

Use of hyaluronan (Gengigel) in the treatment of gingivitis in orthodontic patients: A clinical, biochemical, and microbiological study

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ABSTRACT

Introduction: To compare the effects of hyaluronan (Gengigel) alone and in combination with scaling using clinical, microbial, and lactate dehydrogenase (LDH) parameters. **Materials and Methods:** In this, the three treatment groups included were scaling, scaling plus local application of Gengigel, and Gengigel alone. The 0.2% hyaluronic acid (HA) gel was applied topically and intrasulcularly 0.8% hyaluronan was applied. The clinical parameters, and microbial and biochemical analyses of gingival crevicular fluid (GCF) LDH were assessed. Intragroup comparisons were made by Student's unpaired *t*-test and intergroup comparisons were done using one-way analysis of variance followed by *post hoc* Turkey's test. **Results:** At the end of study period (0-56 day), intergroup comparison demonstrated no significant reduction in plaque index (PI) (0.07 NS), gingival index (GI) (0.99 NS), and LDH (0.70 NS) values. A significant correlation was found between LDH values and bleeding index at all study intervals (0 days, 28 days, and 56 days); gingival index (GI) (0.007 S) was significantly correlated on day 0 and day 56. The microbial reduction was demonstrated. **Conclusion:** These changes in the clinical, microbial, and biochemical parameters reported with the different treatment modalities clearly support that the use of Gengigel would act as an advantageous adjunct to scaling. Further studies are required to confirm the Gengigel effect using histologic methods.

Key words: Gel, gingival crevicular fluid (GCF), gingivitis, hyaluronan, orthodontic brackets, L-lactate dehydrogenase (LDH)

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INTRODUCTION

The retentive areas created by dental restorations and orthodontic appliances could increase the amount of plaque around the teeth, i.e., bacterial colonization, particularly periodontopathogens such as *Treponema denticola*, *Porphyromonas gingivalis*, *Tannerella forsythia* (formerly *Bacteroides forsythus*), *Prevotella nigrescens*, *Prevotella intermedia*, and *Aggregatibacter actinomycetemcomitans* in subgingival dental plaque.^[1]

In the gingival crevicular fluid (GCF), Uematsu *et al.* found several cell mediators such as interleukin1 β , interleukin 6, tumor necrosis factor- α , epidermal growth factor, and β_2 microglobulin, significantly elevated in teeth undergoing orthodontic forces, compared with untreated controls.^[2]

Lactate dehydrogenase (LDH), an enzyme normally limited to the cytoplasm of cells, is only released extracellularly after cell death. In the literature there are various studies, which have demonstrated that the activity of LDH in GCF is significantly correlated with gingival inflammation,^[3-5] and tissue destruction from periodontitis.^[6,7] An increase in LDH activity during the early phases of orthodontic treatment is indicated as a possible diagnostic tool for tissue response during orthodontic treatment.^[7-9]

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The topical application of a high-molecular weight exogenous hyaluronan (HA)-based gel (Gengigel; Ricerfarma, Milan, Lombardy, Italy) has been proposed to induce periodontal healing in patients with inflammatory gingivitis, during both open and randomized, controlled, double-blind studies.^[10-12]

A Medline search including the key words “gingivitis,” “orthodontic appliances,” and “hyaluronic acid” revealed scant results. The intrasulcular and extrasulcular applications of HA gel is used effectively in the treatment of gingivitis;^[13] however, its effectiveness on orthodontic patients with gingivitis has not been tried. A recent study by Pistorius *et al.*^[14] using HA spray for the treatment of gingivitis reported significant improvement in gingivitis. Sapna and Vandana^[13] reported that hyaluronan gel is an effective topical agent for treating gingivitis, along with scaling and intrasulcular application.

Thus, the purpose of the present study was to compare the benefits of hyaluronan (Gengigel) alone and in combination with scaling using clinical, microbial (periodontopathogens), and biochemical (LDH) parameters.

MATERIALS AND METHODS

This randomized, controlled, parallel design study was conducted on patients attending the Department of Periodontics. Thirty-two patients in the age group of 18-25 years undergoing orthodontic treatment with plaque-induced gingivitis with a probing depth <3 mm were selected. Written informed consent was obtained from all patients prior to their participation in this study and was approved by ethical committee.

