

# The Efficiency of Ozonated Gel vs Chlorhexidine Gel as Adjunct Treatment to Control Plaque-Induced Gingivitis Assessed by Interleukin-1 $\beta$ Levels in Gingival Crevicular Fluid (A Comparative clinical trial)

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## Abstract

**Aim:** The aim of this study was to compare the clinical effectiveness of ozonated gel (OZ) or Chlorhexidine Gel (CHX) on patients affected with plaque - induced gingivitis (PIG) based on the evaluation of the interleukin-1 $\beta$  (IL-1  $\beta$ ) cytokine level as a biomarker in Gingival Crevicular Fluid (GCF) after one week of treatment .

**Materials and methods:** Fifty male patients affected with (PIG) (25/group) with an average age of (18-30) years were enrolled throughout the study. They were divided into two groups, (G1) scaling and treated with (OZ) gel and (G2) scaling and treated with (CHX) Gel. Participants were trained to rubbing their gingiva two times a day for 7 days. GCF were collected from four anterior teeth by paper strips (PS) at 0-time and at 7- days after treatments. Cytokine concentrations of IL-1 $\beta$  were determined in the GCF samples using Commercial ELISA diagnostic Kit Salivary (SALIMETRICS, USA. Assay).

**Results:** The median values of the GCF volumes at 0-time showed that there were no statistically significant differences among the two groups. However, at 7-days post treatments there were statistically significant differences with G1 group showed much more effectiveness compared to G2. The values of IL-1 $\beta$  concentration in each group at 0-time revealed no statistically significant differences. While, at 7-days the results revealed that there were statistically significant ( $p < 0.05$ ) differences.

**Conclusion:** GCF volume ( $\mu$ l) and IL-1 $\beta$  concentration (pg/ml) at 7-days post-treatment was directly correlated to the treatment used in each group (G1 and G2). OZ gel treated group showed significantly lower GCF volume and IL-1 $\beta$  concentration. This highlighted the evidence in favor of the OZ gel in the treatment of gingivitis.

**Keywords:** Plaque- Induced Gingivitis, Ozonated Gel, Chlorhexidine Gel, Gingival Crevicular Fluid, interleukin-1 $\beta$  cytokine.

## INTRODUCTION

Dental plaque inducers gingivitis is a revocable inflammatory condition caused by the accumulation and continuity of microbial biofilms (dental plaque) on the teeth. It is characterized by redness and swelling of the gingivae and a tendency for the gingivae to bleed easily. In susceptible individuals, gingivitis may lead to periodontitis and loss of the soft tissue and bony support for the tooth<sup>1</sup>. Since the 1960s when Loe<sup>2</sup> and coworkers established the clear role of dental plaque as the etiological agent of gingivitis, mechanical plaque control has become the cornerstone of periodontal therapy. The widespread prevalence of gingivitis suggests the inefficiency of the mechanical plaque control in preventing gingival inflammation.<sup>3,4</sup> To prevent gingivitis and its progression to periodontitis, daily and effective supra-gingival plaque control methods are considered the most effective methods to regain and maintain dental and periodontal health.<sup>5</sup> The difficulty in achieving an "ideal" mechanical plaque control has led scientists and clinicians to seek chemical antimicrobial agents that could help inhibit biofilm formation on the tooth surface.<sup>6</sup>

Chlorhexidine (CHX) is considered the most widely used adjunctive antiseptic in periodontal treatment and it is the "gold standard" agent for chemical plaque control methods, as it is a broad-spectrum antiseptic<sup>7,8</sup>. The results of Mariotti and Rumpf<sup>9</sup>; Mohammedi<sup>10</sup> suggested that the substantivity of chlorhexidine lasts for up to 12 weeks, moreover, even low concentrations of CHX may be toxic to gingival fibroblasts, thereby reducing the production of

collagen and non-collagen proteins and potentially delaying periodontal healing . Furthermore, an increasing number of immediate-type allergies to this agent have been reported, including contact urticaria, occupational asthma, and anaphylactic shock. Because of the possible of dangerous allergic reactions to CHX, topical usage of this drug, particularly to mucous membranes, is restrained<sup>11</sup>.

