ORIGINAL ARTICLE

Periodontal Medicine

Evaluation of hyaluronan gel (Gengigel®) as a topical applicant in the treatment of gingivitis

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Keywords
argyrophilic nucleolar organizer region, inflammatory infiltrate, plaque-induced gingivitis, scaling, topical hyaluronan gel.

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Abstract

Aim: To clinically and histopathologically evaluate the anti-inflammatory effect of 0.2% hyaluronan gel alone and with mechanical therapy on gingivitis. The argyrophilic nucleolar organizer region staining technique was attempted to routinely determine its diagnostic and prognostic dependability for periodontal lesions.

Methods: In each of the 28 gingivitis patients, the four quadrants were subjected to different treatments: scaling, scaling + topical hyaluronan gel, only topical hyaluronan gel, and topical + intrasulcular hyaluronan gel. Clinical parameters were recorded at baseline, and on days 7, 14, and 21. Biopsies were taken from each quadrant, inflammatory infiltrates were graded, and the argyrophilic nucleolar organizer region count was measured before and after treatment.

Results: A significant reduction was seen in clinical parameters, inflammatory infiltrates, and the argyrophilic nucleolar organizer region count within the groups. The effect of topical + intrasulcular gel was equivalent to scaling ($P > 0.05$). Topical + intrasulcular hyaluronan gel application demonstrated a better reduction than topical hyaluronan gel alone.

Conclusions: Hyaluronan gel is an effective topical agent for treating gingivitis, along with scaling and intrasulcular application. The argyrophilic nucleolar organizer region count can be used as a histopathological indicator in cases of non-responsive gingivitis to assess the severity of gingival inflammation.

Introduction

Plaque-induced gingivitis is an inflammation of the gingiva resulting from bacteria located at the gingival margin. As part of non-surgical therapy, local drug therapy is common in the treatment of periodontal diseases, as it provides a high concentration of drugs where needed without exposing the whole body. The side-effects of systemic antibiotic therapy and the possible failing compliance of the patient can be minimized by using locally-applied antibiotics.

Various antibiotics, including minocycline,[1] tetracycline,[2] metronidazole,[3] and hexidine,[4] and anti-inflammatory agents, such as flurbiprofen and triclosan,[5] have been trialed in previous studies as a topical preparation. However, in the dental profession, anti-inflammatory treatment is generally limited to mouthwashes that contain no high-risk, active constituents.

The endogenous hyaluronan (HA) is a high-molecular weight (10 000–10 000 000 Da), non-sulfated polysaccharide component of the glycosaminoglycan family that is present in the extracellular matrices of many tissues, such as skin, synovial joints, and periodontal tissues. It is also a key component in the series of stages associated with the wound-healing process in both mineralized and non-mineralized tissues.

The topical application of a high-molecular weight exogenous HA-based gel (Gengigel®; Ricerfarma, Milan, Italy) has been proposed to have some potential in inducing periodontal healing in patients with inflammatory gingivitis, during both open and randomized, controlled, double-blind studies.[6,7]

The anti-inflammatory effect of HA can be attributed to its action of de-activating bacterial hyaluronidases, normalizing the macro-aggregation of connective tissue proteoglycans, and bonding with free water, thus performing an anti-edema effect. It has also been stated that HA is a natural and essential extracellular matrix substance that encourages healing by stimulating angiogenesis, has bacteriostatic and antiseptic properties, maintains the structural integrity of the tissues, regulates tissue hydration and cell physiology, protects the tissues by forming a barrier, stimulates the production of pro-inflammatory cytokines, regulates the migration of phagocytes, prevents bacterial colonization, stimulates granulation tissue formation and healing, interacts with growth factors for the development of mineralized and non mineralized tissues, and is absorbed locally when applied to tissues.[8]

A Medline search including the key words ''gingivitis'' and ''hyaluronic acid'' revealed scant results. HA has been trialed for topical application, that is, on the outer surface of gingiva (extrasulcular), but intrasulcular application has not been trialed for gingivitis cases.[7,9] A recent study by Pistorius *et al.* using HA spray for the treatment of gingivitis showed significant improvement in clinical parameters.[9] Thus, a first attempt has been made in the present study to use HA alone and with scaling and intrasulcular application.

