Antimicrobial efficacy of 0.8% Hyaluronic Acid and 0.2% Chlorhexidine against *Porphyromonas gingivalis* strains: An in-vitro study

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**ABSTRACT**

**Objective:** The aim of the present in-vitro study was to assess antimicrobial efficacy of 0.8% hyaluronic acid (HA) and 0.2% Chlorhexidine gluconate (CHX) against *Porphyromonas gingivalis* (*P. gingivalis*).

**Methods:** The study was performed between December 2018 and March 2019 at the College of Dentistry at the Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia. The *P. gingivalis* biofilms were formed and grown for 72 hours at 37 °C under anaerobic conditions on glass slides coated with human saliva. The slides were individually positioned and exposed to 0.8% HA or 0.2% CHX. Therapeutically, the biofilms were divided into 3 groups as follows: (a) negative group; (b) 0.8% HA group and (c) 0.2% CHX group. P-values less than 0.05 were considered statistically significant.

**Results:** In the 0.8% HA group, *P. gingivalis* CFUs/ml were significantly higher at baseline than at 24- (P<0.05), 48 (P<0.05) and 72 hours (P<0.05) intervals. In the 0.2% CHX group, *P. gingivalis* CFUs/ml were significantly higher at baseline than at 72 hours interval (P<0.05). In the CHX group, there was no difference in *P. gingivalis* CFUs/ml between baseline, 24- and 48-hours intervals. At 48- and 72-hours intervals, the *P. gingivalis* CFUs/ml were significantly higher in the 0.2% CHX group compared with the 0.8% HA group.

**Conclusion:** In-vitro, 0.8% HA is more effective in reducing the *P. gingivalis* CFUs/ml compared with 0.2% CHX.

**Keywords:** Bacteria, Chlorhexidine gluconate, Hyaluronic acid, In-vitro, *Porphyromonas gingivalis*.

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**INTRODUCTION**

Periodontitis is associated with inflammatory conditions including gingival inflammation, increased probing depth, clinical attachment loss; and resorption of supporting alveolar bone. From a clinical perspective, poor oral hygiene maintenance is a well-known and significant risk-factor of periodontitis; however, laboratory-based investigations on oral biofilm samples collected from patients with periodontitis have shown an increased colonization of pathogenic microbes including *Porphyromonas gingivalis* (*P. gingivalis*), *Treponema denticola* (*T. denticola*), and *Tannerella forsythia* (*T. forsythia*), collectively known as Red Complex Bacteria (RCB). The RCB are well-known microbes associated with the etiopathogenesis of periodontal inflammatory conditions, including periodontitis and triggers inflammatory signaling pathways thereby jeopardizing human gingival fibroblasts.
Hyaluronic acid (HA) is a high molecular weight (20,000 kilodaltons) polysaccharide that belongs to the family of glycosaminoglycans. It consists of glucuronic acid, N-acetyl-glucosamine and a basic unit of two sugars. HA commonly exists in the synovial fluid, cartilage, and tissues of the eye and skin. The high molecular weight of HA exerts immunosuppressive and anti-inflammatory effects and promotes wound healing. However, there are no studies that have compared the antimicrobial efficacy of HA with 0.2% CHX against the P. gingivalis. It is hypothesized that HA and CHX exhibit a comparable level of antimicrobial efficacy against RCB. The aim of the present in-vitro experiment was to compare the antimicrobial efficacy of 0.8% HA with 0.2% CHX against P. gingivalis.

**METHODS**

**Ethical statement:** The study protocol was reviewed and approved by the Research Ethics Review committee IRB No. 18-0320 dated November 29, 2018 of the Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia. The study was performed between December 2018 and March 2019 at the College of Dentistry, Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia. Since the present study had an experimental design, a consent form was not warranted.

**Bacterial strain:** The P. gingivalis strains (ATCC®33277“ Manassas, VA, USA) were grown and maintained as described elsewhere. In summary, P. gingivalis W83 biofilms were formed and grown anaerobically on glass-slides, which were coated with human saliva for 96 hours at 37°C. Each slide was positioned in 50 mL tubes containing 45 mL of the bacterial inoculum (1.5 × 108 bacteria/mL) with Brain-Heart-Infusion broth supplemented with 5 µg/mL hemin and 1 µg/mL menadione. The slides were randomly exposed to either 0.8% HA or 0.2% CHX. The culture medium was replaced daily.