Ozone is now being regarded in dentistry as a possible alternative antiseptic agent. Ozone has effective antimicrobial activity against bacteria, fungi, protozoa, and viruses and does not induce microbial resistance.<sup>12,13,14</sup> Recent researches have reported antimicrobial effects on oral pathogens of both gaseous and aqueous forms of ozone, and the effectiveness of ozone in the treatment of oral diseases is now a topic of deep research.<sup>15,16</sup> Additional reports have indicated that aqueous ozone is extremely biocompatible with fibroblasts, cementoblasts, and epithelial cells, suggesting that aqueous ozone would be valuable in treating oral infectious diseases such as periodontal disease, apical periodontitis, and peri-implantitis.<sup>17,16</sup> Ozone can be used in several produces for treatment of periodontal disease: Ozonated water, ozonated oil, and gaseous ozone.<sup>18,19,20</sup>

In this study a clinical comparison between the effectiveness of the OZ gel and CHX Gel on plaque - induced gingivitis PIG has been evaluated based on the evaluation of the IL-1 $\beta$  cytokine concentration as a biomarker in GCF volume.

## MATERIALS AND METHODS

This study was carried out at the University of Baghdad / College of Dentistry / Department of periodontology and Al-Mansour Health Center for Family Medicine /Baghdad Health Administrative Office /Al-Karkh from December 2017 till June 2018. Fifty male patients with PIG, (25/group) with an average age of (18-30) years were enrolled throughout the study. They were divided into two groups, Group1(G1) scaling and OZ gel treatment (Fig.1) and group 2 (G2) scaling and CHX Gel treatment (Fig.2). Full medical history (name, age, smoking, drinking alcohol, antibiotics consumption, allergy, other medications) and dental history was obtained from each subject.



Fig.1 Ozonated gel



Fig.2 Chlorhexidine Gel

### Inclusion criteria: -

1. Male Patients with plaque-induced gingivitis
2. Age range of 18–30 years old
3. Patients exhibited no evidence of clinical attachment loss
4. Ready to give agreement.

### Exclusion criteria

1. Use of antibiotics in the past 3–4 weeks
2. History of dental treatment/use of mouthwash
3. Patient with acute necrotizing ulcerative gingivitis, acute herpetic gingivostomatitis, allergic gingivitis, gingivitis associated with skin diseases, gingivitis associated with endocrine-metabolic disturbances, gingivitis associated with hematologic-immunologic disturbances, gingival enlargement associated with medications, gingival tumors were excluded
4. Individuals with known systemic disease
5. smokers

### Collection of GCF samples

Before sample collection; the weight of four paper strips (W1), (PerioPaper Strips; Oraflow Inc. New York, USA, Fig.3) was predetermined and placed in a 1.5mL Eppendorf tube and weighted on an electronic balance with a readability of  $\pm 0.01$ mg and maximum capacity of 220 g. The main weight of the four (PS) was used as a reference weight for the rest calculation of the main GCF ( $M_{GCF}$ ). Collection of the GCF was performed repeatedly of four different teeth. The PS was transferred into the Eppendorf tube and immediately weighted (W2). The Main weight of GCF absorbed on the four PS was determined by differential weighing according to the equation adopted by Preiano<sup>21</sup>: -

$$MGCF = W2 - W1$$

- M=Main weight of GCF (mg).
- W2 = weight of GCF after GCF collection
- W1= weight of GCF before GCF collection



Fig.3 Paper Strips

### Sample collection:

Samples collection and processing were carried out as described by Preiano.<sup>21,22</sup>

