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Effect of Supraperingival Irrigation with Aerosolized 0.5% Hydrogen Peroxide on Clinical Periodontal Parameters, Markers of Systemic Inflammation, and Morphology of Gingival Tissues in Patients with Periodontitis

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Background: Various studies have shown that non-surgical periodontal treatment is correlated with reduction in clinical parameters and plasma levels of inflammatory markers. The aim of this study was to evaluate the effect of long-term weekly supraperingival irrigations with aerosolized 0.5% hydrogen peroxide as maintenance therapy followed by non-surgical periodontal treatment on clinical parameters, plasma levels of inflammatory markers, and morphological changes in gingival tissues of patients with periodontitis.





Material/Methods: In total, 43 patients with chronic periodontitis were randomly allocated to long-term maintenance therapy. The patients' periodontal status was assessed using clinical parameters of approximal plaque index, modified gingival index, bleeding index, pocket probing depth, and plasma levels of inflammatory markers (high-sensitivity C-reactive protein and white blood cell count) at baseline and after 1, 2, and 3 years. The morphological status of gingival tissues (immediately after supraperingival irrigation) was assessed microscopically.

Results: Complete data were obtained on 34 patients. A highly statistically significant and consistent reduction was observed in all long-term clinical parameters and plasma levels of inflammatory markers. Morphological data showed abundant spherical bubbles in gingival tissues.

Conclusions: 1. The present study showed that non-surgical periodontal treatment with long-term weekly supraperingival irrigations with aerosolized 0.5% hydrogen peroxide improved clinical periodontal status and plasma levels of inflammatory markers and may be a promising method in periodontology. 2. We found that supraperingival irrigation with aerosolized 0.5% hydrogen peroxide created large numbers of spherical bubbles in gingival tissues.

MeSH Keywords: **C-Reactive Protein • Hydrogen Peroxide • Leukocyte Count • Periodontal Diseases • Therapeutic Irrigation**

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Background

Studies have shown the effectiveness of supragingival irrigation applied as monotherapy and in combination with root planing, and revealed that supragingival irrigation with a variety of agents reduced the gingival microbial load and gingival inflammation, and as a method of maintenance periodontal therapy, it plays an important role in the longevity of teeth [1]. This further emphasizes the importance of supragingival irrigation in the treatment of periodontal disease.

Research has begun to examine the utility of low-cost antiseptic agents as adjuncts to mechanical periodontal therapy [2]. Hydrogen peroxide (H_2O_2) has a long history of use as an effective antimicrobial agent with a broad spectrum of activity, including activity against bacterial spores and viruses [3], and is extensively used in applications where its decomposition into non-toxic byproducts (water and oxygen) is important [4].

The delivery of antibacterial agents to gingival pockets colonized by anaerobic organisms presents a challenge. Therapeutic delivery of H_2O_2 requires mechanical access to subgingival pockets [5]. Data in the literature indicate that nebulizers for liquid aerosolization are a better method for delivery of H_2O_2 [6], and similar results can be achieved with lower drug concentrations [7].

It has been noted [3] that the bactericidal efficacy of aerosolized H_2O_2 exceeds the effects of direct soaking in pure H_2O_2 solutions at room temperature. Steindl et al. [8] also noted that aerosolized H_2O_2 effectively removed pathogens from surfaces.

Thus, the future of supragingival irrigations is promising, and should provide a more predictable adjunct for the treatment and maintenance of periodontal patients [1]. However, whether and how this intervention affects long-term periodontal parameters remains to be established [9].

The aim of this study was to evaluate the effect of long-term weekly supragingival irrigations with aerosolized 0.5% H_2O_2 as maintenance therapy followed by non-surgical periodontal treatment on clinical parameters, plasma levels of inflammatory markers, and morphological changes in gingival tissues of patients with periodontitis.

Material and Methods

Reagents

Hydrogen peroxide (30%, analytical grade) was obtained from Reachem, Bratislava, Slovakia. A 6% stock solution was prepared in distilled water. The 0.5% H_2O_2 solution for supragingival

irrigations was prepared daily. Formalin 10% neutral-buffered was obtained from Sigma-Aldrich (St. Louis, MO).

