Neutrophil elastase levels in the gingival crevicular fluid following hyaluronan gel application in the treatment of chronic periodontitis: A randomized split-mouth study

Savita Mallikarjun, Abhilash Neelakanti, Harsha Mysore Babu, Sujatha Ballambettu Pai, Sachin Vaijnathrao Shinde, Swathi Krishnan

ABSTRACT

Background: Neutrophils are the predominant leukocytes in the periodontium, which prevent infection from periodontal pathogens and subsequent tissue destruction. A potentially destructive role has been elucidated, especially due to elastase enzyme. Controlling its levels might be crucial in minimizing the tissue destruction. Hyaluronan, known to inhibit the release of this enzyme from neutrophils, might be a viable option to treat chronic periodontitis.

Aims: The aim of this study is to evaluate and compare the effects of 0.2% hyaluronan gel adjunctive to scaling and root planing on the levels of elastase in gingival crevicular fluid (GCF).

Settings and Design: This split-mouth study included eighty (forty experimental and forty control) sites from twenty patients representing both sexes.

Materials and Methods: GCF samples were collected from all the eighty sites; simultaneously, clinical periodontal parameters were recorded. Enzyme-linked immunosorbent assay was used to determine the levels of elastase at baseline and 6 weeks after therapy, following mechanical debridement and subsequent subgingival placement of the experimental drug.

Statistical Analysis Used: With the aid of statistical software (SPSS Version 13), Student’s t-test and Pearson’s correlation test were performed.

Results: There was a mean reduction in the elastase levels from baseline to 6 weeks after therapy in the experimental group. However, the difference between the groups was not statistically significant.

Conclusions: Adjunctive use of hyaluronan following mechanical debridement resulted in comparable reduction in the elastase levels, suggesting that this substance has an inhibitory effect on elastase, and subsequent tissue destruction. Further long-term studies are mandatory to validate the results of this study.

Key words: Gingival crevicular fluid, hyaluronan gel, neutrophil elastase, root planing, scaling

Periodontitis ultimately results in tissue destruction, due to inflammatory host response against dental plaque-associated pathogens and their products. Mechanical debridement forms the basis of periodontal therapy but is not effective for all sites and forms of periodontal disease. Therefore, local delivery of chemical substances adjunctive to mechanical debridement is practiced, providing additional benefits. Although several agents have been tried earlier, their undesirable side effects necessitated a safer, endogenous substitute.

Hyaluronic acid (HA), a chief component of the mammalian extracellular matrix (ECM), with antibacterial and...
anti-inflammatory properties,\(^4\) can be considered as a viable option. It is a key component in wound healing process;\(^5\) topical application of exogenous HA to inflamed periodontal sites is known to offer beneficial effects in patients with gingivitis.\(^6\) Another important function of HA is the inhibition of release of neutrophil elastase (NE).\(^7\) Since elastase is one of the important enzymes responsible for periodontal destruction,\(^8\) the ability of HA to inhibit its release might help in achieving promising results in periodontal therapy.

However, very scarce literature is published so far, regarding the effects of HA-containing gel on the levels of NE in gingival crevicular fluid (GCF). Hence, an attempt is made in this study to evaluate the effect of 0.2% HA gel adjunctive to scaling and root planing (SRP) on clinical parameters and the levels of NE in the GCF of patients with chronic periodontitis.

**MATERIALS AND METHODS**

**Experimental design, patient selection, randomization**
The GCF samples analyzed in this study were collected in accordance with a previously reported prospective, randomized clinical trial, which determined the effect of application of HA-containing gel on SRP.\(^9\) Our study design, which followed the guidelines of the Declaration of Helsinki (2002), was approved by the Institutional Ethics Committee, and was conducted in the Department of Periodontics, Dayananda Sagar College of Dental Sciences, Bengaluru, India. Eighty sites from twenty patients (11 males and nine females) in the age group of 20–60 years were included in this study after fulfilling the selection criteria. Prior verbal and written informed consent was obtained from them.