Briefly, samples were collected in the morning, 2–3 h after breakfast from 50 patients. The study was approved by the internal Ethical Committee of University of Baghdad, Baghdad, Iraq. A written informed agreement was obtained from all patients prior to the start of the study. Patients were instructed to rub their gingiva twice a day for three minutes (at the morning and night) for seven days. Patients, also were advised to avoid eating, drinking or rinsing for half an hour after gel application. The GCF samples were collected from all patients on the same four sampling tooth sites by PS at baseline (0-time) and at 7-days. Pre-secreted saliva was removed by rinsing the oral cavity with water. Then, the four sampling sites were isolated from residual salivary contamination by using cotton rolls and by gently air-drying the tooth surface for 10 s. The newly formed crevice fluid was collected after 2 min with a  $2 \times 6$ -mm PS, pore size  $0.22 \mu\text{m}$ . Each PS was gently introduced into the gingival crevice until minimum resistance was felt and left in situ for 30 s. The collection was performed at the same four sites of each patient (Fig. 4). The four PS were removed after 30 s and transferred

into the Eppendorf tube which was sealed and immediately weighted. The total weight of GCF absorbed on the four PS was determined by differential weighing and the PS that contaminated with blood were discarded.<sup>22a</sup>



Fig.4 Collection of GCF samples

#### Extraction of the GCF (elution and centrifugal procedure from PS)

In each Eppendorf tube, the four (PS) were incubated with 300  $\mu$ L phosphate buffered saline (PBS). Samples were eluted at 4°C overnight (Refrigerator). Samples were centrifuged at 400 g (2000 RPM) for 4 min. The supernatants were kept frozen at -80°C until analyzed.<sup>23,24b</sup>

#### Determination of the of the GCF volume

All sites from each patient were pooled and used for GCF volume determinations. The mass of the fluid was calculated from the differences between masses of the PS with GCF and dry strips, then the value was divided by four to obtain the mean value per patient. The obtained value, expressed as  $\mu$ g, was converted to volume in  $\mu$ L assuming the density of GCF was 1 mg/ mL (Volume=Mass/Density).<sup>25</sup>

The obtained IL-1 $\beta$  value expressed as Pg/ml was adjusted to the original volume by multiplying the value by the Dilution Factor (DF).<sup>26</sup> [DF=Vf / Vi = Vd+Vi / Vi; Vf =Final volume; Vi =Initial volume; Vd =diluent volume].

#### Determination of IL-1 $\beta$ concentrations

Cytokine concentrations of IL-1 $\beta$  were determined in the GCF samples at 0-time and 7-days according to the manufacture procedure using Commercial ELISA diagnostic Kit Salivary (SALIMETRICS, USA. Assay). Briefly, the kit employs a quantitative “sandwich” enzyme immunoassay technique. A murine antihuman monoclonal

antibody specific for IL1- $\beta$  was precoated onto a 96-well microplate. Any IL1- $\beta$  present was bound by the immobilized antibody. After washing of unbound proteins, an enzyme-linked (horseradish peroxidase) polyclonal antibody (200  $\mu$ L) specific for IL1- $\beta$  (goat antihuman) was added to each well. Then, 200  $\mu$ L of a substrate solution was added and any color developed was proportional to the amount of IL1- $\beta$ , bound in the initial step. The intensity of the color (optical density) was measured using a microplate reader ELISA plate reader with 450 nm within 30 min. A standard curve was prepared by plotting the concentration of the IL1- $\beta$  (standards 200, 100, 50, 25, 12.5, 6.25, 3.12, and 0 pg/mL) against their optical density and the concentration of IL1- $\beta$  was determined.

#### Data processing and statistical analysis

Data analysis was performed using the statistical package SPSS ver. 11.5 (SPSS Inc., Chicago Illsion, USA) and the computer software by David S. Walonick, 2010, Stat Pac Inc.). Parametric or non-parametric statistics were performed after evaluation of the normality of the data, using a Shapiro–Wilk test.