**Results:** The baseline bacterial CFUs/ml were comparable in the study groups. There was no statistically significant difference in the P. gingivalis CFUs/ml in the negative control group at all-time intervals. In the 0.8% HA group, P. gingivalis CFUs/ml were significantly higher at baseline compared with microbial colonization at 24- (P<0.05), 48- (P<0.05) and 72 hours (P<0.05) intervals. In the 0.2% CHX group, P. gingivalis CFUs/ml were significantly higher at baseline compared with microbial CFUs/ml at 72 hours interval (P<0.05). In the CHX group, there was no statistically significant difference in the P. gingivalis CFUs/ml at baseline and at 24- and 48 hours intervals (Table-I). At 48- and 72 hours intervals, the P. gingivalis CFUs/ml were significantly higher in the 0.2% CHX group compared with the 0.8% HA group (P<0.05) (Table-I).
DISCUSSION

It is well-established that CHX exhibits antibacterial properties and is a useful adjunct to traditional treatments of CP, such as scaling and root planning (SRP). However, the present experimental results indicate that the potency of 0.8% HA to reduce the counts of periodontopathogenic microbes is higher than that of CHX. One explanation for this is that hydrop hilicity of HA enhances the receptiveness of coagulum thereby improving cell repair, differentiation and proliferation of basal keratinocytes and mesenchymal cells. Moreover, HA facilitates osseous regeneration via induction of osteogenic proteins such as osteopontin and bone morphogenetic protein-2. Furthermore, the high concentration of medium and lower molecular weight HA exhibits bacteriostatic properties against a variety of pathogenic microbes including Prevotella Oris, Aggregatibacter actinomycetemcomitans and Streptococcus species, and the present in-vitro experiment is among the limited evidence that has shown HA to exhibit antibacterial effect against P. gingivalis.

From a clinical perspective, it is speculated that use of HA-based oral rinses when used as an adjunct to mechanical debridement (synonym, SRP) is more effective in the treatment of CP as compared to when CHX-based mouthwashes are used with SRP. The authors of the present experimental study support the results of a split-mouth randomized clinical trial (RCT) in which, 24 patients with moderate to severe CP were evaluated after SRP. In this study, the test-sites received 0.8% HA gel application as an adjunct to SRP and the control-sites underwent SRP alone. The 12-week follow-up results showed that there was a statistically significant reduction in periodontal inflammation in the test- compared with the control-sites. The authors of the present study hypothesize that the 0.8% HA gel significantly reduced the counts of pathogenic microbes (including P. gingivalis) in the test- compared with the control-sites thereby markedly reducing the clinical markers of periodontal inflammation (including plaque index, gingival bleeding and pocket depth).

The authors also speculate that SRP when used as an adjunct to SRP is more effective in the treatment of CP in medically compromised patients such as individuals with chronic hyperglycemia. It is well-established that persistent hyperglycemia (such as among patients with poorly controlled diabetes mellitus and prediabetes) is a risk factor for periodontal and peri-implant diseases; and a state of chronic hyperglycemia delays wound healing after periodontal therapy. According to Bansal et al., hyaluronate modulates wound healing and its administration in sites with periodontal defects helps. Moreover, in a recent study in diabetic rats, Eliezer et al. showed that cross-linked HA augments osseous wound healing by slowing down collagen membrane degradation. Further well-designed randomized controlled clinical trials are needed to test these aforementioned experimental results.

Limitations of the study: One limitation of the present study is that the results were entirely based on an in-vitro assessment of P. gingivalis strains. This makes it difficult to contemplate these laboratory-based results into a clinical setting in which, confounders such as habitual tobacco smoking and an immunocompromised health status may potentially compromise the efficacy of SRP with or without adjunct HA therapy in patients with CP. Moreover, only one concentration of HA (0.8%) was tested. The minimum concentration of HA that may potentially help reduce periodontal inflammation and augment healing remains to be determined. This warrants additional studies.

CONCLUSION

The 0.8% HA is more effective in reducing the P. gingivalis CFUs/ml compared with 0.2% CHX. Further well-designed RCTs are needed to assess the clinical efficacy of 0.8% HA as an adjunct to SRP in the treatment of CP.

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REFERENCES

1. Javed F, Al-Kheraif AA, Salazar-Lazo K, Yanez-Fontenla V, Aldosary KM, Alshehri M, et al. Periodontal inflammatory conditions among smokers and never-smokers with and without type 2 diabetes mellitus. J Periodontol. 2015;86:839-846. doi: 10.1902/jop.2015.150120

2. Javed F, Naastrom K, Benchimol D, Altamash M, Klinge B, Engstrom PE. Comparison of periodontal and socioeconomic status between subjects with type 2 diabetes mellitus and non-diabetic controls. J Periodontol. 2007;78:2112-2119. doi: 10.1902/jop.2007.070186.

3. Knack C, Eick S, Knoller GU, Purschwitz RE, Jentsch HF. Clinical and microbiological results 12 months after scaling and root planing with different irrigation solutions in patients with moderate chronic periodontitis: A pilot randomized trial. J Periodontol. 2012;83:312-320. doi: 10.1902/jop.2011.110044

4. Suzuki N, Yoneda M, Hirofuji T. Mixed red-complex bacterial infection in periodontitis. Int J Dent. 2013;2013:587279. doi: 10.1155/2013/587279

5. Neuman MG, Nanau RM, Oruna-Sanchez L, Coto G. Hyaluronic acid and wound healing. J Pharm Pharm Sci. 2015;18:53-60.

6. Sukumar S, Drizhal I. Hyaluronic acid and periodontitis. Acta Medica (Hradec Kralove). 2007;50:225-228. doi: 10.14712/18059694.2017.88

7. Vanden Bogaerde L. Treatment of infrabony periodontal defects with esterified hyaluronic acid: Clinical report of 19 consecutive lesions. Int J Periodontics Restorative Dent. 2009;29:515-523.