Subjects were enrolled if they had a medical history showing good general health, at least 24 natural teeth in the mouth excluding the third molars, and also full-mouth fixed orthodontic needed to have at least 50% bleeding sites and 50% dichotomous plaque score. Oral and written information was given to each enrolled subject. Both the parent and subject signed the consent form. The exclusion criteria included

1. Medical history of the liver, heart, kidney, and muscle diseases that are known to affect LDH levels,
2. Patients who had taken antibiotic therapy in the month prior to the commencement of the study, and
3. Periodontal therapy in the last 6 months prior to the commencement of the study
4. smokers,
5. Pregnant and lactating women
6. Advanced periodontitis or rampant dental caries based on a noninvasive examination, and
7. Removable oral prostheses or removable orthodontic appliances.

In this randomized, single-blinded study, 32 patients were included. The different treatment modalities were control group, scaling alone; experimental group A, only topical application of HA gel (Gengigel; Ricerfarma, Milan, Lombardy, Italy); and experimental group B, both scaling and topical applications of HA gel. The allotment of different treatment protocols to different groups 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 the treatment, the indices assessed included clinical parameters: Plaque index (PI),^[15] gingival index (GI),^[16] and gingival bleeding index (GBI);^[17] microbial assessment^[18] and biochemical analysis of GCF LDH.^[9] These were recorded by the main investigator and were repeated on days 0 and day 28. Any adverse reactions to the gel were also recorded.

The duration of the study was 56 days. From day 0 to day 28 day, scaling + gel application was performed in one of the selected groups and in the other group, the application of gel was done. The control group received only scaling. The 0.2% HA gel was applied topically (extrasulcularly) on the gingival surface, intrasulcularly 0.8% hyaluronan was applied by the coinvestigator [Figures 1 and 2]. 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 cotton bud applicator twice daily for 28 days after regular oral hygiene regimen. After application, the patients were instructed to avoid eating, drinking, or rinsing for 1 h. Intrasulcular applications were done with an applicator by the co-investigator during their weekly visit. 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. An observation period up to 56 days was considered for all three treatment groups.



Figure 1: gengigel

Supragingival microbial sample collection was collected at the baseline and 28th day. After recording the clinical parameters, sterile universal curette (Columbia 2R/2L; 4R/4L, Hu-Friedy, Chicago, USA) was used to collect plaque sample and then transferred to 1 mL thioglycolate broth (transport medium).^[19] The vial was sealed tightly to avoid contamination and labeled. The labeled vials were sent to the microbiological laboratory within 24 h of collection. Samples were processed within 2 days of collection. The collected samples were subjected to anaerobic culture method for the detection of periodontopathogens such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, and *Actinomyces* species. The plates were inoculated for 72 h and after this specified period, the plates were taken out of the jar; the colony characters such as size, shape, hemolysis and pigmentation were noted and the numbers of each colony type were counted. The count was multiplied by 200 (dilution factor) to express colony forming units (CFUs).^[7] The CFUs were expressed in the tabular form with grading where the individual pathogens are marked as follows: (–) undetected, which corresponds to the number of bacteria less than 10^3 , (+) slightly positive corresponding to the number of bacteria 10^3 to 10^4 ; (++) positive, which corresponds to the number of bacteria 10^4 to 10^5 ; and (+++) strongly positive, with the number of bacteria higher than 10^5 . The organisms were further quantified by making use of Gram stain and key biochemical reactions as per the standard protocol and expressed in the form of percentages.^[18]

GCF collection and LDH enzyme activity determination were carried out at the baseline and at the 28th day, along with the clinical parameters. The GCF volume in each paper point was calculated and analyzed for LDH enzyme activity.^[7,9]

All the vials containing GCF samples were put in the spectrophotometric automatic apparatus (COBAS INTEGRA 400 plus; Roche Diagnostics, Mannheim, Baden-Württemberg, Germany) to be automatically analyzed without any manual procedures.^[7,9] LDH total unit activity = GCF volume X volume activity X $1/10^6 \mu\text{l}$ was calculated.

RESULTS

Of the 32 patients, seven 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 25 patients; the results were computed including these data. Comparison of PI, GI, gingival bleeding index, microbiological and biochemical evaluations was done from the baseline to the 28th day [Table 2]. The clinical parameters and LDH values were reevaluated after an observational period at on the 56th day. The results are presented in Tables 1-6.

