Comparative evaluation of 0.2% hyaluronic acid gel (Gengigel®) and photodynamic therapy in the Treatment of Chronic Periodontitis: A Clinical Study

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Abstract

Introduction

Periodontal tissues represent a unique system, where epithelial, non-mineralized, and mineralized connective tissues exist in harmony. The integrity, however, is lost during chronic inflammation associated with periodontal disease.1

Periodontitis is an inflammatory disease, which is characterized by the presence of gingival inflammation, periodontal pocket formation and loss of connective tissue and alveolar bone of the teeth, that results from the extent of subgingival inflammation induced by bacteria present in the plaque.2

The main objective of periodontal therapy is to eliminate deposits of bacteria and bacterial niches by removing the supragingival and subgingival biofilm. The gold standard for the non-surgical treatment of periodontal disease remains the mechanical debridement with scaling and root planing (SRP) that aims at removal of plaque, calculus and endotoxins from both supragingival and subgingival root surfaces of the teeth in order to prevent disease progression.3 The usual nonsurgical treatment modalities for periodontitis include scaling and root planning, local drug delivery, lasers, and certain advanced techniques like photodynamic therapy. Though combination of mechanical and chemical treatment provides good recovery from the initial status of the disease.4

As a part of non-surgical therapy, local drug therapy is a common mode of therapy. Topical delivery has the advantage of bringing a high concentration of drug to a particular targeted site. The side effects of systemic antibiotic therapy and the possible failing compliance of the patient can be minimized by using locally applied delivery systems. Therefore, a positive influence on the subgingival biofilm may be accomplished with a local delivery system. 5

In addition to the better-known antimicrobials and biomaterials, there are number of substances which are less well known and less used at present, and at the same
time, have the potential to augment results of periodontal therapy. One such molecule is ‘Hyaluronic acid’.6

Hyaluronic acid (HA) is also known as hyaluronan or hyaluronate. It is a linear polysaccharide found in extracellular matrices of connective tissue, synovial fluid and other tissues. Hyaluronan is produced by fibroblasts in the presence of endotoxins.7

Hyaluronic acid is known to enhance the inflammatory cell and extracellular matrix cell infiltration into the wound site. Also, causes elevation of proinflammatory cytokine production by inflammatory cells and extracellular matrix cells. Most commonly scavenges reactive oxygen species, such as superoxide radical and hydroxyl radical thus preventing periodontal destruction.8

In the last decade interest in hyaluronan has intensified in cell biology, pathology and immunology after it has shown that cells have receptors that can specifically recognize its pure polysaccharide structure. Thus, Hyaluronan administration in periodontal disease sites has resulted in many beneficial effects.6,9

Recent advances in non-surgical therapy also includes photodynamic therapy (PDT) which is emerging novel approach used in the treatment of chronic periodontitis. Photodynamic therapy is an oxygen dependent photochemical reaction that occurs upon exposure to a particular wavelength of light in the presence of a suitable photosensitizer dye. The various photosensitizer dyes used include, a) Tricyclic dyes with different mesoatoms., E.g. Acridine orange, proflavine, riboflavin, methylene blue, toluidine blue, fluorescein, and erythrosine. b) Tetrapyrroles. E.g.: Porphyrins and derivatives, chlorophyll, phylloerythrin, and thalactocyanines, and c) Furocoumarins. E.g.: Psoralen and its methoxy derivatives, xanthotoxin, and bergapteine.10

The various light sources utilized in photodynamic therapy includes lasers of various wavelengths, non-laser light sources like light emitting diodes (LED). The photochemical reaction results in generation of cytotoxic species such as superoxide, hydroxyl radicals, hydrogen peroxide and singlet oxygen. Among these, reactive oxygen species singlet oxygen plays a major role in microbial destruction, as it can interact with large number of biological substrates inducing oxidative damage on the cell membrane and cell wall of bacteria, fungi, and viruses. Photodynamic antimicrobial chemotherapy represents an alternate antibacterial, antifungal and antiviral treatment against drug-resistant organisms. Applications of PDT in dentistry are growing rapidly. PDT is also used in the treatment of oral cancers, bacterial and fungal infections, and also in the photodynamic diagnosis of malignant transformation of oral lesions and also in the non-surgical management of patients with periodontitis.11

Several studies have been done to compare the efficacy of hyaluronic acid as an adjunctive approach to scaling. They revealed positive results with respect to hyaluronic acid based Gengigel.4 Also, there has been significantly good results obtained by the recent studies done on PDT with SRP than SRP alone. But there is paucity of studies which compare the effectiveness of Gengigel with SRP to PDT with SRP.