Argyrophilic nucleolar organizer region (AgNOR) staining is one of the methods that have been employed to indicate proliferative activity in normal tissues and both benign and malignant tumors. It was recently demonstrated that changes in AgNOR number and its distribution indicate the destruction and repairing stages of experimentally-induced marginal periodontitis in dogs.[10] A study by Saluja and Vandana that evaluated the diagnostic and prognostic ability of AgNOR in various neoplastic and non-neoplastic periodontal conditions showed that AgNOR has a limited diagnostic value, with a definite prognostic value in non-neoplastic periodontal lesions.[11]

Thus, the purpose of the present study was to assess and clinically compare the anti-inflammatory effect of HA gel (0.2% hyaluronic acid, Gengigel®) alone and in combination with intrasulcular application and scaling in the treatment of plaque-induced gingivitis. A histopathological evaluation of treated sites by counting inflammatory infiltrates and AgNOR count was also conducted before and after therapy.

Materials and methods

The ethical review committee of Rajiv Gandhi University of Health Sciences, Karnataka, India, approved the study. A split-mouth + cross-over (mixed-design) study was conducted on 32 patients (aged 18–35 years) who had plaque-induced gingivitis with a probing depth <3 mm. Informed consent was obtained from all the patients. The exclusion criteria included patients who had taken antibiotic therapy in month prior to the commencement of the study, and periodontal therapy in the last 6 months prior to the commencement of the study. Patients with known systemic diseases, smokers, and pregnant and lactating women were also excluded.

In this randomized, single-blinded study, the dental arches were divided into four quadrants, and each quadrant received different treatment modalities: control group, scaling; experimental group I, scaling and topical application of HA gel; experimental group II, only topical application of HA gel; and experimental group III, both topical and intrasulcular applications of HA gel. The allotment of different treatment protocols to different quadrants was done randomly, and the main investigator was blinded for the treatment protocol. The randomization code was concealed until the results were analyzed.

Prior to treatment, each quadrant was subjected to assessment of the following clinical parameters: plaque index (PI),[12] gingival index (GI),[13] and gingival bleeding index (GBI).[14] These were recorded by the main investigator and were repeated at days 7, 14, and 21. Any adverse reactions to the gel were also recorded.

The duration of the study was 3 + 3 (6) weeks. In the first 3-week trial, scaling was performed in one of the quadrants of the selected patients. In the second 3-week trial, the application of gel either alone or in combination was done (Figure 1). The HA gel was applied topically (extrasulcularly) on the gingival surface, and intrasulcularly in selected quadrants by the co-investigator. The patients were instructed to brush twice daily with a conventional toothbrush and toothpaste using roll-on technique as part of regular plaque-control measures. They were advised to apply the gel on the gingiva topically with the help of

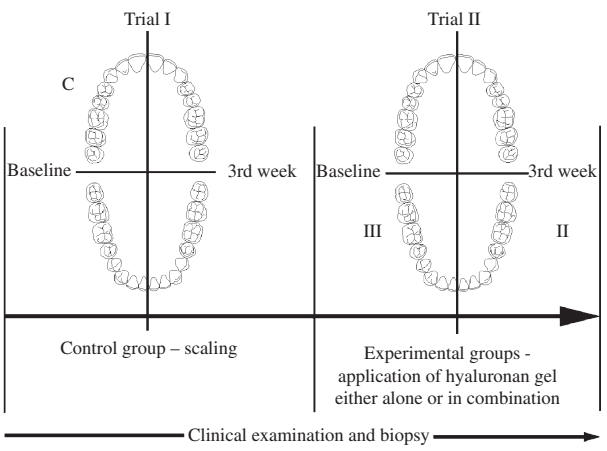

Figure 1. Experimental schedule depicting mixed-design study (split-mouth + cross-over design).

cotton bud applicator twice daily for 21 days after regular oral hygiene regimen. After application, the patients were instructed to avoid eating, drinking, or rinsing for 1 h. Intrasulcular application was done with a 2-mL syringe with a 20-gauze blunt needle on alternate days until the end of the study period by the co-investigator. The gel was placed intrasulcularly until the level of the gingival margin, and it could be retained in the sulcus to a better extent, unlike a solution that flows out of the sulcus.

Histopathology

A biopsy of the gingival papilla was performed at the buccal aspect between the first and second molars in each quadrant for the histopathological study. At baseline, the biopsy was obtained from the quadrant site that underwent scaling, and served as the control for the other experimental sites. The biopsies were repeated at the treated sites at the end of the study period. The specimens were then immediately fixed in 10% buffered formalin, embedded in paraffin, and then 3-μm sections were cut for hematoxylin–eosin staining.