Patient selection

The patients (n=43) for this study were selected from a large number of individuals treated at the Department of Odontology of the Lithuanian University of Health Sciences. Screening examinations included medical history, clinical examinations, full-mouth intra-oral radiographs, and pocket depth measurements to confirm the diagnosis of chronic periodontitis.

To participate in this prospective longitudinal study, patients had to fulfill the following inclusion criteria: age 20–50 years, a minimum of 20 teeth in the mouth, pocket depth ≥ 6 mm on at least 2 teeth, and radiographic evidence of horizontal and vertical bone loss.

We excluded subjects with systemic disorders (diabetes mellitus, rheumatoid arthritis, or osteoporosis), systemic antibiotic use during the study or the preceding 6 months, and smoking or alcohol intake.

Prior to the beginning of the trial, a decision was made that subjects exhibiting marked disease progression (inflammation or suppuration) between annual examinations would be excluded from the study and would receive additional treatment.

All patients received full information about the importance of oral hygiene and the procedures that can be performed at home. All patients were also familiarized with the same Fones tooth-brushing technique. To highlight the influence of weekly supragingival irrigation alone on periodontal tissues, the patients were asked not to alter their usual diet or lifestyle. While at home, the subjects also used their usual oral hygiene procedures.

All patients in this study signed the informed consent form approved by Kaunas Regional Bioethics Committee. All experiments were conducted in accordance with the rules and regulations approved by Kaunas Regional Bioethics Committee (No. BE-2-21).

Data of clinical examination

The 4 clinical indices of periodontal inflammation discussed in this paper are approximal plaque index (API), modified gingival index (MGI), bleeding index (BI), and pocket probing depth (PPD).

Approximal Plaque Index. Oral hygiene was assessed using the API according to Lange et al. [10]. The total percentage of plaque-containing surfaces was calculated.

Modified Gingival Index. To assess gingival inflammation, the MGI [11] was calculated on the facial and lingual surfaces at 2 sites of each tooth (the papillae and the margin). These assessments were performed on all evaluable teeth. Compressed air, water, and mouth mirrors were available to the examiner.

The mean MGI was calculated by dividing the sum of all scores by the total number of the examined surfaces.

Bleeding Index. The Bleeding Index (a modified Index of Saxton [12]) was determined, using the Williams periodontal probe in the evaluation (Hu-Friedy, Chicago, IL).

Pocket probing depth was measured and recorded at 6 sites per tooth (disto-buccal, buccal, mesio-buccal, mesio-lingual, lingual, and disto-buccal) using the Williams periodontal probe (Hu-Friedy, Chicago, IL).

The examiner was trained and calibrated in performing the clinical measurements before the examinations. For the assessment of measurement reproducibility, replicated periodontal measurements performed with a 2-day interval were used. At the site level, reproducibility was assessed, and the result was 0.82. The procedures were performed manually by a single experienced periodontist (R.S.).

Following the baseline examination, all patients underwent a non-surgical periodontal debridement procedure.

Treatment procedure

Following the completion of the non-surgical periodontal debridement procedure, all subjects were enrolled in a maintenance periodontal therapy – the weekly supragingival irrigation procedure with 0.5% aerosolized hydrogen peroxide. We selected this concentration because irrigation with higher concentrations (1% or 3%) of aerosolized H₂O₂ caused gingival pain in the studied patients.

For the irrigation procedure, the dental unit Knight Asepsis (Midmark Corp., Versailles, OH) angular air and water dental syringe was used along with a reservoir for hydrogen peroxide solution, which could be adjusted for the pressure of about 60 psi and the water outflow of 200 mL.

The solution was applied with the irrigator on the gingival margins at the angle of 45 degrees [1]. The temperature of the hydrogen peroxide used during the procedure was 37°C. Each irrigation procedure required about 5 min.

The procedure of the assessment of supragingival irrigations on the clinical indices of periodontal inflammation, PPD, and

plasma levels of inflammatory markers (hs-CRP and WBC count) was then repeated after 1 year, 2 years, and 3 years.

In the absence of positive response (PPD ≥ 5 mm and with inflamed sites), non-surgical treatment was repeated and performed under anesthesia.

Blood samples and laboratory analyses

Twelve-hour fasting blood samples were taken from every participant from the antecubital vein for hs-CRP and WBC count measurements. The hs-CRP level in the plasma was assessed by using particle-enhanced turbidimetric assay on a Cobas Integra 400 Plus analyzer (Roche Diagnostics, Mannheim, Germany). The precipitate was determined turbidimetrically at 552 nm. WBC count (10⁹/L) was obtained by using a hematology analyzer ADVIA 2120 (Siemens Healthcare Diagnostics, Dublin, Ireland).