**Inclusion criteria**
Each participant had a minimum of twenty teeth (five per quadrant) and a diagnosis of moderate to advanced chronic periodontitis.\(^10\) The participants had a probing pocket depth (PPD) of at least 5 mm and clinical attachment loss (CAL) of minimum 3 mm in at least two nonadjacent sites in the maxillary arch. All participants were in good general health.

**Exclusion criteria**
Patients with systemic diseases and immunocompromised conditions that could influence the outcome of therapy, those under medication such as anticoagulants, antibiotics, steroids, or anti-inflammatory drugs in the past 3 months, those who received periodontal therapy in the past 6 months, smokers, and pregnant and/or lactating women were all excluded from the study.

**Oral hygiene regime**
All patients were enrolled in an individualized oral hygiene regime at the beginning of the study. They had been advised to use a soft manual toothbrush. The use of any kind of mouthwash was discouraged as it would interfere with the results of the study. Oral hygiene instructions were provided at each examination and treatment visit.

**Periodontal treatment protocols**
Using a split-mouth design, the sites with deepest periodontal pocket in either of the maxillary quadrants of each patient were allocated to experimental or control groups by simple randomization. Only posterior teeth were selected, so as to prevent cross-contamination of other sites with the experimental drug, whereas only maxillary teeth were selected because of the technical difficulties associated with collection of GCF samples, as well as contamination with saliva, owing to saliva pooling in the mandibular arch. Both experimental and control sites were treated with SRP. The experimental sites additionally received local drug delivery (LDD) in the form of subgingival application of 0.2% hyaluronan gel [Figure 1].

The following periodontal clinical parameters were recorded at baseline and after 6 weeks: Plaque index (PI),\(^11\) gingival index (GI),\(^12\) PPD; measured using The University of North Carolina-15 periodontal probe) and relative attachment level (RAL). At baseline, clinical parameters were assessed [Figure 2] and recorded in a special pro forma sheet.

**Gingival crevicular fluid sample collection**
Each of the two sites was sampled at two-time points, before treatment (baseline) and 6 weeks after therapy. GCF samples were collected on the following day of clinical examination to avoid contamination of the samples with bleeding induced by probing.

On the following day, after removing the supragingival plaque with cotton and properly isolating the area, sterile capillary tubes were placed near the entrance of the pocket at the predetermined sites, until three microliters of GCF was procured [Figure 3]. The GCF samples so collected were...
immediately transferred to plastic vials containing 200 µL of phosphate-buffered saline solution and refrigerated at −70°C until further analysis.

After the assessment of clinical parameters and collection of GCF samples at baseline at the selected sites, full mouth scaling was performed using piezoelectric ultrasonic scalers. Further, root planing was performed at the experimental and control sites using area-specific Gracey curettes.

Local drug delivery (0.2% hyaluronan)
The experimental sites were isolated with cotton rolls to prevent contamination with saliva. The test product (GENGIGEL) was carried in a syringe with an attached blunt cannula, to the experimental site. About 0.3–0.5 ml of the gel was deposited into the periodontal pocket, ensuring that the entire pocket is loaded with the drug [Figure 4]. The pocket entrance was finally covered with a periodontal dressing to retain the material in the pocket, as well as to prevent the ingress of oral fluids. The cases were advised to maintain the experimental site clean, by wiping the periodontal dressing gently with a moist gauze pad. The disintegration time for gengigel has been given in different periods in different studies. Hence, to standardize the duration, we have opted for 1 week for protection of the experimental site by a periodontal dressing. The cases were recalled after a week for the removal of the dressing.

Next recall visit was scheduled 6 weeks after therapy to reassess the clinical parameters and recollect the GCF samples (following the similar procedure at baseline) for biochemical analysis.

