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The Effect of Super Oxidized Water Mouthwash on the Level of IL 1β in Gingival Crevicular Fluid for Patients with Gingivitis Randomized Clinical Trial

Ali Jawad M Ali, Saif S Saliem*

Department of Periodontics, College of Dentistry, University of Baghdad, Iraq

ABSTRACT

Background: One of the most predominant periodontal diseases is the plaque induced gingivitis. For the past 20 years, super-oxidized solutions have been shown to be powerful antimicrobials and disinfectants via oxidative damage. The taste is better than chlorhexidine and does not stain the teeth. Microsafe®, a recently presented super-oxidized solution for gingival care, offers a completely improved approach to treatment of gingivitis. Interleukin 1 beta is one from interleukins family and it is released by many cells such as macrophage to control immune response.

Aim: The aim of the study is to determine the effect of super oxidized water mouthwash on the pro inflammatory cytokines (IL_1β) in the gingival crevicular fluid compared to chlorhexidine in participants who continue to perform regular mechanical oral hygiene.

Materials and methods: Forty-five adult male patients with generalized gingivitis participated in the double blinded randomized controlled parallel study divided into three groups, two mouth rinses and distilled water(negative control) used during seven days periods as adjunctive to regular mechanical oral hygiene(brushing and flossing), one group received super oxidized water mouth rinses (Microsafe®) three times daily and the second group received Alcohol-free chlorhexidine 0.12% solution(kin gingival®) twice daily and the third group received distilled water (negative control). In the first visit GCF collected from targeted sites (upper incisors, labial side) and then scaling and polishing were done. In the second the GCF was collected again from the same teeth.

Results: The level of IL-1β showed a highly significant differences in CHX group in the 2nd visit after treatment (46.062 versus 22.958 p=0.005) when compared to D.W and Microsafe® groups which also showed a significant changes(65.517 versus 50.277 P=0.026, and 55.920 versus 46.351 p=0.010 respectively). Significantly decreased between the study groups in level of IL_1β between the 1st visit and the 2nd visit (P=0.039)

Conclusion: According to the results of our study, CHX was better than SOW and this was improving by immunological marker (IL-β). There was a positive reduction in the IL_ 1β after topical application of Microsafe® for one week but the duration of application must be continued more than 1 week to reach a good decrease of IL-1β in GCF which is a sign of less periodontal destruction.

Key words: Super oxidized water, Chlorhexidine, Interleukin1_beta, Gingival crevicular fluid

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Corresponding author: Saif S Saliem
e-mail ✉: ali.mario28@yahoo.com
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INTRODUCTION

Regardless to the gender, age, or race, about more than 90% of the population have plaque-induced gingivitis which is the most common periodontal diseases. Gingivitis is initiated by the noxious substance's resultant from the accumulation of microbial plaque at or near the gingival sulcus [1]. The important thing of gingivitis is that it can lead to periodontitis, though not all gingivitis will lead to the progress of periodontitis [2], so the importance of avoiding gingivitis and periodontitis is further highlighted. The maintenance of good oral hygiene is then of dominant importance, despite epidemiological studies representing that most part of the people does not reach adequate mechanical hygiene control. This fact has led to the increase in attention in the expansion of oral hygiene products based on chemical plaque control, mainly those effective antimicrobial mouth-rinses that may prevent the development of gingivitis by generating an effect on the supragingival and subgingival establishing of teeth by oral bacteria [3]. Microsafe®, a recently available super-oxidized solution for gingival care, offers a completely improved approach for treatment of gingivitis. The neutral pH, super oxidized water was certified as an antiseptic in México in 2004 [4]. The active components of this solution include 99.97% super oxidized water and <0.03% of numerous reactive species of chlorine and oxygen including sodium chloride 0.023%, hypochlorous acid

0.003% and sodium hypochlorite 0.004%. However, the total content of free available chlorine is low and its ranges between 50 and 80 ppm. This SOW has bactericidal, fungicidal virucidal, and sporicidal actions by oxidative damage and it is ready to use with no additional mixing or dilution. It does not need special disposal or handling. Super-oxidized solutions have been shown to be powerful antimicrobial agents and disinfectants through oxidative damage [5]. Chlorhexidine is the typical mouthwash used for chemical plaque control [3]. Inappropriately, CHX has some unwanted effects such as extrinsic stain on the teeth and dental restorations, interfering with function of the taste, it has a bitter taste, increasing calculus formation [6]. CHX mouth rinses holding anti discoloration agents were reported to have no reliable useful effects on plaque control and gingivitis [7]. This inspires many researchers to find replacements for CHX. Interleukin-1β is a pro inflammatory cytokine which is one from the family of interleukins. It is secreted by many cells in the body (mainly T cells and macrophages), frequently in response to a stimulation and involved in the beginning of effective stages of inflammation and immunity [8]. GCF is predominantly formed by serum transudate and/or inflammatory exudate derived from the periodontal tissues. Therefore, the analysis of biomarkers in GCF is a widely used non-invasive method to study the status of inflammation of periodontal tissues and the host response to different periodontal therapies [9].

