

# COMPARATIVE EVALUATION OF HYDROGEN PEROXIDE AND CHLORHEXIDINE MOUTHWASH ON SALIVARY INTERLEUKIN-1 $\beta$ LEVELS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND CHRONIC PERIODONTITIS: A RANDOMIZED CONTROLLED CLINICAL TRIAL

Evaluación comparativa de enjuague bucal con peróxido de hidrógeno y clorhexidina sobre los niveles de interleucina-1 $\beta$  salival en pacientes con diabetes mellitus tipo 2 y periodontitis crónica: ensayo clínico controlado aleatorio

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## ABSTRACT

**Introduction:** Periodontal inflammation causes dysbiosis and change in the microbiota. Nonsurgical periodontal therapy (NSPT) helps in removal of plaque and restoring periodontal health. Various adjunctive therapy like use of mouthwash helps in maintenance of periodontal health and reducing inflammatory load.

**Materials and Methods:** A total of 108 subjects diagnosed with type 2 diabetes mellitus and periodontitis were divided into three groups: Group 1 received NSPT and rinsing with 0.2% chlorhexidine mouthwash for 3 months, Group 2 received NSPT and rinsing with 1.5% hydrogen peroxide mouthwash for 3 months, Group 3- received NSPT only (control group). The clinical parameters measured included Plaque Index (PI), Gingival Index (GI), Bleeding on probing (BOP) and probing (PD) at baseline, 1, 2, 3 months follow up. Salivary interleukin 1 $\beta$  levels were measured at baseline and 3 months interval.

**Results:** Group 1, 2 and 3 showed significant reduction in PI, GI, BOP and PD at 1 and 3 months follow up ( $p < 0.05$ ). However, Intergroup comparison of clinical parameters showed significant reduction in group 1 and 2 when compared with group 3 ( $p < 0.05$ ). Salivary interleukin 1- $\beta$  levels showed significant reduction from baseline to 3 months in all the three groups and intergroup comparison didn't show any significant changes, ( $p > 0.05$ ).

**Conclusions:** Hydrogen peroxide mouthwash as an adjunct to NSPT can be considered as a safe and effective measure to reduce periodontal inflammation in type 2 diabetes mellitus patients with chronic periodontitis.

**Keywords:** Chlorhexidine; Hydrogen peroxide; Mouthwashes; Periodontitis; ultrasonics; dental scaling.

## RESUMEN

**Introducción:** La inflamación periodontal causa disbiosis y cambios en la microbiota. La terapia periodontal no quirúrgica (NSPT) ayuda a eliminar la placa y restaurar la salud periodontal. Diversas terapias complementarias, como el uso de enjuague bucal, ayudan a mantener la salud periodontal y reducir la carga inflamatoria.

**Materiales y Métodos:** Un total de 108 sujetos diagnosticados con diabetes mellitus tipo 2 y periodontitis se dividieron en tres grupos: el grupo 1 recibió NSPT y enjuague bucal de clorhexidina al 0,2% durante 3 meses, el grupo 2 recibió NSPT y enjuague bucal de peróxido de hidrógeno al 1,5% durante 3 meses, y el Grupo 3 recibió NSPT únicamente (grupo de control). Los parámetros clínicos medidos fueron el índice de placa (PI), el índice gingival (GI), el sangrado al sondaje (BOP) y al sondaje (PD) al inicio del estudio, 1, 2, y 3 meses de seguimiento. Los niveles de interleucina 1 $\beta$  en saliva se midieron al inicio y a los 3 meses.

**Resultado:** Los grupos 1, 2 y 3 mostraron una reducción significativa en IP, GI, BOP y PD al mes y 3 meses de seguimiento ( $p < 0,05$ ). Sin embargo, la comparación intergrupala de los parámetros clínicos mostró una reducción significativa en los grupos 1 y 2 en comparación con grupo 3 ( $p < 0,05$ ). Los niveles de interleucina 1- $\beta$  salival mostraron una reducción significativa desde el inicio hasta los 3 meses en los tres grupos y la comparación entre grupos no mostró ningún cambio significativo ( $p > 0,05$ ).