Biochemical analysis of gingival crevicular fluid samples
The test kit employed for this study includes a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) test kit (Human NE ELISA, KINESIS Dx, Los Angeles, CA, USA) [Figure 5] to determine the levels of NE present in the GCF samples.

Enzyme-linked immunosorbent assay procedure
All samples and reagents were brought to room temperature prior to use. Five standards were prepared according to the manufacturer instructions. Fifty microliters of standards and 40 µl of GCF samples were pipetted out into the
Hyaluronan gel and neutrophil elastase
Mallikarjun, et al.

respectively. Care was taken to avoid the addition of the samples, biotin conjugate, or streptavidin–horseradish peroxidase (HRP) conjugate to the blank wells. Ten microliters of biotin conjugate was pipetted out into each sample well. The blank and standard wells were avoided. Fifty microliters of streptavidin–HRP conjugate was pipetted out into each sample and standard well. Blank wells were avoided. The plate was covered and incubated at 37°C for 1 h in an incubator. At the end of an hour, the plate was removed from the incubator and washed four times with the wash buffer. Any residual buffer was blotted by firmly tapping the plate upside down on an absorbent paper. Then, 50 μL each of the substrates A and B were added to all the wells on the plate, including the blank wells. The plate was covered, gently shaken to mix the constituents, and reincubated at 37°C for 10 min in dark (wrapped in a dark paper). After completion of the incubation, 50 μL of stop solution was added to all the wells, to stop any further reaction between the constituents. The absorbance of the plate was read at 450 nm within 15 min after adding the stop solution.

Statistics
The findings, thus, obtained were averaged (mean ± standard deviation) for each continuous parameter, and categorical values were presented as numbers and percentages in tables and graphs. Data so collected were subjected to statistical analysis with the help of Statistical Package for Social Sciences version 13.0 software (SPSS Inc., Chicago, USA). Intragroup comparison of the clinical and biochemical parameters, i.e., PI, GI, PPD, CAL, and NE levels at baseline and after 6 weeks, was carried out using paired t-test. Intergroup comparison of the clinical and biochemical parameters between the experimental and control groups at baseline and after 6 weeks was performed using unpaired t-test. Correlation between different parameters in the experimental and control groups at baseline and after 6 weeks was determined using Pearson’s correlation test. In all the above tests, the level of significance was set at α = 0.05, i.e., P < 0.05 was considered statistically significant.

RESULTS
PI scores are shown in Table 1 and Graph 1. The difference in PI scores between control and experimental sites was not statistically significant, either at baseline or after 6 weeks (P > 0.05). However, the difference within both control and experimental groups was statistically highly significant (P < 0.001).

GI scores are shown in Table 2 and Graph 2. The difference in GI scores between control and experimental sites was not statistically significant, either at baseline or after 6 weeks (P > 0.05). However, the difference within both control and experimental groups was statistically highly significant (P < 0.001).

PPD measurements are given in Table 3 (intergroup comparison), Table 4 (test group), Table 5 (control group), and Graph 3. The difference in PPD measurements between control and experimental sites was not statistically significant, either at baseline or after 6 weeks (P > 0.05). However, the difference within both control and experimental groups was statistically highly significant (P < 0.001).

CAL measurements are given in Tables 4-6 and Graph 4. The difference in CAL measurements between control and experimental sites was not statistically significant, either at baseline or after 6 weeks (P > 0.05). However, the difference within both control and experimental groups was statistically highly significant (P < 0.001).