Therefore, present study evaluates the efficacy of Gengigel with SRP, Photodynamic therapy with SRP, SRP alone in the nonsurgical treatment of chronic periodontitis.

Materials and Methodology

20 Subjects were selected from those diagnosed as having chronic periodontitis (based on the 1999 Classification of Periodontal Diseases and Conditions). The Ethical clearance for the study was obtained from the ethical committee and review board of the institution.

Study Protocol
The nature of the study was explained verbally in a language comprehensible to the patient, information sheet was given and informed consent was obtained from the patient. This clinical study was conducted from October 2017 to October 2018.

**Inclusion criteria**
- Chronic periodontitis patients having minimum of six teeth with periodontal disease.
- Pocket depth equal to or greater than 5mm.
- Age between 30 and 65 years.
- Both sexes are included.
- No systemic conditions that would contraindicate routine periodontal procedures.
- Subjects who are able to attend the hospital at regular intervals.

**Exclusion criteria**
- Subjects who have received periodontal therapy within the past 6 months.
- Pregnant and lactating patients.
- Patients who have taken antibiotics within 6-month period preceding study.
- Teeth exhibiting class II and class III mobility.
- Smokers.
- Subjects undergoing orthodontic therapy
- Acute oral infections
- Subjects with known allergy to toluidine blue dye.

**Study design:**
A split mouth study was designed among all the selected subjects by categorizing randomly into three different groups in such a way that each group consisting of one quadrant of every subject.

Group A (N= 20): Those to be treated with Scaling and Root planning only.

Group B (N=20): Those to be treated with Scaling and Root planning + Photodynamic therapy (0.05% toluidine blue)

Group C (N=20): Those to be treated with Scaling and Root planning + Gengigel (0.2% hyaluronic acid)

In Group B participants after thorough SRP, safety goggles were provided to the patient, operator, and the assistant to prevent damage to the eyes by laser.

**Clinical parameters**
The clinical parameters recorded were Plaque Index (Silness and Loe 1964), Gingival Index (Loe and Silness 1963), and Sulcus Bleeding Index (SBI). Also, Pocket Probing Depth (PPD) and Clinical attachment loss (CAL) were recorded using UNC 15 Probe and customized acrylic stent( as shown in fig.3,4,5). All the clinical parameters were recorded at baseline, 30 days and 90 days.

**Treatment procedure**
At the first appointment, brief case history of the patient and full mouth periodontal examination was conducted. Before any treatment, all clinical parameters were recorded. After which scaling and root planning was performed to all twenty patients. After one week, each quadrant was allocated to one of the groups.

In group A, subjects received only scaling and root planing (SRP).

In group B, after thorough scaling and root planing (SRP) a 26-gauge needle was taken and the bevel of the needle was cut to remove the sharp edge and made blunt and using this needle the toluidine blue photosensitizer solution with a concentration of 0.05% was placed into the pocket, starting from the apical end and moving coronally to avoid entrapment of air bubbles. Three minutes later all pockets were thoroughly rinsed with sterile saline to remove the excessive photosensitizer. Immediately after
rinsing, the LED light of PDT unit was focussed at the depth of the pocket and moved circumferentially in sweeping motion around the teeth for one minute (as shown in fig.1).

Figure 1 (Application of Photosensitising agent Toluidine Blue 0.005%)
In group C after thorough scaling and root planing (SRP) Gengigel (0.2% hyaluronic acid gel) was placed intrasulcularly into the periodontal pocket (as shown in fig.2).

Figure 2 (Application of Gengigel)

Results
There were no dropout cases and all the 20 subjects maintained their appointments.

In Group A the mean plaque index scores at baseline, 1 month and 3 months were 1.95 ±0.29, 1.39±0.18 and 1.15±0.09 respectively. In Group B the mean plaque index scores at baseline, 1 month and 3 months were 2.12±0.40, 1.34±0.14 and 1.10±0.07 respectively. In Group C the mean plaque index scores at baseline, 1 month and 3 months were 2.19±0.41, 1.33±0.17 and 1.10±0.07 respectively. The mean PI was found to be statistically significant at baseline, 1 month and 3 months (P<0.001) between Group A, Group B and Group C. The reduction of mean plaque index score in group C was slightly higher than that of Group A and B.