**Conclusión:** El enjuague bucal con peróxido de hidrógeno como complemento de la NSPT puede considerarse una medida segura y eficaz para reducir la inflamación periodontal en pacientes con diabetes mellitus tipo 2 y periodontitis crónica.

**Palabras Clave:** Clorhexidina; Peróxido de hidrógeno; Anti-sépticos bucales; Periodontitis; Ultrasonido; Raspado dental.

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## INTRODUCTION

Dental plaque is detrimental for oral health. It consists of microorganisms which cause dental caries and periodontal diseases. Meticulous removal of dental plaque can help in oral health maintenance. In-office and home-care agents can be used for reducing plaque formation. Adjunctive agents can be mechanical or chemical agents which assist in plaque removal along with traditional plaque removal agents or mechanism. Various mechanical agents like toothbrush, interdental aids are useful agents in plaque removal. Chemical plaque controlling agents like mouthwash can help in disrupting the plaque biofilm.<sup>1</sup>

Scaling and root planing (SRP) removes plaque mechanically using hand or ultrasonic instruments. Mouthwashes are also used as an adjunct to SRP for periodontal health maintenance.<sup>2</sup>

Chlorhexidine gluconate (CHX) is a broad-spectrum antimicrobial agent which helps in reducing bacterial, viral and fungal load. It has substantivity which is attributed to presence of  $\beta$  cyclodextrin rendering control release of CHX.<sup>3</sup> Chlorhexidine has antiplaque action; it has bactericidal action at higher concentration and bacterio-static at low concentration. Although the effect of CHX on mature plaque is less as bacterial enzymes hinders CHX action.<sup>4</sup> Haydari *et al.*,<sup>5</sup> studied different concentration of CHX (0.06%, 0.12% and 0.2%) in a gingival model and found that all the concentration were effective in reducing plaque.

Hydrogen peroxide (HP) has been used in periodontal surgeries and maintenance therapy to reduce plaque and it was used at 3 % concentration to deliver the antimicrobial effects.<sup>6</sup> It has both gram-positive and gram-negative action and releases the free radicals

(e.g., superoxide anion  $[O_2^{\cdot-}]$  and the hydroxyl radical  $[OH^{\cdot}]$ ). It has been used as a bleaching agent for up to 35 % concentration. HP at 1.5% is used as mouthwash and it has shown oxygen releasing cleansing action. Jhingta *et al.*,<sup>7</sup> assessed hydrogen peroxide combination with CHX (chlorhexidine 0.2% followed by hydrogen peroxide 1.5%) on stains and plaque and found significant reduction in plaque formation compared to CHX rinsing alone.

Diabetes mellitus has implications on oral health. Periodontitis is considered as the sixth complication of diabetes mellitus.<sup>8</sup> Type 2 diabetes mellitus patients need regular dental visits to prevent oral complications. Nonsurgical periodontal therapy (NSPT) helps in preventing periodontal infections. However, oral hygiene reinforcement is an essential aspect in oral health maintenance among diabetes.

Use of mouthwash as an adjunct to oral care can be beneficial in these populations. Sedigh-Rahimabadi *et al.*,<sup>9</sup> used golnar (Punica granatum var pleniflora, a Persian medicine formulation done at pharmacy department) a traditional mouthwash for diabetes patients with gingivitis and found improvement in plaque index. Badooee *et al.*,<sup>10</sup> compared ginger and aloe vera use (ginger 25%, aloe vera 50%, prepared by BarijEsans Company) on xerostomia in type 2 diabetes mellitus patients mouthwash and found reduction in symptoms of xerostomia post mouthwash rinsing.

