

Clinical and microbiological efficacy of hyaluronic acid gel compared to chlorhexidine in the treatment of gingivitis

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Abstract

Background and objectives: Chemotherapeutic agents have the potential to inhibit plaque growth, reduce gingivitis and improve oral health beyond tooth brushing alone. The aim of this study is to compare the therapeutic effectiveness of 0.2% hyaluronic acid-containing gel (Gengigel) with a 0.2% chlorhexidine containing gel effect, in the treatment of plaque induced gingivitis.

Methods: This cross-sectional analytical case control study involved 150 generally healthy patients, age ranging 20-40 years, with gingivitis. The patients were divided in two groups, after scaling and polishing, 75 patients in each. The first group was given 0.2% hyaluronic acid containing gel and the second group was given 0.2 % chlorhexidine containing gel. Clinical examination, which included bleeding on probing, plaque index at the baseline, after 7 days and after 21 days were calculated. Antimicrobial properties of both gels in the saliva were measured by agar diffusion method.

Results: No significant difference was detected between the two groups regarding the mean ranks of the Plaque index difference after a week (chlorhexidine group = 0.81, hyaluronic acid group = 0.89) and after three weeks (0.58, 0.54 respectively). The mean of the difference in Bleeding index after one week in the chlorhexidine group (39.44) were significantly higher than those (32.87) of the group of hyaluronic acid, But the difference was not significant after three weeks (37.40 and 37.27 respectively). The chlorhexidine group inhibition zone (19.2) was significantly higher than the hyaluronic acid group (10.6)

Conclusions: in this study, the adjunctive use of 0.2 percent hyaluronic acid gel is comparable to 0.2 percent chlorhexidine gel for gingivitis treatment.

Keywords: Gingivitis; Chlorhexidine; Hyaluronic acid.

Introduction

Gingivitis generally considered a site-specific inflammatory condition initiated by dental biofilm accumulation and characterized by gingival redness and edema and therefore the absence of periodontal attachment loss¹. Chemotherapeutic agents along with mechanical plaque control have the ability to inhibit plaque growth, reduce gingivitis and improve oral health beyond tooth brushing alone². Chlorhexidine is the gold

standard anti-plaque agent². it prevents plaque accumulation that's why it is anti-plaque and anti-gingivitis, it has defense against a wide variety of gram-positive and gram-negative bacteria³ and one of the most important properties of chlorhexidine is substantivity⁴. Substantivity means oral retentiveness. Despite that it has some side effects including brown discolorations of teeth and restorative material, taste perturbation, oral and mucosal erosion, and

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bitter taste⁵. This is why its use for the long-term is not advocated. Hyaluronic acid (HA) is a linear polysaccharide formed naturally in the extracellular matrix of connective tissue, synovial fluid, and other tissues. It has various functions, including cellular and extracellular interactions, interacting with growth factors, regulation of the osmotic pressure, and tissue lubrication. All these functions help in maintaining the tissues structural and homeostatic integrity⁶. Hyaluronic acid is a key element in constituent of periodontal tissues, gingiva, periodontal ligament, alveolar bone, and cementum⁷. In the therapy of periodontal disease HA has anti-inflammatory, anti-edematous, and anti-

Materials and Methods

This study involved 150 generally healthy patients, age ranging 20-40 years, referred to Khanzad teaching center of dental specialties, from November 2019 to March 2020 for the treatment of gingivitis, enrolled in this study. Informed consent obtained from each patient before initiation of treatment after acceptance of the ethical committee of Kurdistan Board of Medical Specialties (KBMS). The included patient should have at least 24 natural teeth of at least 30% bleeding site on probing¹. Patients excluded if they had a systemic disease or those who have receive a periodontal and antibiotic therapy during the last month, if they were pregnant and/or lactating women, smokers, those individuals who wear removable prosthetic and those who did not attend for follow up visits. The patients were divided into two groups, 75 patients in each. The first group was given 0.2% hyaluronic acid gel (Gengigel; Ricerfarma, Milan, Lombardy, Italy) and the second group was given 0.2% chlorhexidine gel (Denta Perio CJSC "VITEX" Republic of Belarus,) twice daily after they had scaling and polishing. The patients instructed to brush their teeth with the modified bass method¹³ and randomly (by coin toss) were assigned to each