At the end of study period (0-56 days) [Table 3], intergroup comparison demonstrated no significant reduction in PI (0.07 NS), GI (0.99 NS), and LDH (0.70 NS) values. The GBI demonstrated significant reduction; the highest reduction



Figure 2: application of gengigel

Table 1: Inter group comparison: Baselines values

Parameter		Gel alone	Gel + Scaling	Scaling	P* Value*, sig
Plaque Index	Mean	1.791	1.807	1.725	0.71 NS
	SD	0.159	0.242	0.284	
Gingival Index	Mean	1.725	1.672	1.748	0.76 NS
	SD	0.240	0.279	0.186	
Gingival Bleeding Index	Mean	89.687	84.075	89.262	0.56 NS
	SD	12.153	12.575	14.126	
LDH Values	Mean	72.400	59.800	60.300	0.43 NS
	SD	24.740	28.732	17.733	

*Oneway ANOVA test; **Tukey's post hoc test; $p < 0.05$ = significant; NS = Non Significance; S = Significance.

Table 2: Inter group comparison: Clinical parameters and LDH values at 0-28 days

Parameter		Gel alone	Gel + Scaling	Scaling	P* Value*, sig	Significant pairs**
Plaque Index	Mean	0.166	0.243	0.183	0.07 NS	–
	SD	0.206	0.141	0.144		
Gingival Index	Mean	0.271	0.181	0.273	0.46 NS	–
	SD	0.206	0.043	0.152		
Gingival Bleeding Index	Mean	34.381	67.044	59.978	0.004 S	Gel alone & Gel + Scaling; Gel alone & Scaling
	SD	19.250	8.111	17.067		
LDH Values	Mean	31.800	27.200	34.600	0.16 NS	–
	SD	10.581	13.903	11.098		

*Kruskal Wallis test; ** Mann Whitney U test; $p < 0.05$ = significant; NS = Non Significance; S = Significance.

Table 3: Inter group comparison: Clinical parameters and LDH values at 0-56 days

Parameter		Gel alone	Gel + Scaling	Scaling	P* Value*, sig	Significant pairs**
Plaque Index	Mean	0.264	0.546	0.147	0.07 NS	–
	SD	0.271	0.460	0.305		
Gingival Index	Mean	0.173	0.204	0.151	0.99 NS	–
	SD	0.267	0.166	0.298		
Gingival Bleeding Index	Mean	40.089	68.819	61.109	0.007 S	Gel alone & Gel + Scaling; Gel alone & Scaling
	SD	19.627	9.831	16.752		
LDH Values	Mean	39.400	34.100	29.900	0.70 NS	–
	SD	20.359	19.661	12.922		

*Kruskal Wallis test; ** Mann Whitney U test; $p < 0.05$ = significance; NS = Non Significance; S = Significant**Table 4: Inter group comparison: Clinical parameters and LDH values at 28-56 days**

28-56 Days					
Parameter		Gel alone	Gel + Scaling	Scaling	P* Value*, sig
Plaque Index	Mean	0.098	0.303	-0.036	0.24 NS
	SD	0.258	0.451	0.350	
Gingival Index	Mean	-0.098	0.023	-0.122	0.53 NS
	SD	0.310	0.161	0.307	
Gingival Bleeding Index	Mean	5.708	1.775	1.131	0.12 NS
	SD	6.595	4.321	2.909	
LDH Values	Mean	7.600	6.900	-4.700	0.06 NS
	SD	15.981	12.627	8.056	

*Kruskal Wallis test; ** Mann Whitney U test; $p < 0.05$ = significant; NS = Non Significance; S = Significance**Table 5: Correlation of various parameters with LDH values**

Baseline values	Correlation coefficient*	P Value
Gingival Index	0.54	0.002 S
Gingival Bleeding Index	0.38	0.04 S
28 Days		
Gingival Index	0.33	0.07 NS
Gingival Bleeding Index	0.37	0.04 S
56 Days		
Gingival Index	0.57	0.001 S
Gingival Bleeding Index	0.48	0.04 S

* Karl Pearson's coefficient of correlation; $p < 0.05$ = significant; NS = Non Significance; S = Significance**Table 6: Quantitative evaluation of the micro-organisms at different time interval**