8. Chen M, Li L, Wang Z, Li P, Feng F, Zheng X. High molecular weight hyaluronic acid regulates p. Gingivalis-induced inflammation and migration in human gingival fibroblasts via mapk and nf-kappab signaling pathway. Arch Oral Biol. 2019;98:75-80. doi: 10.1016/j.archoralbiol.2018.10.027

9. Vianna ME, Gomes BP, Berber VB, Zaia AA, Ferraz CC, de Souza-Filho FJ. In vitro evaluation of the antimicrobial activity of chlorhexidine and sodium hypochlorite. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004;97:79-84. doi: 10.1016/s1079210403003603

10. Papaioannou W, Papagoupolous A, Koletsi-Kounari H, Kontou E, Makou M. Adhesion of porphyromonas gingivalis and biofilm formation on different types of orthodontic brackets. Int J Dent. 2012;2012:471380. doi: 10.1155/2012/471380

11. Re ACS, Bonjovanni MC, Ferreira MP, Freitas O, Aires SA and MB. Adhesion of periodontal inflammatory conditions among smokers and never-smokers with and without type 2 diabetes mellitus. J Periodontol. 2015;86:839-846. doi: 10.1902/jop.2015.150120

12. Kaur A, Bhavikatti SK, Das SS, Khanna S, Jain M, Kaur A. Efficacy of ozonised water and 0.2% chlorhexidine glucinate in the management of chronic periodontitis when used as an irrigant in conjunction with phase I therapy. J Contemp Dent Pract. 2019;20:318-323.

13. Toole BP. Hylauronaln in morphogenesis. Semin Cell Dev Biol. 2001;12:79-87. doi: 10.1006/scdb.2000.0244

14. Bansal J, Kedige SD, Anand S. Hyaluronic acid: A promising mediator for periodontal regeneration. Indian J Dent Res. 2010;21:575-578. doi: 10.4103/0970-9290.74232

15. Xie Z, Meng K, Yang X, Liu J, Yu J, Zheng C, et al. Identification of a quorum sensing system regulating capsule polysaccharide production and biofilm formation in streptococcus zooepidemicus. Front Cell Infect Microbiol. 2019;9:121. doi: 10.3389/fcimb.2019.00121

16. Al-Shammari NM, Shafshak SM, Ali MS. Effect of 0.8% hyaluronic acid in conventional treatment of moderate to severe chronic periodontitis. J Contemp Dent Pract. 2018;19:527-534.

17. Javed F, Ahmed HB, Mehmoed A, Bain C, Romanos GE. Effect of nonsurgical periodontal therapy (with or without oral doxycycline delivery) on glycemic status and clinical periodontal parameters in patients with prediabetes: A short-term longitudinal randomized case-control study. Clin Oral Investig. 2018;14:1963-1968. doi: 10.1007/s00784-014-1185-6

18. Javed F, Al-Askar M, Al-Rasheed A, Babay N, Galindo-Moreno P, Al-Hezaimi K. Comparison of self-perceived oral health, periodontal inflammatory conditions and socioeconomic status in individuals with and without prediabetes. Am J Med Sci. 2012;344:100-104. doi: 10.1097/MAJ.0b013e31823650a7

19. Abduljabbar T, Vohra F, Javed F, Akram Z. Antimicrobial photodynamic therapy adjuvant to non-surgical periodontal therapy in patients with diabetes mellitus: A meta-analysis. Photodiagnosis Photodyn Ther. 2017;17:138-146. doi: 10.1016/j.pdpdt.2016.11.008

20. Javed F, Al Amri MD, Al-Kheraif AA, Qadri T, Ahmed A, Gharem A, et al. Efficacy of non-surgical periodontal therapy with adjunct nd:Yag laser therapy in the treatment of periodontal inflammation among patients with and without type 2 diabetes mellitus: A short-term pilot study. J Photochem Photobiol B. 2015;149:230-234. doi: 10.1016/j.jphotobiol.2015.06.013

21. Eliezer M, Sculean A, Miron RJ, Nemcovsky C, Weinberg E, Weinreb M, et al. Hyaluronic acid slows down collagen membrane degradation in uncontrolled diabetic rats. J Periodontal Res. 2019. doi: 10.1111/jpr.12665

Authors’ Contribution:

MB and SA conceived and designed the study and edited the manuscript; and are responsible and accountable for the accuracy or integrity of the work.

KA wrote the methods and did statistical analysis.

FA, MA & AA did data collection and manuscript writing.

SA and MB did review and final approval of manuscript.