bacterial effects⁶, it has a bacteriostatic effect against periodontal pathogen⁸. Hyaluronic acid antimicrobial/antiadhesive barrier was verified by Romanò⁹. Hyaluronic acid has been compared to chlorhexidine by Chauhan¹⁰ for chronic periodontitis, while de Araujo Nobre¹¹ compared hyaluronic acid with chlorhexidine in Peri-implant maintenance. However, hyaluronic acid was not compared to chlorhexidine in the treatment of gingivitis. This study aims to compare the therapeutic efficacy of 0.2% hyaluronic acid-containing gel (Gengigel) with the effect of 0.2% chlorhexidine containing gel, in the treatment of plaque induced gingivitis.

treatment group then placed 1ml of the gel over the gingival surface, by advising them to take 1.5 cm of the gel which was found to correspond to 1ml gel. After tooth brushing; eating was avoided at least one hour after gel application. Clinical examination, which included bleeding on probing (BOP) (yes/no) at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual), plaque index¹² at the baseline, after 7 days and after 21 days. A saliva sample (One mL of unstimulated saliva) was taken before examination and send for microbiological examination in a laboratory of Rizgary teaching hospital within less than 3 hours. Both gels were tested for Antibacterial activity using agar well diffusion method on the blood agar medium¹⁴. The saliva sample was diluted in sterile physiological saline (85% NaCl) in test tubes to 0.5 McFarland standards (1.5×10^8 organisms/ml), with a sterile cotton swab the diluted saliva is cultured on the blood agar, two wells were made in the blood agar plate using a sterile Cork borer measuring 4 mm depth and 6 mm in diameter on the same plate, one well filled with (100 µl of chlorhexidine gel) and then another one with (100 µl of hyaluronic acid gel) with

the help of micropipette, after that the plates were incubated in 37°C for 48 h. After incubation of the plates the inhibition zone around the well were observed and the diameters of the inhibition zones was measured in mm¹⁵. Data were analyzed using the Social Sciences Statistical system (IBM, SPSS, version 25). Association check chi-square was used to compare the proportions. The test of Fisher's exact was used when the predicted amount was more than 20 percent of the cells of the table was

less than 5. A normality test (Shapiro-Wilk) was used to demonstrate whether the data was normally distributed or not. Its finding showed that normally the data were not distributed. Therefore, non-parametric studies have been used. The Mann Whitney test was used to compare the mean ranks of the two groups, and the Wilcoxon signed ranks test was used to compare the medians before and after the intervention. Statistically significant was considered a p-value of ≤ 0.05 .

Results

The total number of the studied sample was 150 patients. They were divided into two groups, 75 patients in each. The first group was given hyaluronic acid and the second group was given chlorhexidine. Table (1) shows that 41.3% of the first group was aged ≥ 35 years compared with 8% in the second group (p-value < 0.001). It is evident in the table that 58% of the sample were females, but there was no significant difference between the two groups

regarding the gender distribution (p-value = 0.137). The majority of the sample (95.3%) used to brush their teeth, but there was no significant difference between the groups in the brushing practices (p-value = 0.442). Around half (51%) of those who brush their teeth, they do it once daily, and 23.8% brush their teeth less frequently, but there was no significant difference between the two groups in the frequency of brushing (p-value = 0.353).

Table (1): Basic characteristics of the study samples.

	Hyaluronic		Chlorhexidine		Total		p-value
	No.	(%)	No.	(%)	No.	(%)	
Age							
20-24	23	(30.7)	24	(32.0)	47	(31.3)	
25-29	12	(16.0)	27	(36.0)	39	(26.0)	
30-34	9	(12.0)	18	(24.0)	27	(18.0)	
≥ 35	31	(41.3)	6	(8.0)	37	(24.7)	$<0.001^\dagger$
Gender							
Male	36	(48.0)	27	(36.0)	63	(42.0)	
Female	39	(52.0)	48	(64.0)	87	(58.0)	0.137 †
Brushing							
Yes	70	(93.3)	73	(97.3)	143	(95.3)	
No	5	(6.7)	2	(2.7)	7	(4.7)	0.442*
Frequency of brushing (n = 143)							

Sometimes	15	(21.4)	19	(26.0)	34	(23.8)	
Once daily	40	(57.1)	33	(45.2)	73	(51.0)	
Twice daily or more	15	(21.4)	21	(28.8)	36	(25.2)	0.353†
Total	75	(100.0)	75	(100.0)	150	(100.0)	

*By Fisher’s exact test. †By Chi square test.