Morphological findings of periodontal tissues

If teeth with inflamed sites and PPD ≥ 5 mm were detected during the yearly evaluations of the efficiency of the long-term maintenance periodontal therapy with 0.5% aerosolized H₂O₂, immediately after supragingival irrigation, the patients underwent gingivectomy under local anesthesia, and the specimens of the gingival tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, and then sectioned in 3-mm sections and stained with hematoxylin and eosin.

The specimens of the gingival tissue for morphological investigation were obtained in the following sequence: 3 control samples were obtained at baseline, and the other 9 samples were obtained after 1 year, 2 years, and 3 years (in total, 12 control samples). The control samples were obtained before the irrigation procedure (at least 1 week after the previous irrigation procedure). Nine study samples were obtained after 1 year, 2 years, and 3 years of supragingival irrigations immediately after the irrigation procedure because the cavitation bubbles exist for a very short time.

The histological evaluation of the samples was performed with a cold light microscope Olympus BX40F4 (Olympus Optical Co., Ltd., Japan) under 7× and 10× magnification using CellSens Dimension 1.9 Digital Imaging Software for Research Applications (Olympus Corporation of the Americas, USA).

Statistical analysis

Statistical analysis of the data was performed by using the statistical software package SPSS 20.0 for Windows. Descriptive parameters were shown as the mean, standard deviation, median, and interquartile range. Every data set was tested for

normality with the Kolmogorov-Smirnov test. The Wilcoxon signed-rank test was used for intergroup comparison. The difference was considered to be statistically significant when the level of significance p was less than 0.05.

Results

Baseline examination. In total, 43 patients with chronic periodontitis (21 females and 22 males) aged between 25 and 50 years were examined.

Already in the first year of the study, 9 subjects withdrew from the trial because of irregular attendance. Therefore, the results at baseline and at the re-examination after 1 year are discussed only for 34 patients. The patients' baseline characteristics are presented in Table 1.

The clinical findings of the baseline examination are presented in Table 2.

The distribution of teeth with different pocket depth categories (<4 mm, 4–5 mm, 5–6 mm, and >6 mm) in the subject sample is presented in Table 3.

The baseline blood WBC count and plasma hcCRP levels are presented in Table 4.

Re-examination after 1 year. The reduction in API, MGI, BI, and PPD between baseline and after 1-year maintenance care was statistically significant (Table 2).

During this first 12-month interval, a significant increase in the number of teeth with shallow pockets (<4 mm) and a significant decrease in the number of teeth with deep pockets (4–5 mm, and with 5–6 mm) were observed (Table 3). The number of teeth with pocket depth >6 mm remained unchanged.

The data from the re-examination conducted after 1 year also showed a significant decrease in the plasma levels of inflammatory markers (Table 4).

Re-examination after 2 years. During the entire 2-year period, the plaque score continued to decrease significantly, compared to the analogous data collected after 1 year (Table 2). The number of sites that exhibited bleeding on probing and MGI also decreased significantly (Table 2).

The mean PPD values demonstrated only minor changes over time (Table 2). This decrease in the mean PPD value was not statistically significant. However, the number of teeth with shallow (<4 mm) pockets (Table 3), which was established during the examination after 1 year and was recorded during the

Table 1. Patients' baseline characteristics (mean \pm SD, median, and interquartile range).

Characteristics	Periodontitis patients (n=34)
Age (years)	
Mean \pm SD	41.2 \pm 6.0
Median	40.0
Interquartile range	39.8–44.1
Sex	
Male (n (%))	15 (44.1)
Female (n (%))	19 (55.9)
Number of teeth	
Mean \pm SD	24.9 \pm 2.2
Median	24.8
Interquartile range	23–26
API (%)	
Mean \pm SD	35.9 \pm 4.3
Median	35.8
Interquartile range	29.1–42.2
MGI (points)	
Mean \pm SD	2.6 \pm 0.6
Median	2.6
Interquartile range	2.1–3.1
BI (%)	
Mean \pm SD	37.2 \pm 4.1
Median	37.2
Interquartile range	35.2–40.4
PPD (mm)	
Mean \pm SD	4.6 \pm 0.4
Median	4.6
Interquartile range	4.4–4.9
Percent of teeth with PPD	
\leq 4 mm	27.4
4–5 mm	42.0
5–6 mm	22.4
>6 mm	8.2