Raman *et al.*,<sup>11</sup> studied effect of NSPT versus oral hygiene instructions on type 2 diabetes patients with periodontitis and suggested that oral hygiene instructions alone was not beneficial in controlling inflammation and both NSPT and oral hygiene instructions can help in reducing periodontitis. Based on these findings, we hypothesised that hydrogen peroxide mouthwash can act as antiplaque agent and reduce periodontal inflammation in diabetes

mellitus subjects. We aimed at evaluating the effect of hydrogen peroxide and Chlorhexidine mouthwash in diabetic patients with periodontitis. The objectives of the study were to determine salivary interleukin-1 $\beta$  levels in patients with type 2 diabetes mellitus and chronic periodontitis post chlorhexidine and hydrogen peroxide mouthwash use. To determine changes in clinical parameters in type 2 diabetes mellitus patients before and after administration of chlorhexidine and hydrogen peroxide outhwash.

## MATERIALS AND METHODS

This study was approved by the Institutional Ethical Committee and registered as CTRI/2022/01/039686. The study was in accordance with the Helsinki declaration. Written informed consent was taken from all the study participants before enrolment. This was a double-blinded randomised control trial conducted at the Department of Periodontology between December 2020 to August 2021. Subjects included as diabetic had controlled diabetic levels diagnosed for the last 5 years and under medication for the last 5 years. HbA1c levels were measured for each subject before enrolment.

### Sample Preparation

The sample size was calculated by using G Power software (version 3.1.9.2). At a significance level of .05, a power of 0.90, the sample size of 34 participants in each group was required. Considering an attrition rate of 20%, a total of 41 participants in each group were recruited. At the baseline, 41 subjects were recruited in each group, at 3 months follow up 5 subjects in each group had dropped out.

So, a total of 36 subjects in each group was considered for evaluation. (Figure 1). The details of group division and mouthwash prescribed are given in Table 1.

### Mouthwash rinsing instructions

Each subject was instructed to use 10 ml of mouthwash rinse for 60 seconds twice daily for three months. No intake of food and water for half an hour post rinsing.

### Randomization, Blinding, and Allocation Concealment

Randomization was done using GraphPad Prism software and a dentist not involved in any examination or treatment was assigned for randomisation of the subjects. Mouthwash solutions were re-labelled with a black paper and marker to conceal the colour of the bottle and contact details of the principal investigator were mentioned for reporting any emergency or adverse reactions. Clinicians and participants were blinded about the allocation.

### Inclusion criteria of the study was

- 1) Subjects with age 35-55 years;
- 2) Subjects with controlled type 2 diabetes mellitus *i.e.* defined as fasting blood sugar in the range of 80 to 130 mg/dl and hemoglobin A1c of <7.0%;
- 3)  $\geq 20$  teeth;
- 4) Subjects diagnosed with Periodontitis stage II, Grade B , grade 2 according to new classification for periodontal disease.<sup>12</sup>

### Exclusion criteria

- 1) Systemic disease other than type 2 diabetes mellitus;
- 2) History of periodontal surgery in last 12 months;
- 3) History of antimicrobial, anti-inflammatory drug in past 3 months;
- 4) Pregnant /lactating mother;
- 5) Any known allergy to mouthwash;
- 6) Smokers.

### Clinical Measures

Before recruitment of study subjects, a detailed

medical history of the patients was obtained using a questionnaire. Periodontal parameters included Plaque index,<sup>13</sup> Gingival Index(GI),<sup>14</sup> bleeding on probing (BOP),<sup>15</sup> probing depth (PD) (Table 2). Periodontal parameters were assessed at baseline day 0, day 30, day 60, day 90. Based on inclusion criteria selected subjects were instructed about oral hygiene maintenance and a dental kit consisting of soft bristle toothbrush and toothpaste (Colgate total).

