

# Effect of Continuous Chelation Irrigation Using DualRinse HEDP+3% NaOCI with or without High-power Sonic Activation on Debris and Smear Layer Removal

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## ABSTRACT

**Objective:** This study aimed to assess the effect of sodium hypochlorite (NaOCI) combined with a novel chelating agent DualRinse HEDP (Medcem GmbH, Weinfelden, Switzerland), a product consisting of 0.9 g of 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP) powder, with or without high-power sonic activation on debris and smear layer removal.

**Methods:** Seventy-five mandibular premolars were divided into 5 groups (n=15) and treated with different irrigation protocols: group 1 (D3N), DualRinse HEDP+3% NaOCI without activation; group 2 (D3NA), DualRinse HEDP+3% NaOCI with activation (EDDY, VDW, Munich, Germany) during the final irrigation; group 3 (3NE), 3% NaOCI+17% Ethylenediaminetetracetic acid (EDTA)+3% NaOCI without activation; group 4 (3NEA), 3% NaOCI+17% EDTA+3% NaOCI without activation; group 4 (3NEA), 3% NaOCI+17% EDTA+3% NaOCI with activation during the final irrigation; group 5 (NC), negative control group, 0.9% saline. Samples were analysed by scanning electron microscopy (SEM) to evaluate residual debris and smear layer at 3 levels of the root canal: coronal, middle, and apical. Statistical analysis was performed with a level of significance set at p<0.05. The normality distribution of scores within each group was assessed using Kolmogorov-Smirnov and Shapiro-Wilk tests. A Kruskal-Wallis test followed by multiple comparison tests was used to compare scores among the 5 groups on the apical, middle, and coronal levels of the root canal. A Friedman test followed by multiple comparison tests was used to compare scores within the apical, middle, and coronal levels for each treatment group.

**Results:** Debris score was significantly the lowest for D3NA, followed by D3N, 3NEA and 3NE at all root levels (p<0.05). The smear layer score was significantly the lowest for D3NA, followed by D3N, 3NEA and 3NE only at the apical level, while no significant difference was found in the middle and coronal levels between the groups (p<0.05). DualRinse HEDP resulted in less debris and smear layer compared to the classic approach of NaOCI without activation. Implementing sonic activation further improved debris and smear layer removal.

**Conclusion:** DualRinse HEDP+3% NaOCI improved debris removal at all levels and smear layer elimination at the apical level of the root canal. These results were further enhanced when adding high-power sonic activation.

Keywords: DualRinse, EDTA, HEDP, irrigation, scanning electron microscopy, sodium hypochlorite

### HIGHLIGHTS

- High-power sonic activation with DualRinse HEDP improved debris removal at all levels of the root canals.
- High-power sonic activation with DualRinse HEDP improved smear layer removal at the apical level.
- Soft continuous chelation with DualRinse HEDP provides enhanced removal of debris and smear layer compared to the conventional irrigation protocol.

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### INTRODUCTION

Root canal treatment aims to achieve an adequate disinfection, a bacterial shift away from pathologic biofilm, and to ensure a tridimensional obturation to prevent reinfection of the tooth. It is an essential step before root canal obturation. Hence, an optimised irrigation protocol is important to remove harmful bacteria, biofilm, and debris from non-instrumented areas (1–3).

Due to its excellent antimicrobial properties and unique ability to dissolve soft tissue, sodium hypochlorite (NaOCI) at 1-6%represents the gold standard for root canal irrigation (4). However, shaping instruments also produce inorganic debris and smear layer. Hence, it is essential to remove the smear layer before root canal obturation (5), as it may harbour bacteria responsible for persistent root canal infection.

Ethylenediaminetetraacetic acid (EDTA) is a commonly used irrigant for root canal chelation. However, if applied in alternation with NaOCI, EDTA interferes chemically with NaOCI by reducing its tissue dissolution capability and losing free available chlorine (6). So far, there is not a single solution that fulfils all criteria of an ideal irrigant with sufficient antimicrobial action, tissue dissolution and chelation.