Microorganisms (CFUs)	Gel alone		SRP + gel		SRP alone	
	0 day	28 th day	0 day	28 th day	0 day	28 th day
A a	++	+	++	+	++	–
P g	++	–	++	–	++	+
P i	++	–	++	+	++	+
Actinomycetes	+	+	+	+	+	–
<i>Streptococcus aureus</i>	++	+	+	–	+	+
<i>Staphylococcus mitis</i>	++	–	+++	+	–	–

(-) undetected $< 10^3$; (++) positive - 10^4 to 10^5 ; (+) slightly positive 10^3 to 10^4 ; (+++) strongly positive $> 10^5$.

was in the gel + scaling group followed by the scaling alone and gel alone group. The intergroup comparison (28th-56th day) [Table 4] did not reveal any significant reduction in the clinical parameters and LDH. Regarding the correlation of various clinical parameters with LDH values, a significant correlation was found between LDH values and bleeding

index at all study intervals (0 day, 28 days, 56 days) [Table 5]; the GI (0.007 S) was also significantly correlated on day 0 and day 56 [Table 3].

On quantitative evaluation of microorganisms, the growth of organisms was positive (++) to highly positive (+++) in all groups at the baseline demonstrated reduction of microbes while there was a reduction of these organisms from slightly positive (+) to undetected (–) [Table 6].

DISCUSSION

During orthodontic therapy with fixed appliances, inflammatory reaction of gingival tissue can very often be observed. A variety of studies have reported that temporary changes occurring in the gingiva for an increased accumulation of dental plaque and inflammatory response is due to appearance of new retentive places around the components of fixed appliances attached to the teeth and did not normally result in permanent periodontal losses.^[19-21] It has also been stated that periodontal problems during orthodontic treatment may be primarily attributable to poor oral hygiene. Hence, if good oral hygiene is maintained, orthodontic treatment results in no harmful effects with regard to periodontal health.^[22]

Recently, exogenous hyaluronic acid, known to have an anti-inflammatory effect, was introduced as a 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.^[11,23] It is also beneficial in accelerating the healing of periodontal wounds following surgery.^[24] HA has been used subgingivally in the treatment of chronic periodontitis.^[13] However, it has not been trialed intrasulcularly for the treatment of gingivitis in orthodontic patients. 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 parallel study design in which a total of 25 patients were treated for 28 days and evaluated after an observational period of 56 days.

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.

Scaling as a treatment modality is compulsory for all gingivitis patients and thus, this study focused on comparing its effect with gel application with or without scaling. The study results were evaluated from the baseline to day 28 and day 56.

At the baseline, there was no significant difference in the mean plaque, gingival scores, and gingival bleeding scores between the groups and there was also a constant reduction in all scores from the baselines to the 56th day in each group. The PI reduction was similar in both scaling and scaling + Gengigel group that was similar to the findings of Pagnacco *et al.*^[11] and Sapna and Vandana.^[13] Intergroup comparison showed similar plaque reduction in all treatment modalities. The plaque score reduction can be attributed to adequate oral hygiene maintenance, removal of tooth deposits by scaling, and bacteriostatic effects of hyaluronan as stated by Pirnazar *et al.*^[25] The gingival bleeding score reduction was significant when the scaling + topical HA application was compared with other groups. Pagnacco *et al.*^[11] 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 while Sapna and Vandana^[13] reported a significant reduction in gingival bleeding score in gel + scaling group. The results of these findings can be attributed to the anti-edematous and scavenger effect of hyaluronic acid as stated by Laurent *et al.*^[26]

The anti-inflammatory effect of HA can be attributed to its action of deactivating bacterial hyaluronidases, normalizing the macroaggregation of connective tissue proteoglycans, and bonding with free water, thus performing an anti-edema effect.^[10] It can be said that bleeding on probing is more accurate in predicting gingival health compared to the GI,^[27] 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.^[28]

A study by Yi Xu *et al.* (2004)^[29] concluded that no clinical improvement was achieved by the adjunctive use of hyaluronan 0.2% gel compared to mechanical debridement. However, in their study hyaluronan 0.2% gel was applied only once a week for 6 weeks; a total of seven applications was administered over a 6-week period, compared to the recommended application level of three times daily for at least 4-8 weeks. The absence of observed clinical

improvements contrary to other published studies may indicate that the hyaluronan application used in this study was well below the optimum levels required to achieve a significant clinical improvement.

The baseline GCF LDH values continued to reduce throughout the study period in all the treatment groups, which was not significant between the groups. The Pearson's coefficient of correlation demonstrated that correlations of LDH levels with GI and GBI were significant at the end of study period.

