved by the Marmara University Faculty of Medicine Clinical Research Ethics Committee (approval no: 09.2024.248) and conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all individual participants included in the study.

Consent for publication Not Applicable.

Competing interests The authors declare no competing interests.

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Authors and Affiliations

Anıl Loça¹  · Özlem Erçin¹  · Dilan Kopuz¹  · Özlem Kanar²  · Bora Korkut³  ·
Dilek Tağtekin³  · Funda Yanıkoğlu³ 

✉ Özlem Erçin
ozlem.ercin@kent.edu.tr

Anıl Loça
anil.loca@kent.edu.tr

Dilan Kopuz
dilan.kopuz@kent.edu.tr

Özlem Kanar
oslemelibol@gmail.com

Bora Korkut
bora.korkut@marmara.edu.tr

Dilek Tağtekin
dtagtekin@marmara.edu.tr

Funda Yanıkoğlu
fyanikoglu@marmara.edu.tr

¹ Department of Restorative Dentistry, Faculty of Dentistry, Istanbul Kent University, Siraselviler St. No:71 Beyoğlu, Istanbul, Turkey

² Restorative specialist, Private Clinic, Istanbul, Turkey

³ Department of Restorative Dentistry, Faculty of Dentistry, Marmara University, Istanbul, Turkey