Note: T1 - BL, T2 - Post-Operative 30 Days, T3 - Post-Operative 90 Days
The mean Gingival index scores at baseline, 1 month and 3 months in Group A were 2.47±0.19, 1.57±0.24 and 1.32±0.20 respectively. In Group B, the mean gingival index scores at baseline, 1 month and 3 months were 2.42±0.15, 1.52±0.26 and 1.19±0.11 respectively. In Group C, the mean gingival index scores at baseline, 1 month and 3 months were 2.40±0.15, 1.38±0.19 and 1.09±0.08 respectively. The mean Gingival index (GI) was found to be statistically significant at baseline, 1 month and 3 months (P<0.001) in Group A, Group B and Group C. The reduction of mean gingival index score in group C was slightly higher than that of Group A and B.

The mean SBI scores at baseline, 1 month and 3 months in Group A were 2.59±0.20, 1.60±0.19 and 1.40±0.10 respectively. In Group B, the mean SBI scores at baseline, 1 month and 3 months were 2.45±0.23, 1.52±0.20 and 1.27±0.09 respectively. In Group C, the mean SBI scores
at baseline, 1 month and 3 months were 2.40±0.33, 1.40±0.19 and 1.08±0.05 respectively. The mean Sulcus Bleeding Index (SBI) was found to be statistically significant at baseline, 1 month and 3 months (P<0.001) in Group A, Group B and Group C. However, the mean reduction in Sulcus bleeding index score in group C was slightly higher than that of Group A and B.

* - Statistically Significant

The mean PPD scores at baseline and 3 months in Group A were 7.38±1.17 and 6.65±0.73 respectively. In Group B the mean PPD scores at baseline and 3 months were 7.35±0.97 and 5.78±0.95 respectively. In Group C, the mean PPD scores at baseline and 3 months were 7.70±1.07 and 5.35±0.75 respectively. The difference in mean PPD was found to be statistically significant at all time intervals between all the three groups (P<0.001).

Whereas the mean CAL scores at baseline and 3 months in Group A were 8.08±1.05 and 6.83±0.80 respectively. In Group B, the mean CAL scores at baseline and 3 months were 7.83±0.95 and 6.25±0.88 respectively. In Group C, the mean CAL scores at baseline and 3 months were 8.00±1.01 and 5.85±0.81 respectively. The difference in mean CAL was found to be statistically significant at all time intervals between all the three groups (P<0.001).

* - Statistically Significant

Note: G1 - Group 1, G2 - Group 2, G3 - Group 3
Periodontitis being multifactorial in etiology results in loss of supporting tissues of the periodontium. Bacterial biofilm is the main aetiological factor of periodontitis. This bacterial burden observed in periodontitis is characterised by the increased proliferation of periodontopathogens (e.g., Aggregatibacter actinomycetemcomitans, P. gingivalis) which result in an elevation in the pro-inflammatory mediators such as interleukin (IL)-1β, IL-6 and tumour necrosis factor (TNF)-α. This in turn leads to the deepening of periodontal pockets and subsequent bone loss which further potentiates the spread of periodontitis.

The success of chronic periodontitis treatment depends on removal of periodontopathogens and their toxic products from the root surfaces and periodontal soft tissues, as well as neutralization of host pro-inflammatory cytokines.

Conventional treatment such as scaling and root planning (SRP) is still considered as gold standard, but it does not completely eliminate periodontal pathogens, especially in deep periodontal pockets.

Wu and Savitt (2002) reported that in conjunction with mechanical methods, oral hygiene products containing chemotherapeutic agents with a variety of antimicrobial mechanisms have been desirable and beneficial. Hyaluronic acid, which is an extracellular constituent of the connective tissue, was recently introduced as a local chemotherapeutic agent and has exhibited numerous clinical therapeutic properties.

Hyaluronan, also known as HA, has been identified in all periodontal tissues, such as gingiva and periodontal ligament. Hyaluronic acid has been studied as a marker of inflammation and is a significant factor in the growth, development, and repair of tissues.