re-examination at year 2, increased statistically significantly. The number of teeth with pocket depth of 4–5 mm decreased significantly. The reduction in the number of teeth with pocket depth of 5–6 mm, compared to the examination after 1 year, was not statistically significant, yet it significantly differed from the findings of the baseline examination. The number of teeth with very deep pockets (>6 mm) did not change.

Over the period between year 1 and year 2, the levels of inflammatory markers continued to drop. This decrease was statistically significant for both studied markers (Table 4).

During the second year, the patients lost 2 teeth (0.2%), and 1 patient withdrew from the trial for reasons unrelated to the study.

Table 2. Clinical characteristics of chronic periodontitis patients during different examinations (mean ±SD, median, and interquartile range).

Examinations	Number of teeth	API (%)	MGI (points)	BI (%)	PPD (mm)
Baseline (n=34)	24.9±2.1	35.9±4.3	2.6±0.6	37.2±4.1	4.6±0.4
	24.8	35.8	2.6	37.2	4.6
	23–26	29.1–42.2	2.1–3.1	35.2–40.4	4.4–4.9
Year 1 (n=34)	24.9±2.1	22.7±3.8 [#]	1.2±0.4 [#]	15.7±4.0 [#]	4.0±0.3 [*]
	24.8	22.6	1.1	15.7	4.0
	23–26	20.2–25.4	0.9–1.4	10.9–18.6	3.8–4.2
Year 2 (n=33)	24.9±2.1	17.6±3.2 ^{**}	0.6±0.3 ^{**}	9.0±2.4 ^{**}	3.8±0.3 [*]
	24.8	17.6	0.6	9.0	3.8
	23–26	14.9–19.8	0.4–0.8	7.6–9.7	3.6–3.9
Year 3 (n=30)	24.8±2.1	12.9±2.4 ^{***}	0.3±0.2 ^{**}	4.8±1.1 ^{***}	3.5±0.3 ^{**}
	24.8	13.0	0.3	4.8	3.4
	23–26	11.8–14.6	0.2–0.4	4.2–5.0	3.3–3.9

[#] p<0.01 compared to baseline; ^{*} p<0.05 compared to baseline; ^{**} p<0.05 compared to year 1; ^{***} p<0.05 compared to year 2.

Table 3. Changes in the distribution of teeth with different pocket depth categories between baseline and examinations at years 1, 2, and 3 in patients with periodontitis.

Examinations	Teeth	PPD (mm)				Total
		<4	4–5	5–6	>6	
Baseline (n=34)	Number	235	360	192	70	857
	Percent	27.4	42.0	22.4	8.2	100
Year 1 (n=34)	Number	427	249	111	70	857
	Percent	49.8 [#]	29.1 [#]	12.9 [*]	8.2	100
Year 2 (n=33)	Number	568	120	81	63	832
	Percent	68.2 ^{**}	14.4 ^{**}	9.7 [*]	7.6	100
Year 3 (n=30)	Number	594	45	61	56	756
	Percent	78.6 ^{***}	5.9 ^{***}	8.1 ^{**}	7.4	100

[#] p<0.01 compared to baseline; ^{*} p<0.05 compared to baseline; ^{**} p<0.05 compared to year 1; ^{***} p<0.05 compared to year 2.

Table 4. Evaluation of WBC and hs-CRP values pre- and post-treatment (mean ±SD, median, and interquartile range).