## BIOCHEMICAL MEASURES

### Salivary Interleukin 1 $\beta$ levels

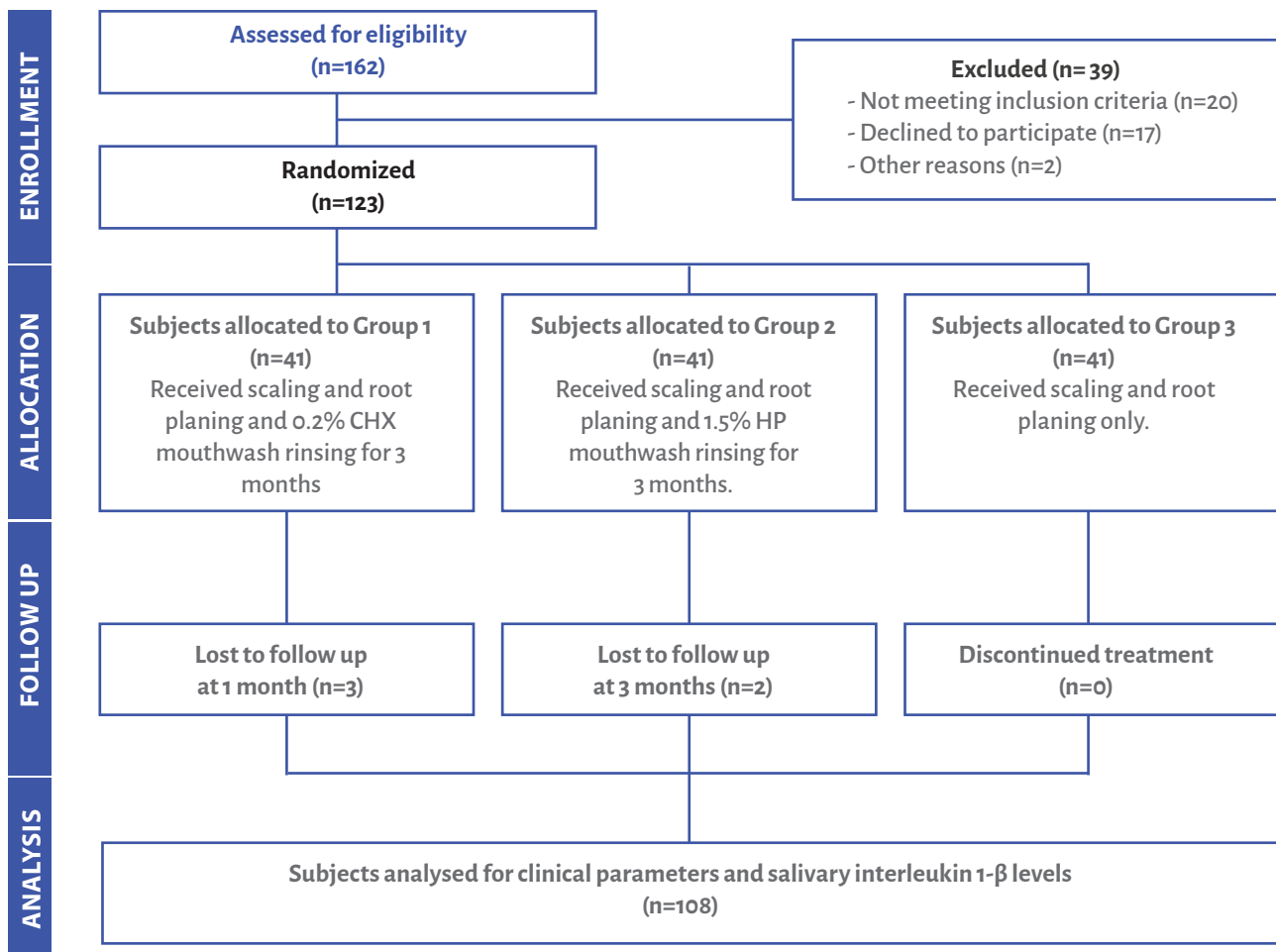
Patients were instructed for overnight fasting and collection of unstimulated saliva was done in polypropylene tubes and the samples were stored at -20°C for further analysis. Salivary sam-

ple was collected at baseline and 3 months post mouthwash rinsing and levels were detected using enzyme-linked immunosorbent assay (by Ray Bio [RayBiotech, Inc. 3607 Parkway Lane, Suite 100, and Norcross GA 30092, USA]).