The grading of the inflammatory infiltrate was established by randomly selecting and counting five fields of lamina propria at ×400 magnification. The inflammatory infiltrate was considered ''absent'' when none of the five fields showed inflammatory cells, ''slight'' when at least two fields contained inflammatory cells that occupied <50% of the field, ''moderate'' when at least two fields showed inflammatory cells that occupied >50% of the field, and ''intense'' when all five fields showed inflammatory cells that occupied >90% of the field.[15]

After histological confirmation by hematoxylin–eosin staining, the other section was stained for the nucleolar organizer region to examine the proliferative activity of the periodontal lesion and for counting the cells. Images were captured using a three-chip charge coupled device camera attached to a trinocular research microscope. Actual counting was done using the Windows-based image analyzer software (Pro Plus, version 4.1.0.0; Media Cybernetics, Houston, TX, USA). For each case, 100 cells were counted, and the AgNOR were visualized as black and brown dots. Images were captured, and manual counting of AgNOR was done as cluster counts.

Statistical analysis

Intragroup comparisons of clinical parameters were made by paired *t*-test. Multiple group comparisons were analyzed by one-way ANOVA, followed by unpaired *t*-test for pairwise comparisons of clinical parameters. Intragroup and intergroup comparisons of the grading of the inflammatory infiltrates was done by calculating the percentage of reduction in the inflammatory infiltrates. For the intragroup comparison of the AgNOR count, Wilcoxon's signed rank test was used, whereas the Mann–Whitney *U*-test was used for intergroup comparisons. The relationship between changes in the GBI, GI, and AgNOR counts were assessed by correlation and regression analyses. A *P*-value of 0.05 or less was considered to be significant.

Table 1. Comparison of various clinical parameters within each group from baseline to day 21 (mean ± SD)

Groups													
		Sc			Sc + TG			TG			TG + IG		
Index	Appointment day	*n*	$\bar{x}^k$	SD	*n*	$\bar{x}$	SD	*n*	$\bar{x}$	SD	*n*	$\bar{x}$	SD
Plaque index	0	28	1.75	0.35	28	1.68	0.40	28	1.76	0.35	28	1.75	0.32
	21	28	0.92	0.30	28	0.70	0.21	28	1.18	0.35	28	1.03	0.30
	t-test		10.40			16.00			9.10			10.20	
	P-value*		<0.001			<0.001			<0.001			<0.001	
Gingival index	0	28	1.88	0.35	28	1.92	0.30	28	1.84	0.36	28	1.88	0.34
	21	28	0.98	0.29	28	0.78	0.23	28	1.19	0.25	28	1.05	0.31
	t-test		13.7			21.30			11.10			13.80	
	P-value		<0.001			<0.001			<0.001			<0.001	
Gingival bleeding index	0	28	91.42	11.1	28	90.66	10.9	28	90.66	12.00	28	89.46	11.40
	21	28	28.41	18.5	28	24.30	18.50	28	43.13	20.50	28	34.21	17.10
	t-test		23.60			19.10			14.20			15.30	
	P-value		<0.001			<0.001			<0.001			<0.001	
Probing depth	0	28	1.72	0.55	28	1.73	0.45	28	1.79	0.48	28	1.64	0.50

Ig, intrasulcular gel; Sc, scaling; SD, standard deviation; TG, topical gel.
*$P < 0.001$, highly significant.

Results

Of the 32 patients, four did not attend clinics at the subsequent visits, and were therefore considered spontaneous dropouts and excluded from the trial. The trial was therefore completed using 28 patients (112 quadrants), and the results were computed including these data.

The PI, GI, and GBI did not differ significantly between the groups at baseline, and significant improvement could be found for all clinical variables in all the groups (Table 1). On intergroup comparison, the scaling + topical gel group showed a maximum reduction in all the clinical parameters (58%, 59%, and 73% for PI, GI, and GBI, respectively), followed by the scaling group (47%, 48%, and 69%, for PI, GI, and GBI, respectively), the topical + intrasulcular gel group (41%, 44%, and 62%, for PI, GI, and GBI, respectively), and the topical gel group (33%, 36%, and 52%, for PI, GI, and GBI, respectively). All the groups showed a significant reduction in the clinical parameters when compared to the topical gel group ($P < 0.01$). However, there was no significant difference when scaling was compared to the topical + intrasulcular gel group ($P > 0.05$) (Table 2).