Hyaluronic acid has essential biological and physiological functions. Beneficial wound healing properties on the effects of exogenous hyaluronan were confirmed in several studies. Hyaluronic acid can influence and enhance tissue regenerative procedure, owing to its capability to preserve large amounts of water. It was recommended that wound healing will be accelerated in the bone matrix due to stimulation of angiogenesis by hyaluronic acid. High molecular weight hyaluronan has

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* - Statistically Significant

Discussion

Periodontitis being multifactorial in etiology results in loss of supporting tissues of the periodontium. Bacterial biofilm is the main aetiological factor of periodontitis. This bacterial burden observed in periodontitis is characterised by the increased proliferation of periodontopathogens (e.g., Aggregatibacter actinomycetemcomitans, P. gingivalis) which result in an elevation in the pro-inflammatory mediators such as interleukin (IL)-1β, IL-6 and tumour necrosis factor (TNF)-α. This in turn leads to the deepening of periodontal pockets and subsequent bone loss which further potentiates the spread of periodontitis.

The success of chronic periodontitis treatment depends on removal of periodontopathogens and their toxic products from the root surfaces and periodontal soft tissues, as well as neutralization of host pro-inflammatory cytokines.

Conventional treatment such as scaling and root planning (SRP) is still considered as gold standard, but it does not completely eliminate periodontal pathogens, especially in deep periodontal pockets.

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Hyaluronic acid, which is an extracellular constituent of the connective tissue, was recently introduced as a local chemotherapeutic agent and has exhibited numerous clinical therapeutic properties.

Hyaluronan, also known as HA, has been identified in all periodontal tissues, such as gingiva and periodontal ligament. Hyaluronic acid has been studied as a marker of inflammation and is a significant factor in the growth, development, and repair of tissues.

Hyaluronic acid has essential biological and physiological functions. Beneficial wound healing properties on the effects of exogenous hyaluronan were confirmed in several studies. Hyaluronic acid can influence and enhance tissue regenerative procedure, owing to its capability to preserve large amounts of water. It was recommended that wound healing will be accelerated in the bone matrix due to stimulation of angiogenesis by hyaluronic acid. High molecular weight hyaluronan has
shown to stimulate osteoinduction during wound healing. It is directly or indirectly associated with various cell functions, such as recognition, locomotion, and cell proliferation, which will provide tissue healing properties.\textsuperscript{17}

Hyaluronic acid also acts as a fundamental element of periodontal ligament matrix and performs essential roles in cell migration, adhesion, and differentiation. The large size and negative charge of HA facilitates the absorption of large amount of hydration water to exert significant pressure on the surrounding tissue. This results in the production of enlarged extracellular space.\textsuperscript{18}

Some systematic reviews have reported that hyaluronic acid can be efficiently used as an adjunct to conventional periodontal treatment.\textsuperscript{19,20,21} Only few studies have examined the effect of hyaluronan on periodontal health. Less is known about the effect of subgingival administration of hyaluronan on periodontitis.

Another adjunctive therapy to mechanical debridement gaining popularity is Photo dynamic therapy (PDT). PDT is a new treatment modality that has been developing rapidly within various medical specialties since the 1960s. It has been defined as “the light induced inactivation of cells, microorganisms, or molecules”.\textsuperscript{22} PDT is a technique combining diode laser energy with a photosensitizer to produce free radicals to destroy targeted cells.

PDT is based on the principle that a dye, as a photosensitizer or photoactivatable agent (i.e, Toluidine Blue-TBO, Methylene Blue, Malachite Green and Indocyanine Green-ICG), binds to the target cells and is activated by light of an appropriate wavelength. By changing the energy status of the molecules in the photosensitizer, free radicals of singlet oxygen are formed, which are toxic to the cell by destroying the membrane.\textsuperscript{23,24} Despite of its short half-life time, singlet oxygen exerts strong cytotoxic effects, destroying cellular constituents such as organelles, proteins, nucleic acids, cholesterol, etc. Microorganisms are killed by singlet oxygen, which include viruses, bacteria, protozoa, and fungi. Current studies on PDT showed that a number of the periodontal pathogens are susceptible to low level laser in the presence of photosensitizer, suggesting that PDT is advantageous for conventional periodontal therapy.\textsuperscript{25,26,27}

Some systematic reviews and meta-analyses conclude that PDT when used as an adjunct to conventional periodontal therapy kills more bacteria than when conventional periodontal therapy is used alone.\textsuperscript{28,29}

Several studies have been conducted previously comparing Gengigel (0.2% hyaluronic acid) with SRP and SRP alone or PDT with SRP and SRP alone but there is paucity of studies conducted to compare PDT+SRP and Gengigel (0.2% hyaluronic acid) + SRP with SRP alone. Hence, the present single blinded randomised clinical study was planned to comparatively evaluate the efficacy of Gengigel (0.2% hyaluronic acid) and photodynamic therapy as an adjunct to scaling and root planing in the treatment of chronic periodontitis.