### Statistical analysis

Demographic details were presented in percentage and standard deviation. Repeated measure analysis of variance was used to measure GI, PI, BOP and PD at different time intervals for intragroup comparison. Intergroup comparison was done using Anova. Level of significance was set at  $p \leq 0.05$ . Statistical analysis was performed using Statistical Package for the Social Sciences, version 22.0. IBM Corp., Armonk, NY, USA was used for all statistical analysis.

**Figure 1.** CONSORT flow diagram of study design.



**Table 1.** Details of study groups and mouthwash prescribed.

Groups	Intervention	Mouthwash trade name
Group 1 CHX	36 subjects were treated with SRP with 0.2% CHX mouthwash.	Colgate-plax, Colgate-Palmolive.
Group 2 HP	36 subjects were treated with SRP with 1.5% HP mouthwash.	Peroxyl, Colgate-Palmolive.
Group 3	36 subjects were treated with ultrasonic scaling and root planing (SRP) (Gracey curette, Hufriedy).	

**Table 2.** Periodontal parameters assessed in the study.

Plaque Index <sup>13</sup>	
Score	Interpretation
0	No plaque in the gingival area.
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may only be recognized by running a probe across the tooth surface, not visible by the naked eye.
2	Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen by the naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface.
Gingival Index <sup>14</sup>	
Score	Interpretation
0	Normal gingiva.
1	Mild inflammation – slight change in color, slight oedema. No bleeding on probing.
2	Moderate inflammation—redness, oedema and glazing. Bleeding on probing.
3	Severe inflammation – marked redness and oedema. Ulceration. Tendency to spontaneous bleeding.
Bleeding on Probing <sup>15</sup>	
Score	Interpretation
1	Bleeding present (+).
2	Bleeding absent (-).
The minus sign indicates the gingival score of 0-1 The plus sign indicates the gingival score of 2-3.	

**Table 3.** Demographic parameters of different groups in the study.

Variables	CHX Group 1	HP Group 2	Group 3
Age in years (Mean± Standard Deviation)	42± 0.74	43±0.34	45± 0.42
Gender	36	36	36
Male	20	17	21
Female	16	19	15

CHX: Chlorhexidine. HP: Hydrogen Peroxide.

**Table 4.** Intergroup comparison of clinical parameters.

Clinical parameters	CHX Group 1 Mean±S.D	HP Group 2 Mean±S.D	Group 3 Mean±S.D	p-value
<b>Plaque index</b>				
Baseline	2.36± 0.42	2.32 ± 0.23	2.34± 0.22	Non Significant
1 month	1.22± 0.12	1.16± 0.21	1.23± 0.13	<0.05
3 month	0.36± 0.18	0.55± 0.12	0.41± 0.21	<0.05
p-value*	<0.05	<0.05	<0.05	
<b>Gingival index</b>				
Baseline	1.81± 0.22	1.83 ± 0.12	1.78± 0.33	Non Significant
1 month	1.02± 0.32	0.98± 0.13	0.91± 0.12	<0.05
3 month	0.35± 0.12	0.34± 0.11	0.33± 0.23	<0.05
p-value*	<0.05	<0.05	<0.05	
<b>Probing Depth</b>				
Baseline	4.25± 0.32	4.67 ± 0.22	4.23± 0.13	Non Significant
1 month	3.15± 0.43	3.17± 0.13	3.27± 0.33	<0.05
3 month	2.11± 0.27	2.61± 0.14	2.34± 0.12	<0.05
p-value*	<0.05	<0.05	<0.05	
<b>Bleeding on Probing (%)</b>				
Baseline	2.21±0.36	2.54± 0.24	2.34± 0.36	Non Significant
1 month	1.13± 0.42	1.33± 0.13	1.23± 0.14	<0.05
3 month	0.83± 0.34	0.71± 0.36	0.67± 0.34	<0.05
p-value*	<0.05	<0.05	<0.05	