Graph 1: Mean plaque index recorded in the groups at two different time intervals

| Time slot | Mean | Standard Deviation |
|-----------|------|--------------------|
| Baseline  | 1.97 | 0.47               |
| Test      | 1.93 | 0.47               |
| Control   | 0.88 | 0.45               |
| 6 weeks   | 0.93 | 0.44               |

Table 1: Comparison of plaque index between baseline and 6 weeks (paired t-test)

| Group | PI | Mean | SD¹ | SEM² | MD³ | t    | P     |
|-------|----|------|-----|------|-----|------|-------|
| Test  | Baseline | 1.97 | 0.47 | 0.10 | 1.094 | 7.467 | <0.001* |
| 6 weeks | 0.88 | 0.45 | 0.10 |
| Control | Baseline | 1.93 | 0.47 | 0.11 | 0.994 | 7.314 | <0.001* |
| 6 weeks | 0.93 | 0.44 | 0.10 |

*Significant difference, ¹Standard deviation, ²Standard error of mean, ³Mean difference. PI=Plaque index

Table 2: Comparison of gingival index between baseline and 6 weeks (paired t-test)

| Group | GI | Mean | SD¹ | SEM² | MD³ | t    | P     |
|-------|----|------|-----|------|-----|------|-------|
| Test  | Baseline | 1.86 | 0.60 | 0.13 | 1.206 | 7.107 | <0.001* |
| 6 weeks | 0.66 | 0.47 | 0.11 |
| Control | Baseline | 1.83 | 0.52 | 0.12 | 1.131 | 6.446 | <0.001* |
| 6 weeks | 0.69 | 0.56 | 0.13 |

*Significant difference, ¹Standard deviation, ²Standard error of mean, ³Mean difference. GI=Gingival index

Table 3: Comparison of probing pocket depth between the groups (in mm)

| Time interval | Group | Mean | SD¹ | SEM² | MD³ | t    | P     |
|---------------|-------|------|-----|------|-----|------|-------|
| Baseline      | Test  | 6.00 | 1.03 | 0.23 | 0.100 | 0.302 | 0.765 |
| Control       | 5.90 | 1.07 | 0.24 |
| 6 weeks       | Test  | 3.10 | 1.07 | 0.24 | -0.150 | -0.505 | 0.617 |
| Control       | 3.25 | 0.79 | 0.18 |

¹Standard deviation, ²Standard error of mean, ³Mean difference

Indian Journal of Dental Research, 27(4), 2016
and experimental sites was not statistically significant, either at baseline or after 6 weeks (P > 0.05). However, the difference within both control and experimental groups was statistically highly significant (P < 0.001).

NE levels in the GCF are given in Tables 4, 5, and 7 and Graph 5. The difference in NE levels between control and experimental sites was not statistically significant, either at baseline or after 6 weeks (P > 0.05). However, the difference within both control and experimental groups was statistically highly significant (P < 0.001).

The correlation between different parameters in the test group after 6 weeks is shown in Table 8. Statistically significant correlations were found between PI and pocket depth (PD) (positive and moderate; P < 0.05), PI and CAL (positive and strong; P < 0.01), GI and PD (positive and strong; P < 0.01), and PD and CAL (positive and very strong; P < 0.001). However, the correlation between NE and all other parameters was negligible (r = −0.19–+0.19).

The correlation between different parameters in the control group after 6 weeks is shown in Table 9. Statistically significant correlations were found between PI and GI (positive and moderate; P < 0.05), PI and CAL (positive and moderate; P < 0.05), and PD and CAL (positive and moderate; P < 0.05). However, the correlation between NE and all other parameters was negligible (r = −0.19–+0.19).

DISCUSSION

Mechanical debridement brings about positive changes in the subgingival microenvironment. However, studies have shown that the subgingival sites get recolonized with potential periodontal pathogens within days to weeks after the treatment. This would imply the necessity to frequently recall the patients who may be periodically at risk and/or unable to maintain a proper standard of oral hygiene.13
In this context, adjunctive application of certain substances that possess anti-inflammatory effect may be the treatment of choice. To serve this purpose, several pharmacological agents have been employed, but focus shifted on to natural substances, more specifically endogenous substances. One such recently emerging substance is hyaluronan, chief component of the mammalian ECM. Its efficacy has already been proved in various medical applications.\cite{2,3}

The usefulness of HA in adjunctive periodontal therapy has been previously reported, but focus was on inflammatory component of the disease.