DualRinse HEDP (Medcem GmbH, Weinfelden, Switzerland) consists of 0.9 g of etidronate powder. Adding etidronate powder to a NaOCI solution results in a mixture of NaOCI + 9% HEDP, which keeps NaOCI active during a 1-hour treatment and adds a chelating action (7). While the chelating effect of DualRinse HEDP has shown to be weaker than conventional chelators, such as 17% EDTA (8), it acts throughout the whole treatment when NaOCI and the chelator are applied together rather than in an alternating fashion.

This clinical concept was introduced as "continuous soft-chelation" (9), and the main benefit is reduced irrigation time since the removal of the smear layer and inorganic debris after instrumentation is not required as the continuous release of the calcium complex prevents debris accumulation (7).

In addition, root canal irrigation can be enhanced by activating the irrigant with high-power sonic energy. EDDY (VDW, Munich, Germany), a flexible polyamide high-powered sonic activation system, has been introduced to create a three-dimensional movement of irrigants that triggers an acoustic current (1, 10, 11).

How soft chelation and high-power sonic activation may interact during root canal irrigation is unclear. Therefore, the current study aimed to investigate the effect of combining DualRinse HEDP + NaOCl irrigation with high-power sonic activation on removing debris and smear layer and comparing it to a standard irrigation protocol using NaOCl during instrumentation and EDTA as a final irrigant with or without highpower sonic activation.

The null hypotheses of this study are that:

1. There is no difference in the effect of the activation of DualRinse HEDP on its ability to remove the smear layer and debris.

2. There is no difference between DualRinse HEDP and the conventional protocol using NaOCI + EDTA + NaOCI in the ability to remove the smear layer and debris.

### **MATERIALS AND METHODS**

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval for the procedures used in the present study was obtained (USJ-2021-39).

### **Specimen Selection and Preparation**

Freshly extracted 75 single-rooted human mandibular premolars were used for this study after collecting 163 teeth (88 were excluded following the exclusion criteria). Sample size estimation was calculated with G\*Power 3.1.9.2 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). Teeth were divided into 5 groups of 15 each to have 80% power and an alpha error probability of 0.05.

Teeth were extracted for orthodontic or periodontal reasons. Periodontal soft tissues were removed from the root surface with ultrasonics, and the teeth were subsequently stored in 0.1% thymol at 4 °C. The selection criteria were: intact teeth with fully formed apices, no previous endodontic treatment, no coronal restoration, no calcification of the root canal, absence of fractures, and a minimum length of 15 mm. Moreover, radiographs were taken in both mesiodistal and buccolingual directions to include teeth having only one straight canal (curvature  $\leq 5^\circ$ , the degree of curvature was verified with the method of Schneider (12)).

Dental crowns were sectioned at the cementoenamel junction with a diamond disc (Komet Brasseler, Lemgo, Germany) to standardise root length at 15 mm. Root canals were accessed and an ISO size #10 K-type file (Dentsply-Maillefer, Ballaigues, Switzerland) was inserted into the canal until the tip of the file was observed at the apical foramen. The working length was determined by subtracting 1 mm from the recorded length. A closed environment was created by sealing off the root tip with wax to mimic clinical conditions and avoid the flow of irrigants through the root apex. Subsequently, the 75 teeth were randomly assigned into 5 groups (n=15) according to the irrigation protocol.

### **Root Canal Instrumentation and Irrigation**

All samples were instrumented with WaveOne Gold Primary 25 (Dentsply-Maillefer, Ballaigues, Switzerland). During instrumentation, the irrigant was delivered using a 27-G sidevented needle (Endo-Eze; Ultradent, South Jordan, Utah, USA) coupled to a 5 mL syringe. The needle was placed as apically as possible at each stage of preparation and then withdrawn approximately 2 mm. Irrigants were delivered with back-and-forth movements of 2–3 mm amplitude.