CHX: Chlorhexidine. HP: Hydrogen peroxide. S.D: Standard Deviation. NS: Non Significant. Statistically significance at  $p < 0.05$ . \*: Repeated measure anova analysis for intragroup comparison. p-probability value,

**Table 5.** Salivary Interleukin 1- $\beta$  levels.

Variable	CHX Group 1	HP Group 2	Group 3	p-value
Saliva Interleukin 1- $\beta$ ( $\mu$ M) baseline	5.27± 0.25	5.22 ± 0.32	5.12±0.32	
3 month	2.28± 0.24	2.27± 0.22	2.23±0.23	<0.05
p-value*	<0.05	<0.05	<0.05	<0.05

CHX: Chlorhexidine. HP: Hydrogen peroxide. \*: Repeated measure Anova Analysis for intragroup comparison.

## RESULTS

Demographic details of the study participants are illustrated in Table 3. The primary outcome measures were PI, GI, BOP and PD and were evaluated at baseline 1, 2 and 3 months post NSPT. The secondary outcome measure was salivary levels of interleukin 1 $\beta$  which was evaluated at baseline and 3 months interval.

### Primary Outcome Measures

In the CHX group, PI from baseline to 3 months the mean change was 2.00. When we compare the mean difference in reduction of gingival index from baseline to 3 months we observed a significant reduction of 1.56.  $p$ -values from baseline to 3 months had a reduction of 2.14. In the HP group, mean PI difference from baseline to 3 months 0.77. GI showed 1.59 mean

reduction from baseline to 3 months. In group 3, mean PI was reduced from 1.93 from baseline to 3 months, GI 1.45 from baseline to 3 months. PI, GI, BOP and PD reduction was significant in all the groups 1, 2 and 3 ( $p < 0.05$ ). Intergroup comparison showed significant reduction in PI, BOP, GI in group 1 and 2 ( $p < 0.05$ ) compared to group 3. However, no significant reduction was observed in between group 1 and 2 in terms of PI and GI ( $p > 0.05$ ). Intergroup comparison in terms of PD reduction was insignificant ( $p > 0.05$ ), Table 4.

### Secondary Outcome Measures

The mean difference from baseline to 3 months for Salivary interleukin  $1\beta$  was 2.99 for Group 1, 2.95 for Group 2 and 2.89 in Group 3. Salivary interleukin  $1\beta$  reduced at 3 months follow in post NSPT in all the three groups as compared to baseline ( $p < 0.05$ ), However intergroup comparison didn't show any significant changes ( $p > 0.05$ ) (table 5).

This result suggests a possible role for CHX and HP as anti-inflammatory agents. However, intergroup comparison between CHX and HP showed no significant difference ( $p > 0.05$ ). Patient satisfaction level was higher in the HP group as compared to CHX (92.6% versus 86.7%). No significant difference in adverse events was observed between group 2 and 3 ( $p > 0.05$ ).

## DISCUSSION

There is a bi-directional relationship between diabetes and periodontitis.<sup>16</sup> Both diabetes and periodontitis share common risk factors and are highly prevalent diseases.<sup>17</sup> There is a need for better management of gingival health in diabetes subjects. Awareness regarding oral health, screening and early diagnosis of gingival problems in diabetic subjects can lower the risk

of progression towards periodontitis. Diabetic subjects are more prone to periodontitis as they harbor higher amounts of bacteria and more bio-film accumulation. This altered biofilm response turn can affect their glycaemic levels.

The present study evaluated antiplaque action of CHX and HP in diabetic subjects having periodontitis and compared salivary interleukin  $1\beta$  levels also. In this study we found that PI, BOP and GI levels were reduced significantly in both CHX and HP groups when compared to control groups.

However, no significant change in PD was seen in intergroup comparison. Intragroup comparison showed significant reduction in all clinical parameters. Similar finding was observed by Jhingta *et al.*,<sup>7</sup> in their study on use of HP as an adjunct to CHX showed significant plaque reduction and stain intensity after 14 and 21 days ( $p$ -values 0.025 and 0.005, respectively).