Parameter	Chronic periodontitis (n=34)				P
	Pre-treatment (n=34)	Post-treatment			
		Year 1 (n=34)	Year 2 (n=33)	Year 3 (n=30)	
	1	2	3	4	
WBC (10 ⁹ /L)	7.40±0.23	6.71±0.31	6.48±0.29	6.40±0.27	p _{1,2} <0.001
	7.39	6.70	6.48	6.40	p _{2,3} <0.01
	7.17–7.49	6.06–7.32	5.99–7.20	5.83–6.96	p _{3,4} >0.05
hs-CRP (mg/L)	2.48±0.45	1.52±0.36	1.31±0.29	1.24±0.26	p _{1,2} <0.001
	2.5	1.52	1.30	1.25	p _{2,3} <0.05
	2.36–2.64	1.27–1.74	1.12–1.50	1.07–1.39	p _{3,4} >0.05

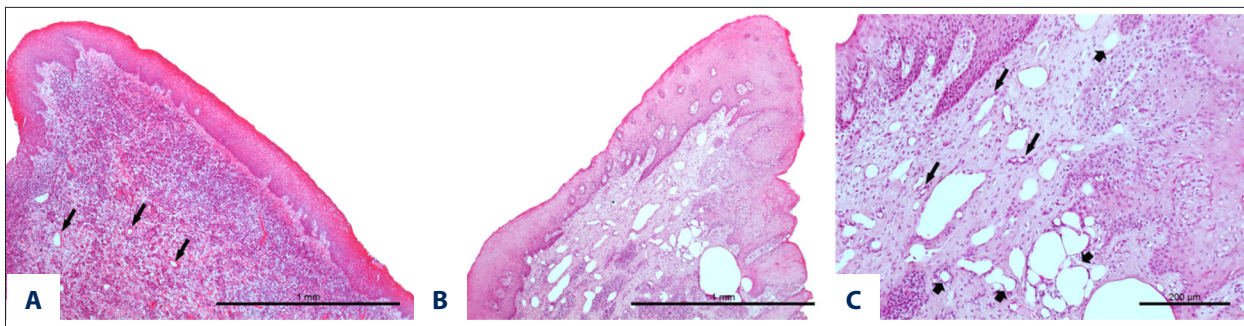


Figure 1. Morphological study of gingival tissues of periodontitis patients. (A) Gingival tissues not exposed to supragingival irrigation. (B, C) Gingival tissues exposed to supragingival irrigation with aerosolized 0.5% H₂O₂. Blood vessels are indicated by arrows. The scale bar in A and B is 1 mm, and in C is 200 µm.

Examination after 3 years. The API scores and the number of sites that exhibited bleeding on probing (BI) between year 2 and year 3 examinations continued to decrease statistically significantly (Table 2). The MGI during the 3-year period of maintenance therapy decreased statistically significantly, compared to the baseline examination (Table 2), but essentially did not differ from the respective data for year 2.

The number of teeth with shallow pocket depth (<4 mm) increased by almost 3 times from baseline examination (Table 3). The number of teeth with 4–5 mm and 5–6 mm pockets also decreased significantly. No significant changes in the number of teeth with very deep pockets (>6 mm) were observed during the 3-year period.

During the third period of the study, the decrease in the levels of inflammatory markers was not as pronounced, and did not differ statistically significantly from the analogous data obtained after year 2 examinations.

Between year 2 and year 3 examinations, the studied periodontitis patients lost 1 multi-rooted tooth with PPD >6 mm at baseline examination. This loss amounted to 0.13% of all teeth. Also, 3 patients withdrew from the trial during the third period of maintenance therapy for reasons unrelated to the study.

None of the patients during the entire 3-year period of maintenance therapy complained of the exacerbation of the disease or suppuration of periodontal tissues, except for 2 patients in whom 2 multi-rooted teeth and 1 premolar (about 0.13% of all teeth per year) were removed due to persistent inflammation and increased tooth mobility. At baseline examination, these teeth had been found to have PPD >6 mm and a furcation.

During the entire 3-year period, 4 patients withdrew from the trial for reasons unrelated to the study.

Morphological data. The morphological study of gingival tissue samples showed a severely damaged extracellular matrix,

disorganized collagen fibers, and many inflammatory cells (Figure 1A). The samples of gingival tissue taken out immediately following supragingival irrigations with aerosolized 0.5% H₂O₂ showed many spherical bubbles (Figure 1B, 1C). There were great variations in bubble size, which most frequently reached 15–50 microns. These bubbles were similar to cavitation bubbles, which form when fluids are exposed to ultrasound.