After each instrumentation step, 2 mL of irrigating solution was used for 30 seconds, for a total of 4 mL during the instrumentation phase, as 2 instrumentation steps with WaveOne Primary Gold were needed to reach full working length. After instrumentation, 6 mL of irrigating solution was used for 3 minutes for the final irrigation. Hence, a total of 10 mL of irrigating solution were used, according to the respective experimental groups as in the study of Kfir et al. (13):

- Group 1 (D3N): DualRinse HEDP+3% NaOCI without activation. The canal was irrigated throughout treatment with a mixture of one capsule of DualRinse and 10 mL of 3% NaOCI as described above (4 mL during the instrumentation and 6 mL for the final irrigation)
- Group 2 (D3NA): DualRinse HEDP+3% NaOCI with activation during the final irrigation. The canal was irrigated as in D3N, adding high-power sonic activation with EDDY during the final irrigation step. EDDY was applied for 3 cycles of 30 seconds each after irrigation with 2 mL of DualRinse for 30 seconds, for a total volume of final irrigation of 6 mL applied for 3 minutes.
- 3. Group 3 (3NE): 3% NaOCI+17% EDTA+3% NaOCI without activation. The canal was irrigated with 4 mL of 3% NaOCI during instrumentation, followed by 2 mL of 3% NaOCI for 1 minute, 2 mL of 17% EDTA for 1 minute and 2 mL of 3% NaOCI for 1 minute, for a total of 6 mL of final irrigation applied for 3 minutes. Distilled water was used between NaOCI and EDTA to prevent interaction.
- 4. Group 4 (3NEA): 3% NaOCl+17% EDTA+3% NaOCl with activation during the final irrigation. The canal was irrigated as in 3NE, adding high-power sonic activation with EDDY during the final irrigation step. EDDY was applied for 3 cycles of 30 seconds each after irrigation for 30 seconds with 2 mL of 3% NaOCl, 2 mL 17% EDTA and again 2 mL of 3% NaOCl, for a total volume of final irrigation of 6 mL applied for 3 minutes. Distilled water was used between NaOCl and EDTA to prevent interaction.
- 5. Group 5 (NC): negative control group. The canal was irrigated with 10 mL of 0.9% NaCl (4 mL during the instrumentation and 6 mL as final irrigation).

All samples received a last flush with 3 mL of distilled water for 1 minute to remove residual root canal irrigants. Canals were then dried with absorbent paper points (Meta Biomed Co, Cheongju, Korea).

#### High-vacuum Scanning Electron Microscopy (SEM) Preparation

After the irrigation protocol, a fine-medium gutta-percha cone was placed inside the canal to estimate the proximity of the canal during cutting and to prevent the penetration of debris into the canal generated during the cutting with the diamond disk.

Two longitudinal grooves were made on each root's buccal and lingual surface with a slow-speed double-sided diamond disc under constant water cooling (22.0 mm diameter, 0.15 mm thick; #984 [Komet Brasseler, Lemgo, Germany]). Cutting with the diamond disc was stopped when the gutta-percha cone became visible by transparency. Next, the two halves of the root were separated using a chisel and a surgical hammer.

One-half of each root was randomly selected for SEM evaluation. Three indentations perpendicular to the longitudinal axis of the root were made on the mesial side of the root (at 3, 6, and 9 mm from the apex) using the diamond disc to standardise the visualisation of the 3 levels of the root canal (coronal, middle and apical).

The samples were dehydrated with a serial concentration of ethanol. After dehydration, root sections were mounted on

metallic stubs using conductive double-coated carbon tape. Then they were sputter coated with a 20 nm thick layer of gold using a Quorum 150 V plus machine and examined under SEM (Mira 3, TESCAN, SEM) (10–20 kV, 8–12 mm working distance). The most defined area at the level of the markings was chosen and viewed under 2 magnifications (×200 for debris evaluation and ×1000 for smear layer evaluation). Six images were obtained for each sample for statistical evaluation from the area having the most residual debris and smear layer. The images taken were analysed blindly by two independent evaluators. The evaluators were endodontists who underwent a training process using the program Image J and the scoring system used.

Debris (dentine chips, pulp remnants, and particles loosely attached to canal wall) were quantified using the following 5-point scoring system adapted from Urban et al. (1): score 1, clean canal wall, only very few debris particles; score 2, few small conglomerations, less than 25% of canal wall covered; score 3, many conglomerations, 25% to 50% of canal wall covered; score 4, 50% to 75% of canal wall covered; and score 5, complete or nearly complete (more than 75%) canal wall covered by debris. The smear layer was evaluated using the following 4-point scoring system adapted from Gambarini and Laszkiewicz, and Kato et al. (14, 15): score 1, opened dentinal tubules without smear layer; score 2, opened dentinal tubules with smear layer covering less than 50% of the examined area; score 3, opened dentinal tubules with smear layer covering more than 50% of the examined area; and score 4, dentinal tubules covered by smear layer in 100% of the examined area. The debris and smear layer percentage were evaluated using the Image J program (Version 1.46; National Institutes of Health, Bethesda, MD, USA) to have a precise percentage for scoring. The mean percentages for debris and smear layer were calculated between the evaluators to have precise scoring. Image J software was selected for measurement of debris and smear layer percentages to allow a quantitative evaluation. To avoid bias and establish reliability for the experiment, inter-examiner agreement was conducted between the 2 blind observers.