A systematic review on chlorhexidine mouthwash as an adjunct to gingival health found that dental plaque reduction was significant in patients using CHX as an adjunct to mechanical oral hygiene methods for 4 to 6 weeks and 6 months. This systematic review assessed the effectiveness of chlorhexidine mouthrinse used as an adjunct to mechanical oral hygiene procedures for the control of gingivitis and plaque compared to mechanical oral hygiene procedures alone or mechanical oral hygiene procedures plus placebo/control mouthrinse.

Since no study has been done comparing CHX and HP mouthwash antiplaque effects in diabetes mellitus patients with periodontitis, direct comparison of results cannot be done. However, a study by Raslan *et al.*,<sup>18</sup> with a one-stage ultrasonic debridement to remove plaque, stain(s), and dental calculus and essential oil



mouthrinse administration for 90-day (twice daily use; 20ml/30s) containing a fixed combination of four EO (eucalyptol 0.092%, menthol 0.042%, methyl salicylate 0.060%, thymol 0.064%), zinc chloride, and sodium fluoride (0.0221%) resulted in better response to gingivitis treatment among patients with diabetes.

Similarly in our present study hydrogen peroxide by releasing free radicals can reduce the inflammatory load in periodontitis patients. Anti-inflammatory action of hydrogen peroxide can be attributed to its scavenging property thereby reducing the oxidative stress.<sup>6</sup>

Sedigh-Rahimabadi *et al.*,<sup>9</sup> studied gingivitis in diabetic patients with Punica granatum mouthwash found that the mouthwash is effective in reducing gingival inflammation without any adverse effect (GBI improved from 13.3% ± 9.72% and 14.76% ± 12.25% at baseline to 3.87% ± 5.7% and 3.42% ± 5.63% in CHX and Punica granatum groups, respectively ( $p < .001$ )).

Hydrogen peroxide can be also considered safe and effective way to reduce inflammation. CHX can cause stains after long term use, unpleasant taste which can be avoided by use of other mouthwashes like hydrogen peroxide.<sup>19</sup> This was the main objective of the study *i.e.* to find an effective mouthwash reducing periodontal inflammation and not having side effects as of CHX.

HP reduces gingival inflammation and studies have shown that long-term adjunct to daily oral hygiene, the results in reduce gingival redness suggesting potential antibacterial property of HP.<sup>20</sup>

HP can mask the bitterness of CHX as suggested by Madhurasai *et al.*,<sup>21</sup> in their study on HP and CHX having synergistic action as mouthwash and masking the bitterness of CHX. Saravanamuttu,<sup>22</sup> suggested use of Hydrogen peroxide mouthwash as a method to reduce

aerosol contamination as it has antimicrobial and virucidal effects. Walsh,<sup>23</sup> suggested safety issues related to use of HP in dentistry and it suggested as the agent is versatile it is used as mouthwash to bleaching agent, it can be used safely with indicated necessary concentration to be maintained for desired action.

A study by Caruso *et al.*,<sup>24</sup> suggested that use of HP can reduce hospitalization rate and complications related to SARS-Cov-2 infection and HP has antimicrobial and virucidal actions. They treated the epithelial of oral mucosa with 3% H<sub>2</sub>O<sub>2</sub> 3% for 6 months and observed no damage on oral mucous membranes or their microvilli. A study by Kamolnarumeth *et al.*,<sup>25</sup> used mixture of HP and CHX mouthwash to reduce plaque and stains in gingivitis patients and found significant reduction in plaque level (CHX 0.64 ± 0.41 *versus* CHX + H<sub>2</sub>O<sub>2</sub> 0.46 ± 0.36,  $p = 0.035$ ).