Moreover, there are hardly many published studies that have reported the effect of HA on NE, which is one of the enzymes responsible for periodontal tissue destruction. Hence, the aim of this study was to evaluate the efficacy of 0.2\% HA gel as an adjunct to SRP and its effect on the levels of NE in the GCF of patients with chronic periodontitis.

In the current study, the clinical parameters such as PI, GI, PPD, and RAL were evaluated at each follow-up visit in both experimental and control sites. Further, biochemical analysis for determination of the levels of NE was done through ELISA.

The findings of our study revealed a statistically significant reduction in mean PI scores in the experimental as well as the control sites from baseline to 6 weeks. However, when comparing between the two groups, no statistically significant difference was observed at baseline or 6 weeks. Similar findings were reported by Johannsen et al.,\cite{14} who used HA gel as an adjunct to SRP, and observed a statistically significant reduction in mean PI scores in the experimental as well as the control sites from baseline to 12 weeks; however, no statistically significant difference in mean PI scores was observed between the groups over a period of 12 weeks. Our findings are also supported by a study by Eick et al.,\cite{9} in which the effect of HA gel adjunctive to SRP was determined on the clinical variables, subgingival periodontopathic bacteria, and local immune responses. Even in this study, the reduction in PI values from baseline to 6 weeks in both groups was statistically significant, but not between the groups at any recall interval.

The findings of all these studies show that the adjunctive use of HA does not provide any additional benefit over SRP in

### Table 7: Comparison of neutrophil elastase levels between the groups (ng/ml)

| Time interval | Group | Mean  | SD  | SEM  | MD  | t  | P   |
|---------------|-------|-------|-----|------|-----|----|-----|
| Baseline      | Test  | 61.56 | 17.74 | 3.97 | 0.292 | 0.054 | 0.957 |
|               | Control | 61.27 | 16.22 | 3.63 | 0.384 | 0.703 |       |
| 6 weeks       | Test  | 12.33 | 8.72 | 1.95 | 0.986 | 0.384 | 0.703 |
|               | Control | 11.34 | 7.46 | 1.67 | 0.940 | 0.940 |       |

*Standard deviation, †Standard error of mean, §Mean difference

### Table 8: Correlation between different parameters in test group after 6 weeks

| Correlations | PI     | GI     | PD     | CAL    | NE     |
|--------------|--------|--------|--------|--------|--------|
| PI           | R      | -      | 0.381  | 0.505  | 0.572  | 0.193  |
|              | P      | -      | 0.097  | 0.023  | 0.008  | 0.415  |
| GI           | R      | 0.381  | -      | 0.641  | 0.397  | 0.109  |
|              | P      | 0.097  | -      | 0.002  | 0.083  | 0.648  |
| PD           | R      | 0.505  | 0.641  | -      | 0.828  | 0.018  |
|              | P      | 0.023  | 0.002  | -      | <0.001 | 0.940  |
| CAL          | R      | 0.572  | 0.397  | 0.828  | -      | -0.103 |
|              | P      | 0.008  | 0.083  | <0.001 | -      | 0.666  |
| NE           | R      | 0.193  | 0.109  | 0.018  | -0.103 | -      |
|              | P      | 0.415  | 0.648  | 0.940  | 0.666  | -      |

*Significant correlation, NE=Neutrophil elastase, PI=Plaque index, GI=Gingival index, PD=Probing depth, CAL=Clinical attachment loss

Graph 4: Mean clinical attachment level recorded in the groups at two different time intervals

| Time slot | Mean | Standard Deviation |
|-----------|------|--------------------|
| Baseline  | Test | 5.05               | 1.15 |
|           | Control | 4.85               | 1.09 |
| 6 weeks   | Test | 2.80               | 1.15 |
|           | Control | 3.05               | 1.00 |