#### **Statistical Analysis**

IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, NY, USA) was used to perform statistical analysis. The level of significance was set at p<0.05. The outcome variables of the study were debris score and smear layer score. In addition, a Kappa score was calculated to assess interobserver reliability (Kappa score=0.82) using Minitab software (Minitab<sup>®</sup> 18.1, Minitab, Inc., Pennsylvania State University, PA, USA).

The normality distribution of scores within each group was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Since variables were not normally distributed, non-parametric tests were used for statistical comparisons.

Kruskal-Wallis test followed by multiple comparisons test were used to compare scores among the 5 groups on the apical, middle and coronal levels of the canal.

Friedman and multiple comparisons tests were used to compare scores within the apical, middle and coronal levels for each treatment group.

	Debris score according to the root level				
	Apical	Middle	Coronal	р	
D3N	2 (1–2) <sup>b</sup>	2 (1–2) <sup>b</sup>	1 (1–2) <sup>b</sup>	0.301	
D3NA	1 (1–2)ª	1 (1–2)ª	1 (1–1)ª	0.214	
3NE	3 (2–4) <sup>c/B</sup>	3 (2–3) <sup>c/A</sup>	2 (2–3) <sup>c/A</sup>	0.003	
3NEA	2 (1–3) <sup>b</sup>	2 (1–3) <sup>b</sup>	2 (1–3) <sup>c</sup>	1.000	
NC	5 (4–5) <sup>d</sup>	5 (4–5) <sup>d</sup>	5 (4–5) <sup>d</sup>	0.184	
р	<0.001	<0.001	<0.001		

Median, minimum and maximum scores for remaining debris. Lowercase superscript letters (a, b, c, d) indicate the presence of a significant difference between treatment groups according to multiple comparison tests. Uppercase superscript letters (A, B) indicate the presence of a significant difference between root levels according to multiple comparisons tests. D3N: DualRinse HEDP+3% NaOCI without activation, D3NA: DualRinse HEDP+3% NaOCI with activation during the final irrigation, 3NE: 3% NaOCI+17% EDTA +3% NaOCI without activation, 3NEA: 3% NaOCI+17% EDTA+3% NaOCI with activation during the final irrigation, NC: Negative control group

#### RESULTS

Debris scores showed significant differences among all 5 treatment groups at all canal thirds (p<0.001) (Table 1). Debris score was significantly lower for D3NA, followed by D3N, 3NEA

**TABLE 2.** Smear layer scores according to the root level

	Smear layer score at the root canal thirds				
	Apical	Middle	Coronal	р	
D3N	3 (2-4) <sup>a,b/B</sup>	2 (2–3) <sup>a/A</sup>	2 (1–2) <sup>a/A</sup>	0.001	
D3NA	2 (2–4) <sup>a/B</sup>	2 (1-2) <sup>a/A</sup>	2 (1–2) <sup>a/A</sup>	0.033	
3NE	3 (2–3) <sup>b/B</sup>	2 (2-3) <sup>a/A</sup>	2 (2–3) <sup>a/A</sup>	< 0.001	
3NEA	2 (2–3) <sup>a,b</sup>	2 (2-3)ª	2 (1–2)ª	0.009	
NC	4 (4–4) <sup>c</sup>	4 (4–4) <sup>c</sup>	4 (4–4) <sup>c</sup>	1.000	
р	<0.001	<0.001	<0.001		

Median, minimum and maximum scores for smear layer. Lowercase superscript letters (a, b, c) indicate the presence of a significant difference between treatment groups according to multiple comparison tests. Uppercase superscript letters (A, B, C) indicate the presence of a significant difference between root levels according to multiple comparisons tests. D3N: DualRinse HEDP+3% NaOCI without activation, D3NA: DualRinse HEDP+3% NaOCI with activation during the final irrigation, 3NE: 3% NaOCI+17% EDTA+3% NaOCI without activation, 3NEA: 3% NaOCI+17% EDTA+3% NaOCI with activation during the final irrigation, NC: Negative control group

and 3NE (Figs. 1, 2). The debris score was significantly higher in the control group compared to all other groups.