Prabhu *et al.*,<sup>26</sup> used both CHX and HP as an adjunct in reducing plaque levels, less stain intensity. They further concluded that rinsing twice daily with 0.2% chlorhexidine and 1.5% hydrogen peroxide can be safely prescribed. Similar findings were obtained in our present study though CHX and HP was used separately. Over the counter mouthwashes and the increase risk of prediabetes or diabetes suggests that long term use of mouthwash has systemic side effects<sup>27</sup> and so the duration of the present study was for 3 months only. HP has short-term inhibitory effects on SARS-CoV-2 virus and is used to reduce viral load.<sup>28</sup>

Similar, antimicrobial action can be expected from HP responsible for reducing periodontal inflammation. A pilot study on effects of HP in reducing viral load suggests significant reduction and it highlights the virucidal effects of HP.<sup>29</sup> Dona *et al.*,<sup>30</sup> studied role of HP as plaque control agent and suggested HP has potential antiplaque activity.



Romesh *et al.*,<sup>31</sup> compared HP and CHX as procedural mouthwash and found both were effective in reducing dental aerosol contamination significantly. Interleukin 1- $\beta$  levels are increased in periodontitis.<sup>32</sup> When we consider periodontal inflammation in type 2 diabetes patients and measure serum or salivary interleukin 1- $\beta$  levels a significant increase in levels is noted and this cytokine can be considered as a biomarker for periodontal infection.<sup>32</sup>

In the present study we measured salivary interleukin 1- $\beta$  levels at baseline and 3 months post NSPT in all the 3 groups significant reduction in interleukin 1- $\beta$  was seen, however intergroup comparison didn't show any significant changes ( $p > 0.05$ ). No study has measured salivary interleukin 1- $\beta$  post NSPT and use of any mouthwash in diabetic patients with periodontitis. A meta-analysis stated that salivary interleukin 1- $\beta$  levels are reduced post NSPT in diabetic patients with periodontitis and can be used as biomarker for periodontal infections.<sup>33</sup> No adverse effects were noted at 3 months interval though long-term studies are suggested to evaluate adverse side effects. Both CHX and HP have anti-inflammatory effects to periodontal tissues and this can help in reducing inflammatory load and restoring the oxidative stress.

### Study Limitations

Longer follow-up would have rendered better comparative data but due to staining effect related to CHX mouthwash after long administration, the study was planned for 3 months. Also, short duration was preferred in relation to patient compliance to study protocols.

All subjects were instructed to follow oral hygiene and brushing technique using a sulcular method was demonstrated, adherence to correct oral hygiene measure by patient is another limitation of the study and the results should be interpreted keeping these limitations. The

patients were instructed to return empty mouthwash bottles to check for compliance but this was just an attempt to reinforce oral hygiene measures.

Antimicrobial evaluation after HP or CHX mouthwash administration was not performed as it would have suggested the efficacy of HP against pathogenic microbes. Further, the short duration of the study cannot give the long-term effect of HP mouthwash use against CHX mouthwash. Cross over study design might have given a better comparative result as many confounding factors like subject oral hygiene methods, food habits can be adjusted.

## CONCLUSION

The present study showed a short term effect of both HP and CHX mouthwash effectively reducing bleeding on probing, plaque and gingival index. However, no significant reduction in probing depth was seen in any groups.

Both the mouthwashes can be used effectively as an adjunct to NSPT in management of periodontitis in diabetic patients. Side effects related to CHX can be avoided by alternative use of HP mouthwash which was well tolerated by the subjects.

## CONFLICT OF INTERESTS

Authors declare no conflict of interest.

## ETHICS APPROVAL

This study was approved by the institutional ethical committee (Haldia Institute of Dental Sciences & Research) and registered as CTRL/2022/01/039686. The study conducted in accordance with Helsinki declaration.

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## AUTHORS' CONTRIBUTIONS

**Nisha S:** Concepts; Design; Definition of intellectual content; Literature search; Clinical studies; Experimental studies; Data acquisition; Data analysis; Statistical analysis; Manuscript preparation; Manuscript editing; Manuscript review.

**Das D:** Concepts, Design, Definition of intellectual content, Literature search, Data acquisition, Manuscript preparation, Manuscript review.

**Kulavi S:** Design, Literature search, Data analysis, Manuscript editing, Manuscript review, Guarantor.


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