Along with measuring changes in clinical parameters for reduction in gingival inflammation, this study included the measurement of changes in total enzyme unit activity of LDH, along with changes in GCF volume. The GCF collection for LDH enzyme activity estimation was carried out using #30 standardized sterile paper point (SS White, New Jersey, USA) inserted 1 mm into the gingival crevice for 30 s, similar to the methodology of Serra *et al.*^[7] However, the LDH enzyme estimation in the present study was performed by a fully automated analyzer (Roche Cobas U 411 Automated), which can be considered as a better method of analyzing LDH enzyme activity than using a manual spectrophotometer as used in previous studies.^[7,8]

The presence of higher LDH enzyme unit activity at the baseline is in accordance with the results of Serra *et al.* (i.e., sites with full-mouth fixed orthodontic appliances in place for a minimum period of 3 months showed a mean total LDH unit activity of 72.40 ± 24.74 IU per sample) who attribute this increased GCF LDH levels to the tissue resorption in both the compressed and tensional sites or even secondary to a possible cell necrosis in the periodontal ligament during the orthodontic treatment.^[8]

These GCF LDH value reduction from the baseline to 28th day are comparable to the mean GCF LDH values for diseased sites (0.129 ± 0.406 IU per site) and healthy sites (0.029 ± 0.035 IU per site), respectively, as observed in a cross-sectional study by Wolff *et al.*,^[5] thus indicating the return of diseased sites to healthy sites following gel application. This posttreatment significant reduction in GCF LDH values can also be correlated to studies showing a significant reduction in GCF LDH activity levels after successful periodontal treatment.^[5,6]

The increased severity of gingival inflammation observed immediately after fixed appliance placement due to the development of a stable pathogenic milieu tips the host-parasite homeostasis in favor of the pathogen and manifests as clinical inflammation.^[30] It also stimulates the growth of periodontopathogenic bacteria but without destructive effects on deep periodontal tissues.^[2,31]

There are various *in vitro* studies^[25] suggesting bacteriostatic or bactericidal effects of various formulations of HA on selected oral and nonoral microorganisms. In the present study, the samples were also quantitatively analyzed the bacteria from undetectable to strongly positive according to grading done by Černochová *et al.* 2008.^[18] There was a quantitative reduction of Ag, Pg, Pi, *Actinomyces*, *Staphylococcus aureus*, *Streptococcus mitis* at the end of 28 days suggestive of the beneficial antibacterial effect of the gel.

Recently numerous studies have been conducted to evaluate the effect of Gengigel in chronic periodontitis patients, which showed a positive response associated with improvement in clinical parameters and reduction in microbial parameters by various authors such as Johannsen *et al.* (2009),^[32] Gonitiya *et al.* (2012),^[33] and Eick *et al.* (2012).^[34]

No adverse effect was observed on clinical examination and according to what was reported by the patients. These findings were similar to the findings of Pagnacco *et al.* (1997)^[11] and Vangelisti *et al.* (1997).^[23] The patients also presented compliance with the regular use of Gengigel. Gengigel as a product for oral use has been evaluated by skin irritation test, sensitizing potentiality, and percutaneous absorption test and has been proved to be a safe nonirritant product.

The important observation made in this study was the effect of Gengigel on reduction of gingival bleeding in patients with orthodontic brackets, which serve as a plaque retentive area. The intrasulcular application of 0.8% Gengigel enhanced the tissue ability to respond to mechanical plaque control procedures. Gengigel application for 28 days was effective in the reduction of clinical parameters and its application is recommended to be continued during orthodontic treatment as a regular oral hygiene maintenance [Table 2].

These changes in clinical, microbial, and biochemical parameters reported with the different treatment modalities clearly support that thorough scaling is effective as initial therapy and the use of hyaluronan gel would act as an advantageous adjunct. The favorable results obtained in this study confirmed that the anti-inflammatory and antimicrobial properties of hyaluronan serve as a promising adjunct to the mechanical therapy of gingivitis with fixed retentive appliances as the retentive factors.

More organized and long-term research is to be carried out to differentiate the specific effect of 0.2% or 0.8% gel, which can support the use of the gel in routine clinical practice. Further studies are required to confirm the Gengigel effect using histologic methods.

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Conflicts of interest

There are no conflicts of interest.

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