Graph 5: Mean neutrophil elastase recorded in the groups at two different time intervals

| Time slot | Mean | Standard Deviation |
|-----------|------|--------------------|
| Baseline  | Test | 61.56              | 17.74 |
|           | Control | 61.27             | 16.22 |
| 6 weeks   | Test | 12.33              | 8.72  |
|           | Control | 11.34             | 7.46  |
It also promotes cell proliferation in gingival epithelial cells, fibroblasts, and lymphocytes, arrests the inflammatory process, and improves periodontal lesions in patients with chronic periodontitis.\[19\] The anti-inflammatory effect of hyaluronan may be due to its action as a scavenger in draining the prostaglandins, metalloproteinases, and other bioactive molecules.\[4\]

Further, in our study, statistically significant reduction in mean PPD was observed in experimental sites as well as the control sites from baseline to 6 weeks. However, when comparing the two groups, no statistically significant difference was observed at baseline or 6 weeks. Similar findings were reported in a study by Xu et al.,\[20\] in which they evaluated the potential benefits of local subgingival application of HA adjunctive to SRP. The results indicated a statistically significant reduction in the mean PPD in both the study groups from baseline to 12 weeks. However, when intergroup comparison was performed, there was no statistically significant difference in the mean PPD scores. Contrasting results were reported by Johannsen et al.,\[14\] where the experimental sites showed greater reduction in PPD compared to the control group, which was statistically significant.

Although statistically insignificant, the results of our study indicated a greater reduction in the PPD in the experimental group (mean reduction = 2.9 mm) compared to the control group (mean reduction = 2.65 mm). This difference could be attributed again to the anti-inflammatory and wound healing properties of hyaluronan, which might have helped in reducing the probing depths either due to resolution of the edematous component, or due to new attachment because of the stimulatory effect of hyaluronan on fibroblasts to synthesize collagen. HA is a candidate for use in the restoration of periodontal integrity due to its complex interactions with the ECM and its components.\[21\]

In our study, statistically significant gain in RAL was observed in the experimental as well as control sites from baseline to 6 weeks, which is a better indicator of periodontal health than the PPD (which might vary due to the changes in the position of gingival margin). However, the difference between the groups was not statistically significant. Similar findings were reported by Eick et al.,\[9\] who noticed significant improvement in terms of CAL in the test and control groups. However, no significant difference was observed between the groups after 6 months. Our treatment outcome in terms of gain in CAL was in accordance with Xu et al.,\[20\] who reported a significant gain in the CAL from baseline to 12 weeks in both experimental and control groups; however, intergroup comparison did not reveal a statistically significant difference. This gain in CAL could be attributed to hyaluronan, which plays an important role in postinflammatory tissue regeneration, facilitating cell migration and differentiation during tissue formation and repair.\[17\]

| Table 9: Correlation between different parameters in control group after 6 weeks |
|-----------------|-----|-----|-----|-----|-----|
| Correlations    | PI   | GI   | PD   | CAL  | NE  |
| R               | -    | 0.515 | 0.341 | 0.507 | 0.209 |
| P               | -    | 0.020 | 0.142 | 0.022 | 0.377 |
| R               | 0.315 | -    | 0.436 | 0.146 | 0.362 |
| P               | 0.020 | 0.055 | -    | 0.539 | 0.117 |
| R               | 0.341 | 0.436 | -    | 0.519 | 0.054 |
| P               | 0.142 | 0.055 | -    | 0.019 | 0.820 |
| R               | 0.507 | 0.146 | 0.519 | -    | 0.068 |
| P               | 0.022 | 0.539 | 0.019 | -    | 0.777 |
| R               | 0.209 | 0.362 | 0.054 | 0.068 | -    |
| P               | 0.377 | 0.117 | 0.820 | 0.777 | -    |

*Significant correlation. NE=Neutrophil elastase, PI=Plaque index, GI=Gingival index, PD=Probing depth, CAL=Clinical attachment loss*

Reducing PI scores. The use of a periodontal dressing could not have been a confounding factor because there was a reduction in the mean plaque scores in the experimental sites from baseline to 6 weeks. Nevertheless, overall reduction in PI scores from baseline might be attributed to good oral hygiene practiced by the patients during the entire study period.