The results obtained from the present study showed that, in Group A the mean plaque index scores at baseline, 1 month and 3 months were 1.95±0.29, 1.39±0.18 and 1.15±0.09 respectively. In Group B the mean plaque index scores at baseline, 1 month and 3 months were 2.12±0.40, 1.34±0.14 and 1.10±0.07 respectively. In Group C the mean plaque index scores at baseline, 1 month and 3 months were 2.19±0.41, 1.33±0.17 and 1.10±0.07 respectively. The mean PI was found to be statistically significant at baseline, 1 month and 3 months (P<0.001) between Group A, Group B and Group C. The reduction of mean plaque index score in group C was slightly higher than that of Group A and B. The results
obtained in the present study in plaque reduction scores were in agreement with the studies conducted by Alwaeli et al., 2015; Campos et al., 2013. However, according to Eick et al (2013) the results of the two therapeutic procedures i.e gengigel and SRP are similar with regard to plaque index and bleeding on probing for which gengigel does not lead to additional benefits.

The results of this study state that, the mean GI scores at baseline, 1 month and 3 months in Group A were 2.47±0.19, 1.57±0.24 and 1.32±0.20 respectively. In Group B the mean gingival index scores at baseline, 1 month and 3 months were 2.42±0.15, 1.52±0.26 and 1.19±0.11 respectively. In Group C the mean gingival index scores at baseline, 1 month and 3 months were 2.40±0.15, 1.38±0.19 and 1.09±0.08 respectively. The mean Gingival index (GI) was found to be statistically significant at baseline, 1 month and 3 months (P<0.001) in Group A, Group B and Group C. The reduction of mean gingival index score in group C was slightly higher than that of Group A and B. The results obtained in the present study in gingival reduction scores were in agreement with the study conducted by Rajan et al. The results of this study state that, the mean SBI scores at baseline, 1 month and 3 months in Group A were 2.59±0.20, 1.60±0.19 and 1.40±0.10 respectively. In Group B the mean SBI scores at baseline, 1 month and 3 months were 2.45±0.23, 1.52±0.20 and 1.27±0.09 respectively. In Group C the mean SBI scores at baseline, 1 month and 3 months were 2.40±0.33, 1.40±0.19 and 1.08±0.05 respectively. The mean Sulcus Bleeding Index (SBI) was found to be statistically significant at baseline, 1 month and 3 months (P<0.001) in Group A, Group B and Group C. However, the mean reduction in Sulcus bleeding index score in group C was slightly higher than that of Group A and B. The results obtained in the present study in gingival reduction scores were in agreement with the study conducted by Rajan et al.

The results of this study state that, the mean PPD scores at baseline and 3 months in Group A were 7.38±1.17 and 6.65±0.73 respectively. In Group B the mean PPD scores at baseline and 3 months were 7.35±0.97 and 5.78±0.95 respectively. In Group C the mean PPD scores at baseline and 3 months were 7.70±1.07 and 5.35±0.75 respectively. The difference in mean PPD was found to be statistically significant at all time intervals between all the three groups (P<0.001). Whereas the mean CAL scores at baseline and 3 months in Group A were 8.08±1.05 and 6.83±0.80 respectively. In Group B the mean CAL scores at baseline and 3 months were 7.83±0.95 and 6.25±0.88 respectively. In Group C the mean CAL scores at baseline and 3 months were 8.00±1.01 and 5.85±0.81 respectively. The difference in mean CAL was found to be statistically significant at all time intervals between all the three groups (P<0.001). The results obtained from this study are in agreement with the study done by Smiley et al whereas Xu et al investigated and found no clinical and microbial improvement between HA gel’s adjunctive use compared with only SRP in relative to bleeding on probing and PDD.

**Conclusion**

The conventional mechanical therapy, scaling and root planing alone have beneficial effect on the non-surgical management of chronic periodontitis. Photodynamic therapy as an adjunct to SRP had added benefit over the conventional scaling and root planing alone. Gengigel (0.2% hyaluronic acid gel) as an adjunct to SRP had added benefit over the conventional scaling and root planing alone. However, Gengigel (0.2% hyaluronic acid gel) +...
SRP showed a better result compared to Photodynamic therapy + SRP and SRP alone.

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