MATERIALS AND METHODS

Study sample

This a double blinded randomized controlled parallel study was conducted from November 2019 to March 2020 at Karbala dental specializing centre. This study approved by ethical committee/ college of Dentistry/ University of Baghdad, follow the guidelines of Helsinki and Tokyo for humans (the reference no. 136619 in 2\12\2019). This study registered at (http:// clinicaltrials. gov. (the reference no. NCT04328753), and followed the consolidation standards of reporting trials (CONSORT 2010).A total of 45 Iraqi male patients aged 15 to 55 years, who met the eligibility criteria were precipitated in this study for the non-surgical treatment of generalized gingivitis.

Inclusion criteria

Patient with generalized gingivitis.

No antibiotic treatment during a

Month period prior to the start of the trial.3.no regular medication with anti-inflammatory compounds.

No history of allergy to oral care products 5.no regular use of oral antiseptics.

Exclusion criteria

Patients who refuse to write an informed consent form.

Smokers.

Female patients because of hormonal disturbances during menstrual cycle, pregnancy, lactation, and menopause.

Collection procedure

The study had been made in Karbala dental specialized center at the morning because the daytime makes a difference on the level of IL-1β, so all samples were taken at the same period from 10 AM-12 PM [10]. The procedure that used in this study was double blinded, parallel procedure, the GCF is collected from the maxillary incisor's sites for all participant in the study. The collection is done by periopaper®. The samples will be collected from the GCF of the included site by an intracrevicular method utilizing periopaper®. The periopaper® should place for 30 sec inside the sulcus. We used a tweezer to insert the periopaper® utilizing a gentle pressure until a minimal resistance is felt to ensure that there was no irritation to the examined site. Periopaper® suspected to be contaminated with saliva or blood were discarded and the procedure repeated. Immediately after collection of the GCF the periopaper® transferred into the pre-weighed Eppendorf tube (Figure 1A and 1B). By assuming that the density of GCF is (1mg/mL) [11,12] and by using the formula (Volume=Mass/Density), the volume will be equal to the mass. After the elution and centrifuging process figure 2 the supernatants produced will be assayed by enzyme-linked immunosorbent assay (ELISA) [13]. The comparison between study groups by clinical marker (IL-1β) before treatment is shown in table 1. In this study, there were no statistically significant differences (P=0.455) between study groups in the immunological marker before treatment.

Table 1: Comparison between study groups by clinical marker before treatment.

		Mean	± SD	P
IL_1β	DW	65.517	± 44.302	0.455
	CHX	46.062	± 39.191	
	Super oxidized water	55.92	± 42.518	

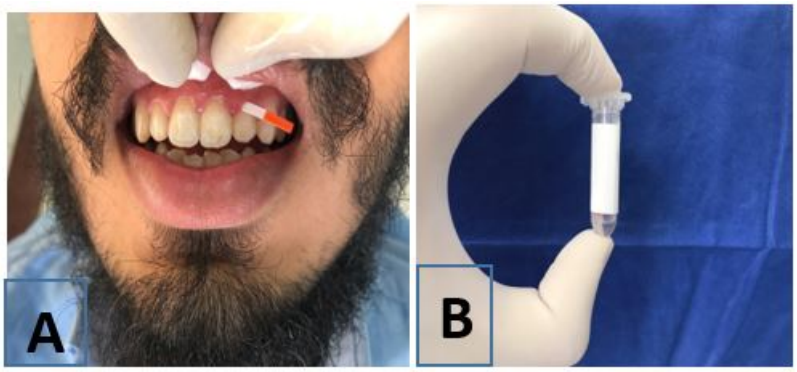

Figure 1A and 1B: show the process of collection with periopaper® and transfer into the Eppendorf tub.

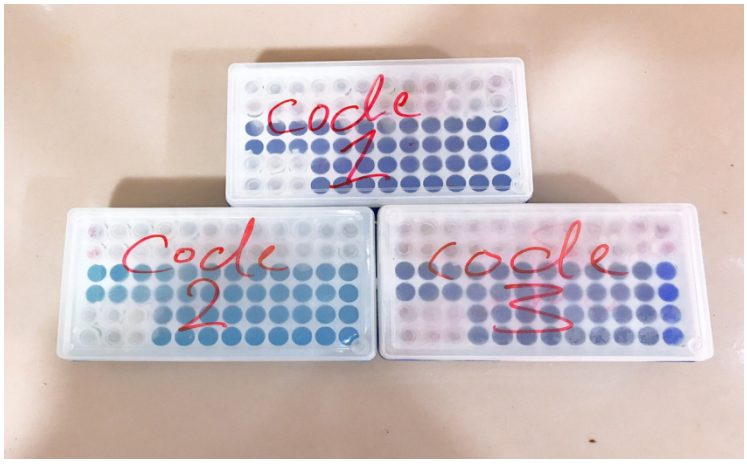

Figure 2: The last stage of defreezing of samples at the room temperature.