It is evident in Table (2) that in each of the study groups, there was significant improvement in the PI and BI after one

week and after three weeks of the intervention ($p < 0.001$).

Table (2): PI and BI parameters one and three weeks after intervention compared with the baseline readings.

	Mean	(±SD)	Median	Min.	Max.	p-value
Hyaluronic acid						
PI baseline	1.97	(±0.62)	2.00	1.00	3.00	
PI 1 week	0.89	(±0.29)	1.00	0.00	1.50	< 0.001*
PI 3 weeks	0.54	(±0.34)	0.50	0.00	1.00	< 0.001*
BI baseline	93.89	(±7.29)	96.00	75.00	100.00	
BI 1 week	61.03	(±20.47)	64.00	25.00	100.00	< 0.001*
BI 3 weeks	37.27	(±13.39)	37.00	14.00	93.00	< 0.001*
Chlorhexidine						
PI baseline	1.90	(±0.59)	2.00	1.00	3.00	
PI 1 week	0.81	(±0.50)	1.00	0.00	2.00	< 0.001*
PI 3 weeks	0.58	(±0.42)	0.50	0.00	1.58	< 0.001*
BI baseline	94.05	(±10.11)	97.00	58.30	100.00	
BI 1 week	54.61	(±15.40)	51.00	30.00	89.00	< 0.001*
BI 3 weeks	37.40	(±14.40)	35.00	17.00	78.00	< 0.001*

*Compared with baseline day zero (By Wilcoxon rank sum test).

The PI and BI readings after one week and after three weeks had been subtracted from the baseline readings in order to calculate the difference. No significant difference was detected between the two groups regarding the mean ranks of the difference of the PI after one week ($p = 0.970$) and after three weeks ($p = 0.318$). The mean and

the mean rank of the difference in BI after one week in the chlorhexidine group (39.44 and 40 respectively) were significantly higher than those (32.87 and 31 respectively) of the hyaluronic acid group ($p = 0.012$), but the difference was not significant after three weeks ($p = 0.669$).

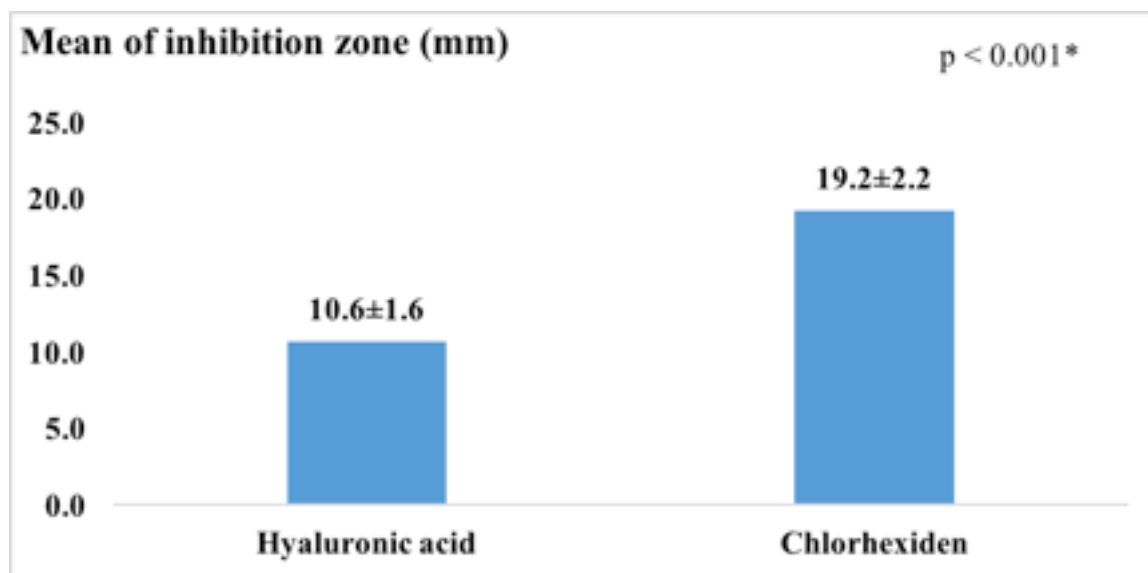
Table (3): Parameters of the difference between zero reading and one and three weeks