Discussion

Changes observed in periodontal parameters

During the 3-year period of maintenance care of periodontitis patients with 0.5% aerosolized H₂O₂, the percentage of tooth surfaces harboring plaque decreased from about 36% to about 13% (Table 2). Other authors [13] also noticed that H₂O₂ is effective in reducing plaque. The effectiveness of H₂O₂ in plaque removal has been ascribed to the physical effect of the stream of H₂O₂ and to the bubbling of the oxygen as it is released from the peroxide [14]. In addition to that, oral hygiene might also have improved because during the extension period, the periodontitis patients maintained a high standard of self-performed oral hygiene developed due to their weekly visits for oral irrigation procedures. Several authors [9,13,15] have noted that repeated motivational interviewing and reinforcement of oral health education may improve oral hygiene knowledge and practices, and may decrease plaque levels and early rate of tooth loss.

In this study, care of periodontitis patients with aerosolized H₂O₂ also prominently decreased MGI and BI from 2.6 to 0.3 points and from 37.2% to 4.8%, respectively (Table 2). MGI and BI represent measures of the severity of the inflammatory burdens within the gingival tissues, and BI is one of the most important periodontal parameters for evaluating the periodontal status of patients with periodontitis [16]. BI is also a very important periodontal parameter that shows a significant relationship with systemic parameters such as CRP [17] and WBC count, as confirmed in a recent study by Bokhari et al. [18].

The reduction in these clinical parameters proves the effectiveness of weekly supragingival irrigations with 0.5% aerosolized H₂O₂ in maintaining gingival health. In a review of H₂O₂ use in dentistry, Marshall et al. [5] concluded that H₂O₂ delivered to deep pockets effectively reduced periodontal disease.

It is also known that oral irrigations with a sole jet stream of water generate benefits for gingival health [19]. This is probably also influenced by the fact that supragingival irrigations have demonstrated their effectiveness in decreasing not only periodontal pathogens [20], but also bacterial toxins [21] and byproducts of the host's inflammatory response associated with periodontitis [22]. The use of oral supragingival irrigation also entails changes in the cytokine levels of IL-1 β and PGE₂, which are associated with destructive changes in the inflamed tissue, as well as with bone resorption [23,24].

In the current study, the analysis of changes in the distribution of teeth with different pocket depth categories (Table 3) further emphasizes the efficacy of supragingival irrigation with 0.5% aerosolized H₂O₂. During the period between the examinations, the percentage of teeth with shallow pockets (<4 mm) increased from 27.4% to 78.6%, the percentage of teeth with pocket depth of 4–5 mm dropped from 42.0% to 5.9%, and the percentage of teeth with 5–6 mm pockets decreased from 22.4% to 8.1%. Long-term and regular supragingival irrigation with 0.5% aerosolized H₂O₂ fundamentally decreased the percentage of teeth with PPD 5–6 mm. However, the number of teeth with very deep (>6 mm) pockets remained virtually unchanged. Residual PPD >6 mm strongly influenced the prognosis for molars [25] and represents a risk factor for disease progression and tooth loss [26].

Pink et al. [27] noticed that WBC count showed consistent long-term associations with PPD. Meanwhile, Skaleric et al. [28] noted that CRP levels also correlated with PPD. These associations of PPD with WBC count and CRP levels may be explained by the fact that in periodontitis patients, PPD results in the formation of large subgingival (about 13 cm²) and inflammatory burden (about 9 cm²) areas [28]. This appears to be the cause of the close association between PPD and plasma levels of inflammatory markers such as CRP and WBC count.

Changes observed in plasma levels of inflammatory markers

We evaluated increased baseline levels of hs-CRP and WBC count in the plasma of patients with chronic periodontitis, and analyzed their long-term decrease following non-surgical periodontal therapy and regular maintenance with weekly supragingival irrigations with aerosolized 0.5% H₂O₂ (Table 4). These results were in accordance with data from clinical trials published by various other authors. Many studies have reported a

reduction in CRP and WBC count after non-surgical periodontal therapy and maintenance therapy in periodontitis patients with and without cardiovascular diseases [29–31] or the metabolic syndrome [32] and advanced periodontitis [33]. The literature data showed that CRP levels increased together with the severity of the periodontal disease, and the presence of *P. gingivalis* in subgingival plaque [34]. The BI showed a much better positive correlation with the CRP levels [17] and with the severity of periodontal disease [35]. Our data (Tables 2, 4) are compatible with the data of the aforementioned authors.