When the percentage of reduction for GBI and GI was compared in each group, there was a correlation between their reductions in all the groups, as shown in Table 3. No adverse effects to the gel were observed on clinical examination and as reported by the patients.

The gingival sections presented chronic inflammatory infiltrates of variable intensity, mainly composed of patches of lymphocytes, plasma cells, and macrophages in the lamina propria. There was a reduction in inflammatory infiltrates from baseline to day 21 in all the groups. The reduction was higher in the scaling + topical HA group (50%), followed by the scaling group (44%), topical + intrasulcular HA group (33.34%), and the topical group (16.67%) (Table 4; Figure 2). Parallel to the reduction of inflammatory infiltrates, a significant decrease in bleeding was recorded at the end of the study.

In the gingival epithelium, the HA significantly reduced the proliferation index (AgNOR count) in all the experimental groups compared to the control group at the end of the study period. On intergroup comparison, the scaling + topical HA experimental group showed a maximum reduction in AgNOR count compared to the other experimental groups (33%, $P < 0.05$) (Table 5; Figure 3).

When the percentage of reduction for GBI and GI were compared with the percentage of reduction for the AgNOR count, there was a positive correlation in the reduction in all the groups, although the reduction was statistically insignificant ($P > 0.05$) (Table 6).

Table 2. Comparison of various clinical parameters between different groups from baseline to day 21 day (mean ± SD)

	Sc		Sc + TG		TG		TG + IG		Significance of difference													
									Sc vs Sc + TG			Sc vs TG			Sc vs TG + IG			Sc + TG vs TG + IG			TG vs TG + IG	
Clinical parameters	Mean reduction	%	Mean reduction	%	Mean reduction	%	Mean reduction	%	*F*-value†	*t*-test	*P*-value*	*t*-test	*P*-value		*t*-test	*P*-value		*t*-test	*P*-value		*t*-test	*P*-value
Plaque index	0.83 ± 0.42	47	0.98 ± 0.32	58	0.58 ± 0.33	33	0.72 ± 0.38	41	0.06	1.52	0.12 NS*	2.49	<0.05 S**		0.95	0.35 NS		4.6	<0.001 HS***		2.69	<0.01 S		1.58	0.12 NS
Gingival index	0.9 ± 0.34	48	1.13 ± 0.28	59	0.66 ± 0.31	36	0.83 ± 0.32	44	11.14	2.88	0.05 S	2.71	0.01 S		0.7	0.49 NS		6.05	<0.001 HS		3.79	<0.001 HS		2.08	<0.05 S
Gingival bleeding index	63.01 ± 14.40	69	66.36 ± 18.40	73	47.5 ± 17.70	52	55.26 ± 19.10	62	6.51	0.76	0.45 NS	3.62	<0.01 S		1.73	0.09 NS		3.9	<0.001 HS		2.22	<0.05 S		1.57	0.12 NS

Ig, intrasulcular gel; Sc, scaling; SD, standard deviation; TG, topical gel.
*$P > 0.05$, not significant (SD), **$P < 0.01$, significant (S), ***$P < 0.001$, highly significant (HS).
†One-way ANOVA, $F = 6.51$.

Table 3. Regression analysis. Comparison of percentage of reduction (days 0–21) in GBI and GI

Groups	Reduction in GBI (%)	Reduction in GI (%)	r†	b‡
Sc	70	47	+0.77	+0.97
Sc + TG	74	59	+0.45	+0.77
TG	53	35	+0.59	+0.89
TG + IG	63	44	+0.86	+1.33

GBI, gingival bleeding index, GI, gingival index; Ig, intrasulcular gel; Sc, scaling; TG, topical gel.
†Pearson's correlation coefficient.
‡Regression coefficient.

Table 4. Comparison of inflammatory infiltrates at day 21 in the different treatment groups (reduction %)

Group	No. sites with intense and medium-grade inflammatory infiltrates		Reduction (%)
	Baseline	Day 21	
Sc	18	10	44
Sc + TG	18	9	50
TG	18	15	16.67
TG + IG	18	12	33.34

Ig, intrasulcular gel; Sc, scaling, TG, topical gel.

Discussion

Recent pharmacological research on periodontal disease intervention has shifted from an antimicrobial to an anti-inflammatory approach to therapy. An elevated prostaglandin E_2 level discovered at inflamed periodontal sites is behind the rationale of non-Steroidal anti-inflammatory drug therapy in treating periodontal diseases.