RESULTS

The comparisons between first visit interleukin-1β levels and in the 2nd visit after treatment according to study groups is shown in table 2 and figure 3. In D.W group, we noticed that the level of IL-1β was significantly decreased in the 2nd visit after treatment compared to the 1st visit (65.517 versus 50.277 P=0.026). In CHX group, highly significant change in IL_1β levels in the 2nd visit after treatment compared to the 1st visit (46.062 versus 22.958 p=0.005) .In SOW group, we noticed that the level of IL-1β was significantly decreased in the 2nd visit after treatment compared to the 1st visit (55.920 versus 46.351 p=0.010).Significantly decreased between the study groups in level of IL_1β between the 1st visit and the 2nd visit (P=0.039) . According to Dunnett's t3 post hoc test, the significant difference was between D.W group and CHX group, p= 0.028 after treatment table 3.

Table 2: Comparisons between first visit interleukin-1β levels and in the second visit according to study groups.

	1st IL_1β			2nd IL_1β			Paired T test	P value
Groups	Mean ± SD	F	p	Mean ±SD	F	p		
DW	65.517 ± 44.302			50.277 ± 32.841			2.494	0.026 Sig.
CHX	46.062 ± 39.191			22.958 ± 17.413			3.35	0.005 HS
Super oxidized water	55.920 ± 42.518	0.803	0.455 NS	46.351 ± 37.587	3.513	0.039 Sig.	2.969	0.010 Sig.
Total	55.833 ± 41.867			39.862 ± 32.217				

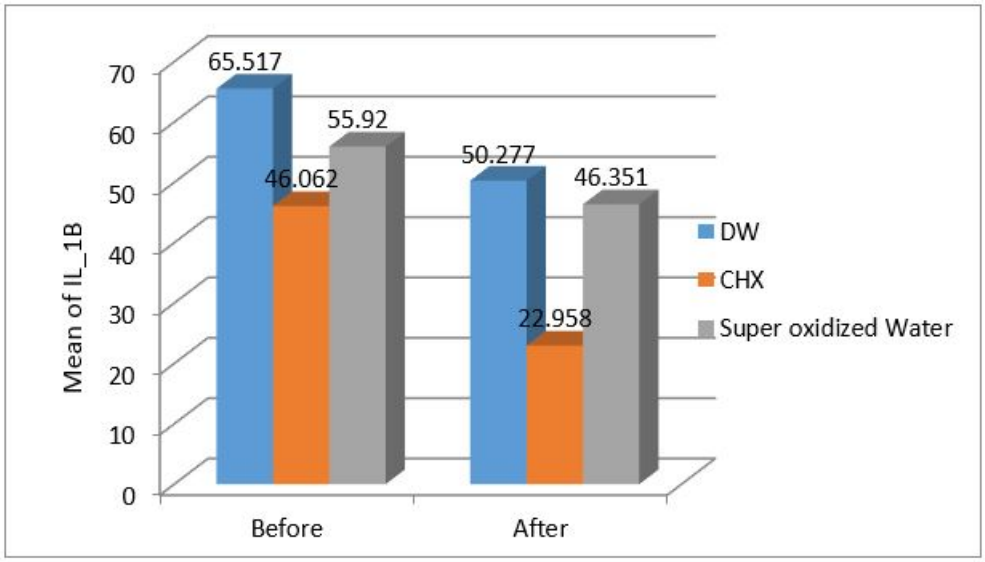

Figure 3: Comparisons between first visit interleukin-1β levels and in the second visit according to study groups.

Table 3: Multiple Comparisons of IL _1 β after treatment between groups using Dunnett's t3 post hoc test.

(I) Groups	(J) Groups	Mean Difference (I-J)	P value	
DW	CHX	27.319	0.028	Sig.
	Super oxidized water	3.926	0.986	NS
CHX	Super oxidized water	-23.393	0.115	NS

DISCUSSION

This study was the first study that deals with the effect of Microsafe® mouthwash (99.97% super oxidized water) on the immunological marker IL-1β in GCF in human with plaque induced gingivitis, a try had been made to check the performance of Microsafe® in the treatment of gingival inflammation as a mouthwash and calculate the level of IL-1β in GCF. The recall period of this study was 1 week. The effect of this biomarker on the progression of gingivitis is confirmed and it has a potential role in disease development. According to Dunnett's t3 post hoc test, the significant difference was between D.W group and CHX group, p=0.028 after treatment. The non-significant differences between Microsafe® group and D.W group (p=0.986) indicate that we need an extended period of follow up to study the influence of Microsafe® on the IL_1β in patient with gingivitis. Microsafe® may offer several advantages over chlorhexidine and other common antiseptic solutions. We also believe the use of Microsafe® as a mouth rinse provides added benefits for the patient. Microsafe® noted that there were no adverse effects on clinical examination as mentioned by the patients. Patients also presented the compliance with the regular use of Microsafe®, proving the practicality of Microsafe® and its excellent acceptability, including acceptability of its sensory characteristics.

CONCLUSION

According to the results of our study, CHX was better than SOW and this was improves by immunological marker (IL-β). There was a positive reduction in the IL_ 1β after topical application of Microsafe® for one week but the duration of application must be continued more than 1 week to reach a good decrease of IL-1β in GCF which is a sign of less periodontal destruction.

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