### RESULTS

#### Comparison of GCF volume ( $\mu$ l) at 0-time (baseline measurement) and 7-days recoded in G1and G2.

The results (Table 1) of the median values of the GCF of the 25 patients recorded in each group (G1 and G2) at 0-time showed that the median values were (0.52 and 0.54 respectively. Comparing the median values, there were no statistically significant ( $p>0.05$ ) differences among the two groups of the GCF, (H-statistics=1.0691 and P-value =0.585). However, at 7-days post treatments (Table 2) the results showed that there were statistically significant ( $p<0.05$ ) differences among the two groups (H-statistics=11.827 and P-value =0.002).

Moreover, the results (Table 3) revealed that the median value in G1 before treatment (0-time) was (0.52) and decreased to (0.34) while in G2 it was (0.54) and decreased to (0.42) after treatment respectively. These reductions were

statistically significant ( $P<0.05$ ), (W-value =37.5 and critical value of W=65) and (W-value = 44.5 and critical value of W=46) in groups G1and G2 respectively. Although the effects of the two post-treatments at 7-days were statistically significant( $P<0.05$ ) compared to 0-time, the OZ gel group (G1) was much more efficient (0.52 to 0.34) compared to G2 (0.54 to 0.42).

Table 1: Comparison of Gingival crevicular fluid (GCF) volume( $\mu$ l) at 0-time recorded in G1, G2 groups.

| Groups<br>(25/group) | Median | GCF( $\mu$ l) at 0-time |         |                  |
|----------------------|--------|-------------------------|---------|------------------|
|                      |        | Kruskal-Willis Test     |         |                  |
|                      |        | H-statistics            | P-value | Result at P<0.05 |
| G1                   | 0.52   | 1.0691                  | 0.585   | NS               |
| G2                   | 0.54   |                         |         |                  |

G1= Ozone gel group, G2= CHX gel group NS= non -significant ( $P > 0.05$ ).

Table 2: Comparison of Gingival crevicular fluid (GCF) volume ( $\mu\text{l}$ ) at 7-days recorded in G1 and G2

| Groups<br>(25/group) | Median | GCF( $\mu\text{l}$ ) at 7-days |         |                  |
|----------------------|--------|--------------------------------|---------|------------------|
|                      |        | Kruskal-Willis Test            |         |                  |
|                      |        | H-statistics                   | P-value | Result at P<0.05 |
| G1                   | 0.34   | 11.8279                        | 0.002   | S                |
| G2                   | 0.42   |                                |         |                  |

G1=Ozone gel group, G2=CHX gel group, S= significant ( $P > 0.05$ ).

Table 3: Effect of different treatments (before & after) on gingival crevicular fluid (GCF,  $\mu\text{l}$ ) at 0-time and 7days recorded in G1 and G2.

| Groups | Median          |                | Wilcoxon Signed-Rank Test |                     | P $\leq$ 0.05 |
|--------|-----------------|----------------|---------------------------|---------------------|---------------|
|        | Before (0-time) | After (7-days) | W-value                   | Critical value of W |               |
| G1     | 0.52            | 0.34           | 37.5                      | 65                  | S             |
| G2     | 0.54            | 0.42           | 44.5                      | 46                  | S             |

G1= Ozone gel group, G2= CHX gel group, S= significant ( $P \leq 0.05$ ).

Table 4: Comparison of IL1- $\beta$  at 0-time recorded in G1 and G2.

| Groups<br>(25 patients /group) | Median | IL-1 $\beta$ (pg/ml) at 0-time |         |                  |
|--------------------------------|--------|--------------------------------|---------|------------------|
|                                |        | Kruskal-Wallis Test            |         |                  |
|                                |        | H-statistics                   | P-value | Result at P<0.05 |
| G1                             | 137    | 4.149                          | 0.125   | NS               |
| G2                             | 140    |                                |         |                  |

G1= Ozone gel group; G2= CHX gel group; NS= non -significant ( $P > 0.05$ ).