Recently, exogenous hyaluronic acid, known to have an anti-inflammatory effect, was introduced as topical applicant for the treatment of gingivitis. The topical application of a high-molecular weight, HA-based gel (Gengigel®) has been proposed to have some potential in inducing periodontal healing in patients with inflammatory gingivitis.[7,16] It is also beneficial in accelerating the healing of periodontal wounds following surgery.[17]

HA has been used subgingivally in the treatment of chronic periodontitis.[18] However, it has not been trialed intrasulcularly for the treatment of gingivitis. In the present study, an attempt has been made to evaluate the effectiveness of Gengigel (0.2% hyaluronic acid) in the treatment of plaque-induced gingivitis with or without scaling when applied topically and intrasulcularly. A randomized, single-blind study was designed in which a total of 112 quadrants from 28 patients were treated for 6 (3 + 3) weeks. In each patient, all four quadrants received different treatment modalities, which were assigned randomly. The split-mouth design used in this study has the advantage of allowing paired comparisons to be made. It also has the additional advantage over two groups of

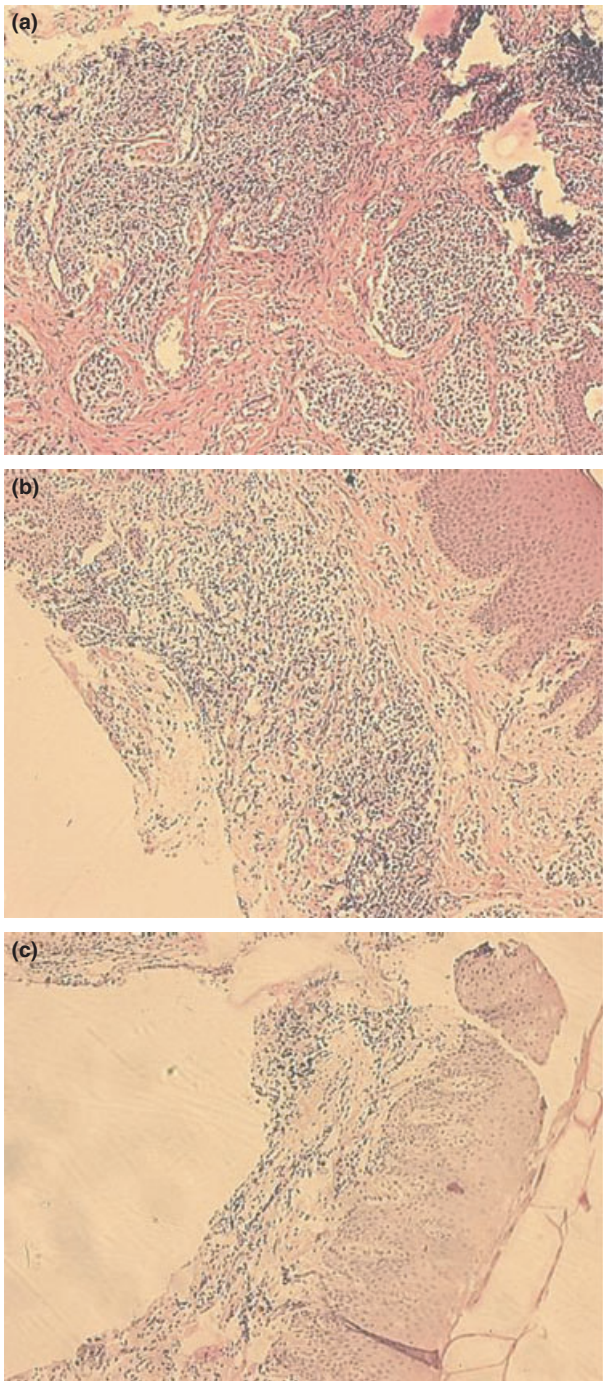

Figure 2. Histological section (hematoxylin–eosin staining) showing (a) intense grade of inflammatory infiltrate, (b) medium grade of inflammatory infiltrates, and (c) slight grade of inflammatory infiltrates.