Intragroup comparisons revealed no significant differences regarding debris score among the different root levels for all groups



Figure 1. SEM images at the apical level (×200) for the evaluation of debris removal in D3N (a), D3NA (b), 3NE (c), 3NEA (d) and NC (e) SEM: Scanning electron microscopy, D3N: DualRinse HEDP+3% NaOCl without activation, D3NA: DualRinse HEDP+3% NaOCl with activation during the final irrigation, 3NE: 3% NaOCl+17% EDTA +3% NaOCl without activation, 3NEA: 3% NaOCl+17% EDTA+3% NaOCl with activation during the final irrigation, NC: Negative control group



Figure 2. SEM images at coronal and middle levels for the evaluation of debris and smear layer removal (×200, ×1000) in D3N (a), D3NA (b), 3NE (c), 3NEA (d) and NC (e)

SEM: Scanning electron microscopy, D3N: DualRinse HEDP+3% NaOCl without activation, D3NA: DualRinse HEDP+3% NaOCl with activation during the final irrigation, 3NE: 3% NaOCl+17% EDTA+3% NaOCl with activation, 3NEA: 3% NaOCl+17% EDTA+3% NaOCl with activation, NC: Negative control group

except 3NE, where the debris score was greater on the apical level, and the difference was not significant between the middle and the coronal levels (p>0.05). Therefore, using DualRinse HEDP resulted in less debris in all canal thirds compared to the classic approach, and implementing sonic activation improved it even more.

Moreover, the smear layer scores were significantly different among the 5 treatment groups at all root levels (p<0.001) (Table 2). At the apical level, the score was lower for D3NA, followed by D3N, 3NEA and 3NE, and it was the greatest for the control group (NC) (Figs. 2, 3). At the middle and coronal levels, the score was statistically different for NC only, while the difference was not significant among the other 4 groups (p>0.05).

Intragroup comparisons revealed significant differences only for D3N and 3NE (p<0.001). For these groups, the smear layer score was greater at the apical level. No significant differences could be detected for all other groups, D3NA, 3NEA, and NC. Together this data showed that using DualRinse HEDP resulted in less smear layer in the apical third compared to the conventional method, and applying sonic activation removes the smear layer even further.





#### DISCUSSION

The creation of the smear layer and debris results from the mechanical instrumentation of root canals. There is no evidence that removing the smear layer or debris is mandatory for clinical success. However, it can ensure better cleaning and penetration of the sealer (16). Sonic activation has been evaluated as a method of irrigant activation with promising results on the smear layer and debris removal (17).

DualRinse HEDP combined with NaOCI aims to be used throughout the root canal treatment and to overcome the incompatibility between NaOCI and EDTA. It combines the disinfection and dissolving properties of NaOCI and the chelating ability of HEDP (18). This combination may lead to a continuous chelating effect whereby the generation of smear layer and debris during instrumentation can be reduced (9). According to the study of Ballal et al. (19), the fresh mixture of HEDP and NaOCI did not show increased toxicity compared to NaOCI alone. Also, the 24 h old mixture was less toxic and statistically similar to pure etidronate, which showed little cytotoxicity and no genotoxicity at the tested dilutions.

The present study showed that DualRinse HEDP+3% NaOCI improved debris removal. DualRinse+3% NaOCI with high-power sonic activation (D3NA) had significantly less debris at all root

canal levels than the other groups, followed by DualRinse HEDP+3% NaOCI without activation (D3N) and NaOCI-EDTA-NaOCI group with activation (3NEA). Groups D3N and 3NEA had comparable percentages of residual debris and significantly less remaining debris compared to NaOCI-EDTA-NaOCI without activation (3NE). Moreover, all the groups showed significantly less debris than the control group (NC).