Table 5: Comparison of IL-1 $\beta$  (pg/ml) at 7-days recorded in G1and G2 groups.

| Groups | Median | IL-1 $\beta$ (pg/ml) at 7-days |         |                  |
|--------|--------|--------------------------------|---------|------------------|
|        |        | Kruskal-Wallis Test            |         |                  |
|        |        | H-statistics                   | P-value | Result at P<0.05 |
| G1     | 97     | 11.183                         | 0.003   | S                |
| G2     | 125.5  |                                |         |                  |

G1=Ozone gel group; G2= CHX gel group, S= Highly significant difference ( $P < 0.05$ )

Table 6: Effect of different treatments (before & after) on IL-1 $\beta$  (pg/ml) at 0-time and 7-days recorded in G1 andG2 groups.

| Groups | Median          |                | Wilcoxon Signed-Rank test |                     | P $\leq$ 0.05 |
|--------|-----------------|----------------|---------------------------|---------------------|---------------|
|        | Before (0-time) | After (7-days) | W-value                   | Critical value of W |               |
| G1     | 137             | 97             | 27                        | 71                  | S             |
| G2     | 140             | 125.5          | 91                        | 89                  | NS            |

G1=Ozone gel group; G2= CHX gel group; S= significant ( $P \leq 0.05$ ) within a group, NS= non-significant ( $P > 0.05$ ).

#### Comparison of IL1- $\beta$ concentration (pg/ml) at 0-time and 7-days recorded in groups G1 and G2.

The results (Table 4) of the median values of IL-1 $\beta$  concentration recorded in each group (G1 and G2) at 0-time were (137 and 140) respectively. Comparing the median values, the results revealed no statistically significant ( $p > 0.05$ ) differences among the two groups (H – statistics = 4.149 and p – value = 0.125). However, at 7-days the results (Table 5) showed that the median values of IL-1 $\beta$  concentration recorded in each group (G1 and G2) were (97 and 125.5) respectively. The results revealed that there were statistically significant ( $p < 0.05$ ) differences among the two groups (H – statistics = 11.183 and p – value = 0.003).

Moreover, the results (Table 6) revealed that the median value of IL-1 $\beta$  level in G1 was 137 before treatment (0-

time) and decreased to 97 after treatment with OZ gel (7-days); this reduction was statistically ( $p < 0.05$ ) significant (W- value = 27 and critical value of W= 71). While, the median value of IL-1 $\beta$  level in G2 before treatments (0-time) was (140), decreased to (125.5) after CHX treatments (7-days), this reduction was statistically ( $p > 0.05$ ) not significant (W- value = 91 and critical value of W= 89).

#### DISCUSSIONS

##### Comparison of GCF volumes ( $\mu\text{l}$ ) at 0-time and at 7-days recorded in G1 and G2 groups.

GCF is a biological exudate and determination of its constituents is a present method to identify specific biomarkers with reasonable sensitivity.<sup>27</sup> The analysis of GCF is a very valuable diagnostic technique in periodontology.<sup>28,29</sup> Several investigators have suggested

that the quantity of GCF increases during gingivitis.<sup>30</sup> and periodontitis.<sup>31</sup> The results of this study were in agreement with Armitage<sup>32</sup> who demonstrated that clinically and histometrically production of GCF strongly increases when a gingiva is severely inflamed (Tables 1). In a study by Nagayoshi et al.<sup>12</sup> found that dental plaque samples were treated with 4 mL of ozonated water for 10 s., and detected that ozonated water was efficient in eliminated gram-positive and gram-negative oral microorganisms and oral *C. albicans*. This reveals its capability to control infectious microorganisms in dental plaque. Furthermore, Filippi<sup>13</sup> suggested that OZ water used on the everyday basis can enhance the healing rate in oral mucosa and the effect is more evident in the first two postoperative days. Moreover, Montevecchi et al.<sup>33</sup> found that ozonated oil is a more effective antiseptic than CHX and povidone-iodine against *S. aureus* and the periodontal pathogen *P. gingivalis*. Furthermore, Indurkar & Verma<sup>5</sup> concluded that gingival massage with ozonated oils can be used as an effective alternative to gingival massage with chlorhexidine gel against plaque induced gingivitis. The significant decreased in the median value of GCF in favor to G1 (Ozone gel) as compared to G2(CHX gel) at 7 days (Tables 2 & 3) could be explained as suggested by Safinaz<sup>34</sup> due to the antibacterial effect of ozone on the plaque microorganisms, since scaling concurrently performed with ozone application enable Ozone gel to react directly with the bacterial plaque allowing it to exert its optimal bactericidal effect during exposure and subsequently reduce gingivitis<sup>33</sup>. These results were further advocated by Katti and Chava<sup>35</sup>, Dhingra and Vandana<sup>36</sup>, Montevecchi., et al.<sup>32</sup>