Table 5. Post-treatment changes in AgNOR counts and comparisons between various groups (mean ± SD)

			Sc + TG		TG		TG + IG		Comparison of reduction between groups	
Groups	Particulars	Baseline	Day 21	Difference from baseline	Day 21	Difference from baseline	Day 21	Difference from baseline	Groups compared	*P*-value†
Cluster (n = 15)	Mean	2.29	1.53	0.76	1.85	0.44	1.79	0.50	Sc + TG *vs* TG	0.03 S*
	SD	0.37	0.22	0.38	0.15	0.36	0.18	0.31		
	Reduction (%)	–	–	33	–	19	–	22	Sc + TG *vs* TG + IG	0.05 S
	t/z‡	–	–	3.41	–	3.13	–	3.41	TG *vs* TG + IG	0.65 NS**
	P-value	–	–	<0.01 (S)	–	<0.01 (S)	–	<0.01 (S)		

AgNOR, argyrophilic nucleolar organizer regions; Ig, intrasulcular gel; Sc, scaling; TG, topical gel.
*$P < 0.05$, significant (S), **$P > 0.05$, not significant (NS).
†Mann–Whitney *U*-test.
‡Wilcoxon signed rank test.

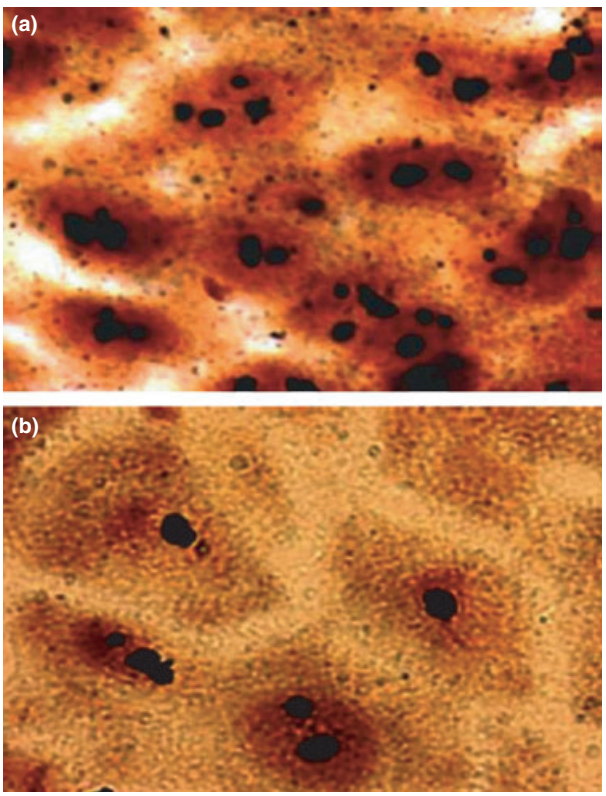

Figure 3. Histological section (argyrophilic nucleolar organizer region [AgNOR] staining) showing increased AgNOR count pretreatment (a) and post-treatment (b).

unmatched patients where subject variation would otherwise play a large role. However, in the split-mouth design, there is concern regarding the independence of samples when both treatments are done in the same patient; there is also concern over spillover or cross-over effects. To avoid this effect, the combination of a split-mouth + cross-over design was used.[19] The need for a positive control in terms of scaling was compared to three different modes of HA gel application to three different quadrants, that is topical gel alone, extrasulcularly; a combination of topical + intrasulcular gel application; and a combination of scaling + topical gel application. Scaling as a treatment modality is compulsory for all the gingivitis patients, thus, this study focused on comparing its effect with gel application using experimental groups. The study results were evaluated from baseline to day 21. There was no significant difference for recorded parameters at baseline for all the quadrants that required different types of treatment. This is suggestive of the equal distribution of clinical parameters, which is an essential component of a split-mouth study design.

The mean and percentage of reduction of the PI, GI, and GBI reached maximum in the combination of scaling + topical gel group, and minimum in the topical gel application groups. The descending order in which the clinical parameter reduction observed was: scaling + topical gel, scaling alone, combination of topical + intrasulcular gel, and topical gel application quadrants. The reduction in the plaque score was not significant when comparing scaling versus scaling + topical HA application, which is similar to the findings of Pagnacco *et al.*[7] In the present study, the mechanical debridement that served as the control group was known to reduce the clinical inflammation markers (the PI, GI, and GBI).[20–23] The enhancement of this effect was observed by the application of topical gel. Both the treatment protocols are regular possibilities in the non-surgical modalities of gingivitis cases. Intergroup comparison of other treatment modalities was also not significant. However, the plaque reduction was significant when various treatment groups were compared to topical HA application. The plaque score reduction can be attributed to adequate oral hygiene maintenance, removal

Table 6. Comparison and correlation between GBI and GI AND AgNOR (reduction %) on days 0–21