These results also indicate that high-power sonic activation with EDDY of the novel DualRinse HEDP mixed with 3% NaOCI contributes to better canal wall cleaning at all root levels. This is in accordance with other studies using EDDY with other irrigants (1, 20, 21).

Besides improving debris removal, the present study also found that DualRinse HEDP+3% NaOCI can enhance smear layer removal at the apical level. D3NA had significantly less smear layer at the apical level than the other groups, followed by D3N and 3NEA. Furthermore, groups D3N and 3NEA had comparable percentages of smear layer and significantly less remaining smear layer than 3NE. Moreover, all the groups showed significantly less smear layer than the control group (NC). Together, these results suggest that activation with EDDY of DualRinse HEDP mixed with 3% NaOCI delivered the irrigant more efficiently to the apical level and hence removed the smear layer more effectively. Improved efficacy of smear layer removal with sonic activation has been previously reported by several studies, such as those by Rödig et al. (2), Urban et al. (1) and Kharouf et al. (22), which showed that upon using sonic activation (EQ-s) and ultrasonic irrigation (Endoultra), more smear layer is removed at the apical third which is comparable to the results of our study. However, until now, no studies evaluated the improved efficacy of irrigant activation using DualRinse HEDP.

In this study, it has been successfully demonstrated that activation with EDDY at a frequency of 6000 Hz enhances DualRinse HEDP action against debris at all levels of the canals and smear layer at the apical level, leading to more open dentinal tubules. However, the results of this study are not in accordance with the findings of Kfir et al. (13), which did not detect any significant difference in debris and smear layer removal between DualRinse HEDP+NaOCI and NaOCI-EDTA-NaOCI without activation. In contrast, studies by Ulusoy et al. and Erik et al. (23, 24) confirmed that the use of 9% HEDP and 18% HEDP resulted in increased removal of the smear layer than EDTA at the apical third, which is comparable to the results of the present study. Additionally, it is important to note the limitations present in this study. The debris and smear layer removal at 3 root levels was evaluated using SEM by 5-grade score at ×200 magnification and 4-grade score at ×1000 magnification, respectively. In the literature, the methodology of evaluation differs between studies from the 3-grade score (25) to 5- grade score (1,2) systems. Also, SEM magnification varies between 35 and 2000× for debris evaluation (2, 26, 27) and between 300 and  $3000 \times$ for smear layer evaluation (27, 28). Therefore, results may vary according to the methodology used in the different studies. Moreover, it must be taken into consideration that SEM images are limited to some areas of the canals. To standardise the areas inspected, marks were made on the mesial side of the root at 3, 6 and 9 mm from the apex to evaluate the apical, middle and coronal areas, respectively. Another limitation of the SEM methodology is that it provides a two-dimensional image that does not allow the measurement of the thickness of the smear layer and debris (29).

This study was also limited by the generation of microscopic debris during the cutting step with the diamond disk, which might raise the percentage of debris in the results. However, this limitation was minimised with the use of gutta-percha inserted into the canal during the cutting, preventing the debris from entering the canal during the cutting procedure.

Lastly, the percentage of the smear layer might vary with the shaping instrument that touches the canal walls depending on the canal's anatomy. For example, instruments could have a round cross-section, while canals might have an oval cross-section; hence, there will always remain some parts of the canal walls untouched during the shaping. According to the study of Peters et al. (30), all instruments left 35% or more of the canals' surface area untouched.

In conclusion, the current study aimed to investigate the effect of combining a soft continuous chelation protocol with highpower sonic activation on the accumulation of debris and smear layer during root canal treatment of extracted human teeth. It has been reported that continuous chelation and activation do not interfere with each other. On the contrary, the combination of both elements was either equal or superior to conventional irrigational protocols with or without activation. Using 3% NaOCI in combination with DualRinse HEDP for root canal irrigation resulted in lower amounts of residual debris at all levels of the root canal and enhanced smear layer removal at the apical level, in comparison with the conventional protocol using NaOCI-EDTA-NaOCI. These results were further enhanced when adding high-power sonic activation.

#### Disclosures

Conflict of interest: The authors deny any conflict of interest.

**Ethics Committee Approval:** This study was approved by The Saint Joseph University Ethics Committee (Date: 16/02/2021, Number: USJ-2021-39).

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