Behle et al. [36] showed that non-surgical periodontal therapy without maintenance therapy resulted in a short-term overall reduction of systemic inflammation, but the responses were inconsistent across subjects and were largely not sustainable. The importance of regular maintenance therapy was especially prominent in a 5-year prospective study by Costa et al. [37], which established that among irregular compliers, tooth loss was as high as 0.36 teeth lost/year, while it was significantly lower among regular compliers (0.12 teeth lost/year). Our data coincide with the findings of the aforementioned authors.

Our findings on the effectiveness of the adjunctive use of regular local-delivery bactericidal drugs such as aerosolized 0.5% H₂O₂ along with mechanical oral hygiene confirmed the data of other authors [38], indicating that the adjunctive use of a local drug provides a clear beneficial response to periodontal treatment.

In addition, human periodontal ligament fibroblasts are sensitive to mechanical stimulus [39], and mechanical stress (supragingival irrigation) can regulate the expression of some inflammation-related cytokines [40].

Thus, non-surgical periodontal therapy in combination with regular long-term maintenance therapy resulted in a significant decrease in hs-CRP and WBC and an improvement in clinical periodontal parameters such BI, MGI, and PPD, and seems to have a potential benefit for overall health.

Observed morphological changes in gingival tissue

Our study showed that following supragingival irrigations with aerosolized 0.5% H₂O₂, abundant thin-walled spherical bubbles formed in the gingival tissues (Figure 1B, 1C), the size of the bubbles ranging from 10 μ m to 100 μ m. The cavities were round in shape, in some places becoming confluent and forming larger irregular cavities. No such changes in the structure of the gingival tissues were observed in control samples (Figure 1A). We failed to find any analogous data in the medical literature.

Cavitation bubbles are known to form following acoustic cavitation [41], hydrodynamic cavitation [42], and spray [43].

Cavitation is a complex phenomenon that involves creation, oscillation, growth, and collapse of bubbles within a liquid medium depending on local pressure variation [44]. It is a well-known fact that during the collapse of cavitation bubbles, radicals such as $\text{OH}\cdot$, $\text{H}\cdot$, and $\text{HOO}\cdot$ are generated [42,45]. In all cases, hydroxyl radical is the main oxidant produced in the bubble [45]. Yasui et al. [46] noticed that a bubble produces not only an $\text{OH}\cdot$ radical, but also O atoms and H_2O_2 .

We hypothesized that our discovered spherical bubbles that formed in the gingival tissue following supragingival irrigations with aerosolized 0.5% H_2O_2 could produce cavitation bubbles. The size of most of these bubbles was 15-50 μm , which is in accordance with the description of bubbles found in the literature [41].

It is unclear how the cavitation bubbles reached the deeper layer of the gingival tissue (Figure 1B, 1C) because no visible damage to the extracellular matrix of the gingival tissue was detected.

The generation of reactive oxygen species following the collapse of the cavitation bubbles may create an antibacterial medium in the gingival tissues, and thus may contribute to the improvement of all periodontal parameters.

However, $\text{H}\cdot$ created during the collapse of cavitation bubbles [42,45] is capable of neutralizing the cytotoxic ROS selectively, rectifying abnormalities in the apoptotic cascades and attenuating the related inflammatory responses [47].

The application of the cavitation phenomenon is a relatively new field in medical therapy [48], where cavitation in ultrasound treatment is used for drug delivery in selectively permeable regions of tissues [49,50]. Cavitation bubbles can be used to track the drug carrier [51,52], and materials whose release is triggered by ultrasound are known to become entrapped in microbubbles [53].

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Conclusions

It is yet unclear whether spray-induced cavitation bubbles also have these characteristics. Further studies with larger samples of patients with periodontitis and/or gingivitis would be necessary to substantiate this method for the treatment and prevention of periodontal disease.

- The present study showed that non-surgical periodontal treatment with long-term maintenance therapy (weekly supragingival irrigations with aerosolized 0.5% hydrogen peroxide) improved clinical periodontal status and plasma levels of inflammatory markers and may be a promising method in periodontology. However, these findings need to be confirmed in larger and more profound studies to allow for any generalization regarding the potential benefits of this therapy.
- We found that supragingival irrigation with aerosolized 0.5% hydrogen peroxide created many spherical bubbles in gingival tissues. These findings require more accurate monitoring of morphological changes in periodontal tissues and the potential (positive or negative) effect of irrigation with aerosolized 0.5% hydrogen peroxide.

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