We reported a statistically significant reduction in the mean GI scores in the experimental as well as the control sites from baseline to 6 weeks. However, when comparing between the two groups, no statistically significant difference was observed at baseline or 6 weeks. Similar findings were reported by Rajan et al.,\[15\] who used 0.2% HA gel as an adjunct to SRP and evaluated its effects on various periodontal parameters. They too reported a statistically significant reduction in mean GI scores in the experimental as well as control groups from baseline to 12 weeks. Our results were in contrast to those of Pilloni et al.,\[16\] who reported a greater reduction of GI scores in the HA group compared to the control group by the end of 21 days, which was statistically significant.

The results of our study suggest that both HA gel and SRP are equally efficacious in reducing the signs of gingival inflammation over a period. Although there was no statistically significant difference in mean GI scores between both groups at the end of 6 weeks, slightly greater reduction was observed in the experimental sites (GI score = 0.66 ± 0.47) than in control sites (GI score = 0.69 ± 0.56). This reduction in the HA group could be attributed to the anti-inflammatory and wound healing properties of hyaluronan.\[17\] It also promotes migration of various cells including fibroblasts at the site of injury and, thus, results in reduction of vascularityization by bringing about fibrosis of connective tissue.\[18\]

Different mechanisms have been proposed to explain the effect of HA on the inflammatory process. HA gel reduces
The aim of our study was to evaluate the GCF levels of NE. The findings of our study indicate a highly statistically significant reduction in the NE levels from baseline to 6 weeks in both groups. However, the difference between the groups was not statistically significant. Interestingly, to the best of our knowledge, only one study evaluated the activity of this enzyme in the past. In contrast to our findings, this study conducted by Eick et al. reported a statistically significant increase in the NE activity from baseline to 6 months in both test and control groups, albeit no statistically significant difference between the groups after 6 months.

The sequential treatment protocol in the present study could have produced the beneficial effect on periodontal health. Our findings warrant a more intensive and additional application of HA gel to bring about long-term beneficial effects as an adjunctive periodontal therapy.

To the best of our knowledge, very few published studies have evaluated the effect of hyaluronan on the enzyme NE. Moreover, the results of our study suggest that although statistically insignificant, hyaluronan brings about the inhibition of NE, in comparison with the control sites. The association of elastase with periodontal destruction has long been reported. Thus, the significant finding of elastase inhibition by hyaluronan in this study might open new avenues as an LDD agent for the management of chronic periodontitis. However, long-term studies with larger sample size are needed to evaluate the efficacy of hyaluronan against elastase to affirm the findings of our study.

CONCLUSIONS

Adjuvant use of hyaluronan following mechanical debridement resulted in comparable reduction in the elastase levels, suggesting that this substance has an inhibitory effect on NE, and subsequent tissue destruction. However, the major limitation of this study is its short duration. Moreover, the use of a positive control in this study would have eliminated the chances of bias in results due to confounding variables. Hence, further long-term studies are mandatory to confirm these results.