Diff†	Hyaluronic acid				Chlorhexidine				p-value*
	Mean	(±SD)	Median	Mean rank*	Mean	(±SD)	Median	Mean rank*	
Diff. PI 0-1WK	1.08	(±0.64)	1.00	75.63	1.08	(±0.59)	1.00	75.37	0.970
Diff. PI 0-3WK	1.44	(±0.65)	1.34	79.03	1.32	(±0.65)	1.50	71.97	0.318
Diff. BI 0-1WK	32.87	(±19.13)	31.00	66.61	39.44	(±16.17)	40.00	84.39	0.012
Diff. BI 0-3WK	56.62	(±12.02)	56.00	73.99	56.65	(±14.45)	58.00	77.01	0.669

readings of the two study groups.

*By Mann Whitney test comparing the mean ranks of the two groups. †Diff: Difference between baseline zero reading minus one and three weeks readings

It is evident in Figure 1 that the mean (19.2 mm) and the mean rank (113) of the inhibition zone of the chlorhexidine group was significantly ($p < 0.001$) higher than the mean and mean rank of the hyaluronic acid group (10.6 and 38 respectively).



*By Mann Whitney test comparing the mean rank of inhibition zone of the hyaluronic acid (38) and that of the chlorhexidine (113).

Figure (1): Parameters of the inhibition zone of the two study groups.

Discussion

Plaque control is the cornerstone of periodontal disease prevention, recovery and maintenance. Mechanical tooth cleaning is not sufficient to clean all tooth surfaces alone, chemotherapeutic agents have the ability to inhibit plaque growth, reduce gingivitis and improve oral health². Pharmacological intervention on periodontal disease shifted from antimicrobial to anti-inflammatory approach⁸. Hyaluronic acid has shown to have anti-inflammatory properties and decrease gingivitis⁶. In the early stage of inflammation, bind hyaluronic acid with fibrin clot and stimulate cytokine production by fibroblasts, ameloblasts, osteoblast, and keratinocytes. This promotes inflammation and activate inflammatory cells, stimulates migration toward the lesion, phagocytosis, increasing osteoblast activity by stimulating differentiation and migration of mesenchymal cells. And holding growth factors responsible for restoring tissue¹⁰. In this study, the performance of 0.2% hyaluronic acid gel (Gengigel) on gingivitis was evaluated and compared with the performance of 0.2% chlorhexidine gel, which applied topically by the patient twice daily after dental prophylaxis. Clinical

Conclusion

Adjunctive use of 0.2% hyaluronic acid gel was used in this study is comparable to 0.2% chlorhexidine gel in the treatment of gingivitis, and it's more effective at the first

Conflicts of interest

There were no conflicts of interest.

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parameter was calculated (BOP% and PI) at the baseline, 7th day, and 21 days. After one week, the plaque index in both groups improved significantly, while after three weeks of the intervention, no significant difference was found in all examination between study groups regarding the plaque index. This result was identical to that of Batavia¹⁶ and Pagnacco¹⁷. The plaque score reduction can be due to adequate oral hygiene maintenance, proficiently removing of all deposits by scaling and polishing and antibacterial effect of both gel^{3, 18}. The gingival bleeding score was reduced in both groups, and was significantly higher in the group of hyaluronic acid than group of chlorhexidine after one week and no substantial difference found after three weeks between both groups¹⁰, that finding was similar to the results of Chauhan¹⁰ and de Araujo Nobre¹¹. This finding can result from anti-inflammatory, anti-edematous, and scavenger effect of hyaluronic acid⁶. Both gels showed antibacterial action by the agar diffusion method. The inhibition zone of chlorhexidine was significantly higher than hyaluronic acid, that was consistent with both YI¹⁵ and Pirnazar¹⁸ results.

week of treatment while after three weeks it's just like chlorhexidine when topically applied to inflamed gingivae in the reduction in PI and BOP.

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