	Sc + TG		TG		TG + IG	
Correlation	Decreased (%)	Spearman's ρ*	Decreased (%)	Spearman's ρ	Decreased (%)	Spearman's ρ
GBI and AgNOR (cluster)	63.2 ± 19.8	–	51.1 ± 15.6	–	52.6 ± 21.4	–
	32.0 ± 13.2	0.30	17.3 ± 13.3	0.36	20.7 ± 10.1	0.05
		0.28		0.19		0.87
GI and AgNOR (cluster)	57.3 ± 10.3	–	34.6 ± 13.3	–	41.9 ± 16.0	–
	32.0 ± 13.0	0.27	17.3 ± 13.3	–0.24	20.7 ± 10.1	0.08
		0.33		0.38		0.79

AgNOR, argyrophilic nucleolar organizer regions; GBI, gingival bleeding index; GI, gingival index; Ig, intrasulcular gel; Sc, scaling, TG, topical gel.
*$P > 0.05$, not significant.

of tooth deposits by scaling,[20–23] and the bacteriostatic effects of HA, as stated by Pirnazar *et al.*[24]

The reduction in the gingival score was significant between all the treatment groups, except when comparing the scaling versus topical + intrasulcular HA groups. The gingival bleeding score reduction was significant when the scaling + topical HA application was compared with other groups, except with the scaling group, which was in contrast to the findings of Pagnacco *et al.*,[7] who reported a significant difference in the reduction of the scaling versus the topical HA groups in a double-blind, parallel group study of 29 patients who were examined for a period of 4 weeks. The reduction in the bleeding score was also significant between the scaling and the topical HA group. The results of these findings can be attributed to the anti-edematous and scavenger effect of hyaluronic acid[25] on prostaglandins and metalloproteinases, as stated by Jentsch *et al.*[6]

The anti-inflammatory effect of HA can be attributed to its action of de-activating bacterial hyaluronidases, normalizing the macro-aggregation of connective tissue proteoglycans, and bonding with free water, thus performing an anti-edema effect.[8]

The combination of scaling + topical gel application demonstrated highest gingival inflammation reduction, substantiating the anti-inflammatory effect of HA gel that enhanced the scaling effects. This observation recommends the application of HA gel routinely to gingivitis patients following scaling. The comparison of scaling versus topical gel application revealed a significant reduction in the scaling group, suggesting that mechanical debridement is better than topical gel. This comparison has been tried for the first time in this study. The topical application in combination with the intrasulcular application of gel was better than topical application alone. This placement of HA gel within the sulcus could have served as a better mode of application, wherein the active ingredient HA comes in direct contact with the inflamed tissues so that all the potential actions of HA were possible. Based on the observations of this study, topical gel alone and in combination with intrasulcular application could be utilized as non-surgical modalities for patients where scaling is contraindicated, and to those who are on supportive therapy. The comparison of scaling versus topical gel + intrasulcular gel revealed no significant difference in the reduction of all the clinical parameters. This was the most surprising observation of this study: the anti-inflammatory and anti-edematous ability of the HA gel equated to the clinical response brought about by scaling. According to the authors of the present study, intrasulcular gel application proving its efficiency in gingivitis cases has been trialed for the first time. The intrasulcular application served as the better mode of application for the HA gel so that all the beneficial actions of HA was potentiated by its close contact with the inflamed sulcular tissue.

One of the components in this commercial formulation of HA gel is xylitol, which is used as a non-cariogenic sweetener. Studies have shown that xylitol when used alone or in combination with other antiplaque agents, such as chlorhexidine, causes a significant reduction in the PI,[26–29] GI,[27,29] and GBI.[29]

There was a positive correlation of GBI reduction with that of GI reduction in all the groups, proving that both the GBI and GI can be used to assess the severity of gingival inflammation. Since there was a greater percentage of reduction for the GBI compared to the GI, it can be said that bleeding on probing is more accurate in predicting gingival health compared to the GI,[30] and also GBI obviates the problems associated with subjective interpretations of visual changes of gingiva, such as the presence or degree of erythema and edema.[31]

No adverse effects were observed on clinical examination, and as reported by the patients. These findings were similar to those of Pagnacco *et al.*[7] and Vangelisti *et al.*[16] Patients also demonstrated compliance with the regular use of HA gel, proving the practicality of HA and its excellent acceptability, including acceptability of its sensory characteristics.