Acknowledgments

We would like to thank Dr. Riyaz Ahmed Shah, PhD., Department of Microbiology and Cell Biology, Indian Institute of Science, Bengaluru, for his excellent technical guidance during the storage and biochemical analysis of this research work.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Bascones-Martínez A, Muñoz-Corcuera M, Noronha S, Mota P, Bascones-Ilundain C, Campo-Traperjo J. Host defence mechanisms against bacterial aggression in periodontal disease: Basic mechanisms. Med Oral Patol Oral Cir Bucal 2009;14:e680-5.
2. Reddy MS, Geurs NC, Gunsolley JC. Periodontal host modulation with antiproteinase, anti-inflammatory, and bone-sparing agents. A systematic review. Ann Periodontol 2003;8:12-37.
3. Vandekerckhove BN, Quirynen M, van Steenberge D. The use of tetracycline-containing controlled-release fibers in the treatment of refractory periodontitis. J Periodontol 1997;68:353-61.
4. Laurent TC, Laurent UB, Fraser JR. Functions of hyaluronan. Ann Rheum Dis 1995;54:429-32.
5. Cantor JO, Cerreta JM, Armand G, Turino GM. Aerosolized hyaluronic acid decreases alveolar injury induced by human neutrophil elastase. Proc Soc Exp Biol Med 1998;217:471-5.
6. Pagnacco A, Vangelisti R, Erra C, Poma A. Double-blind clinical trial Vs. placebo of a new sodium-hyaluronate-based gingival gel. Attual Ter Internazionale 1997;15:1-7.
7. Akatsuka M, Yamamoto Y, Tobetto K, Yasiu T, Ando T. Suppressive effects of hyaluronic acid on elastase release from rat peritoneal leucocytes. J Pharm Pharmacol 1993;45:110-4.
8. Cox SW, Eley BM. Detection of cathepsin B- and L-, elastase-, trypsin-, trypsin-, and dipeptidyl peptidase IV-like activities in crevicular fluid from gingivitis and periodontitis patients with peptidyl derivatives of 7-amino-4-trifluoromethyl coumarin. J Periodontal Res 1989;24:353-61.
9. Eick S, Renatus A, Heiniche M, Pfister W, Stratul SI, Jentsch H. Hyaluronic acid as an adjunct after scaling and root planing: A prospective randomized clinical trial. J Periodontol 2013;84:941-9.
10. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999;4:1-6.
11. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:121-35.
12. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963;21:533-51.
13. Mombelli A, Van Winkelhoff AJ. The systemic use of antibiotics in periodontal therapy. In: Lang NP, Karring T, Lindhe J, editors. Proceedings of the Second European Workshop on Periodontology. London: Quintessence; 1997. p. 38-77.
14. Agricultura A, Tellefsen M, Wilkesjö U, Johannsen G. Local delivery of hyaluronan as an adjunct to scaling and root planing in the treatment of chronic periodontitis. J Periodontol 2009;80:1493-7.
15. Rajan P, Baramappa R, Rao NM, Pavaluri AK, Indeevar P, Rahaman SM. Hyaluronic acid as an adjunct to scaling and root planing in chronic periodontitis. A randomized clinical trial. J Clin Diagn Res 2014;8:ZC11-4.
16. Pilloni A, Annilabi S, Dominici F, Di Paolo C, Papa M, Cassini MA, et al. Evaluation of the efficacy of an hyaluronic acid-based biogel on periodontal clinical parameters. A randomized-controlled clinical pilot study. Ann Stomatol (Roma) 2011;2:3-9.
17. LeBeouf RD, Gregg R, Weigel PH, Fuller GM. The effects of hyaluronan on the conversion of fibrinogen to fibrin and on fibrin gel structure. J Cell Biol 1985;101:340-5.
18. Bartold PM. Page RC. The effect of chronic inflammation on gingival connective tissue proteoglycans and hyaluronic acid. J Oral Pathol 1986;15:367-74.
19. Mesa FL, Aneiros J, Cabrera A, Bravo M, Caballero T, Revelles F, et al. Antiproliferative effect of topic hyaluronic acid gel. Study in gingival biopsies of patients with periodontal disease. Histol Histopathol 2002;17:47-53.
20. Xu Y, Hölling K, Fimmers R, Frenzten M, Jervoe-Storm PM. Clinical and microbiological effects of topical subgingival application of hyaluronic acid gel adjunctive to scaling and root planing in the treatment of chronic periodontitis. J Periodontol 2004;75:1144-8.
21. Moseley R, Waddington RJ, Embrey G. Hyaluronan and its potential role in periodontal healing. Dent Update 2002;29:144-8.