#### Comparison of IL1- $\beta$ concentration (pg/ml) at 0-time and 7-days recorded in G1 and G2 groups.

PIG is the common form of periodontal disease, results from interactions of the immune system and existing biofilm. Gingivitis increases blood flow, vascular permeability, and inflammatory cell migration (neutrophils and macrophages) from peripheral blood to the crevicular fluid. Subsequently, T and B-lymphocytes appear at the injury site. Host cells produce and release IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , and prostaglandins.<sup>37,38</sup> The correlations between the quantities of many host GCF biomarkers and periodontal diseases have been widely studied.<sup>39</sup> The results of this study suggested that IL-1 $\beta$  concentrations in GCF from patients in groups G1 and G2 could be supportive in assessment of gingivitis disease. IL-1 $\beta$  concentrations have been shown to be very high in inflamed gingival tissues and in the GCF of diseased implant sites when compared to healthy implant sites.<sup>40</sup> The results also showed the total amount of IL-1 $\beta$  was significantly higher in G2 (CHX) compared to G1 (Ozone). Gingivitis treatments showed a significant reduction in IL-1 $\beta$  levels in G1 at 7 days (Table 5 & 6). These results could be explained by the reduction of GCF volume following successful treatment. These results supported the conclusions reached by Bocci<sup>41</sup> who found that the ability of ozone to induce cytokines production is significant and that the induction of TNF- $\alpha$ , IFN- $\gamma$ , IL-2 and IL-8 explained at least the enhancement of immune function that

has been reported following ozone administration. Furthermore, Huth *et al.*<sup>14</sup>, created a condition under which ozonated water exerts inhibitory effects on the nuclear factor-kappa (NF-kappa) B system, indicating that it has an anti-inflammatory capability. Additionally, the inhibitory effect of ozone on NF-kappa B system rendering it a powerful anti-inflammatory agent and can prevent disease progression.<sup>42</sup> On the other hand, Patel *et al.*<sup>43</sup> showed that, the adjunctive use of the Ozonated olive oil gel (OZO) with scaling and root plan (SRP) in treatment of chronic periodontitis resulted in a significant improvement ( $P < 0.001$ ) of clinical parameters as well as microbiological parameters over the time and in comparison, to the control groups. Moreover, the *in vitro* study carried out by Eyck *et al.*<sup>44</sup> showed that ozone has a powerful antibacterial activity against putative periodontopathogenic microorganisms and recommended that it may have capability to acts as an adjunctive application to (SRP) in periodontitis patients. The results of this study further coincided with Nagayoshi *et al.*<sup>45</sup> who found that ozonated water could be valuable in minimizing the infections caused by oral microorganisms in dental plaque and coincided with Kshitish and Laxman<sup>46</sup> who suggested that, ozone has a powerful capability to inactivate periodontal microorganisms as compared to CHX.

#### CONCLUSIONS

GCF volume ( $\mu$ l) and IL-1 $\beta$  concentration (pg/ml) at 7-days post-treatment were directly correlated to the treatment used in each group (G1 and G2). OZ gel treated group showed significantly lower GCF volume and IL-1 $\beta$  concentration. This highlighted the evidence in favor of the OZ gel in the treatment of gingivitis.

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