In this study, the histological measure of treatment response was evaluated in terms of inflammatory infiltrate grades. Basically, the clinical response to any treatment

could be enhanced by using an assessment of gingival crevicular fluid (GCF) markers and histological parameters. Routine GCF markers have been attempted; however, histology evaluation has been scant. In a study by Mesa *et al.*, the results showed that there was a higher reduction in inflammatory infiltrates in group receiving scaling + topical HA compared to the scaling group, which is similar to the findings of our study. This reduction can be attributed to the removal of tooth deposits by scaling and by blocking of the CD44 receptor by the HA ligand of hyaluronic acid.[15] The scaling + topical HA group also showed a reduction when compared to other groups in this study. Although topical HA gel application showed a significant reduction in all the clinical parameters, histologically it showed least reduction in inflammatory infiltrates. Thus, we suggest using topical gel for a longer duration to reduce the inflammatory infiltrates effectively. There was also a greater reduction in inflammatory infiltrates in the group receiving topical + intrasulcular HA compared to only topical HA. This demonstrates the additional benefit of the intrasulcular application. The order of reduction of clinical parameters coincided with the inflammatory infiltrate reduction, which substantiated all the potential actions of HA gel.

The AgNOR count is a new dimension in periodontal diagnostic criteria, which is widely used in the diagnosis of proliferative lesions. It is more commonly used for diagnostic purposes, as compared to prognostic determination. However, limited literature is available about AgNOR counts of periodontal tissues in healthy and diseased patients. In a study by Saluja and Vandana, a preliminary attempt was made to show the AgNOR count as a good tool to compare treatment protocols and to enhance clinical diagnosis.[11] In our study, the reduction was more significant in the experimental group receiving scaling + topical HA compared to other groups. There was a definite reduction in the AgNOR count after scaling in plaque-induced gingivitis cases.[11] Since the AgNOR count depicts the underlying proliferative activity of the tissues, the reduction in AgNOR count at the end of the treatment period might indicate the underlying repair process of the gingival tissues.[10]

Since the GBI and GI are used as clinical prognostic markers (indices), a first attempt has been made to compare the GBI and GI with the AgNOR count. Considering the results of the study by Saluja and Vandana, who reported that AgNOR has a definite prognostic value with limited diagnostic ability,[11] AgNOR has been trialed instead of the routine use of GCF biomarkers of inflammation. There was a positive correlation in the reduction of the GBI, GI, and AgNOR count, showing that the AgNOR count can also be used as a histopathological indicator, along with various clinical parameters, to assess the severity of gingival inflammation. Thus, the prognostic value of AgNOR appears to be dependable, as indicated by the results of this study.

The present study is the first of its kind to evaluate the efficacy of topical and intrasulcular applications of hyaluronic acid in treating plaque-induced gingivitis. The results of the study strongly recommend the clinical utility of HA gel in periodontal therapy on a regular basis, considering the multipotential actions of hyaluronic acid. Further randomized, controlled trials are required to build evidence and to support the use of hyaluronic acid gel.

The bacteriostatic and anti-inflammatory properties of hyaluronic acid (Gengigel) can be used for the treatment of plaque-induced gingivitis, either alone or with scaling. The combination of intrasulcular and extrasulcular applications of HA gel is beneficial to patients in whom scaling is contraindicated. The AgNOR count as a histopathological indicator is not recommended in routine cases, as it is an invasive procedure. However, it might be suitable for cases that are non-responsive to the conventional procedures.

The possible limitation could be the study design, which appears complicated. However, by careful execution of treatment sequences in uniformly-distributed, diseased quadrants, and by the application of required statistics for the split-mouth + cross-over design, we were able to assess the effectiveness of four treatment protocols from the same individual who served as his own control.

The beneficial actions of HA and its tissue compliance could be tried in periodontitis patients. The formulation of gel into a slow and sustained release delivery system could be ideal in periodontal treatment to combat the exaggerated effects from inflammation and to facilitate faster healing.

In the present study, taking into consideration the parameters describing the clinical features of gingivitis, the results suggest that scaling, in conjunction with topical application of HA, provides a more favorable approach in the treatment of plaque-induced gingivitis. The intrasulcular application of HA is recommended to have additional effects of hyaluronic acid on inflamed tissues. Additionally, the prognostic value of the AgNOR count can serve as an indicator of proliferative activity of periodontal tissues. The combination of a split-mouth and cross-over design is a good study design and is recommended for further clinical trials utilizing therapeutic agents.

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