

*Research Article***Management of black triangle of interdental papilla using injectable carboxymethyl chitosan hydrogel with and without hyaluronic acid gel
(Comparative Study)****Eman M. Abdelkader* and Mohamed A. Ebrahim***

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Abstract

Open gingival embrasures or “Black triangles” are created due to incomplete fill of interdental papilla.⁽¹⁾ **Aim of the study:** To compare the effect of hyaluronic acid gel alone versus hyaluronic acid gel combined with carboxymethyl chitosan hydrogel In the Management of the black triangle of interdental papilla. **Patients and methods:** 40 deficient dental papilla sites that were randomly assigned into two equal groups. Group (A) using hyaluronic acid gel only. Group (B) using carboxymethyl chitosan hydrogel with hyaluronic acid gel. Following parameters will be evaluated before treatment and recorded at baseline and after 6 months Plaque index (PI), Bleeding index (BI), Gingival recession (GR), Probing depth (PD), Clinical attachment level (CAL), and height of the black triangle. **Results:** Both groups showed improvement in clinical parameters although they are statistically insignificant. Upon comparing the results of both treatment modalities, No statistically significant difference was detected regarding BI, PI, PD, CAL, or GR scores along the whole studied period with a p-value>0.05. **Conclusion:** treatment of black triangle of interdental papilla using injectable carboxymethyl chitosan hydrogel and hyaluronic acid gel show more improvement in clinical outcomes. The reduction of the black triangles after 6 months was not statistically significant between both groups.

Keywords: hyaluronic acid gel (HA), black triangle, chitosan.**Introduction**

The esthetic composition of the smile is vital for interpersonal relationships and strongly affects patient self-esteem. To obtain an esthetic and harmonious smile, the shape, position, and color of teeth, as well as the gingival tissue must be considered. In the gingival tissue, the interdental papilla should stand out for representing a small visible area between the teeth with pink color, firm consistency, and triangle shape⁽²⁾

The presence or absence of the interdental papilla was and still is a great concern to periodontists and patients. Interdental “black triangles” were considered as the third less attractive esthetic problem after caries and crown margins. Conventional

treatment to interdental papilla loss was purely surgical by using connective tissue grafts. This approach with all its sophistication has its drawbacks because of the procedure itself and the need for strict oral hygiene protocols. Other non-surgical approaches were proposed including injection of various fillers such as Hyaluronic acid (HA).and chitosan gel.⁽³⁾

Hyaluronic acid is a polysaccharide (glycosaminoglycan) present in body tissues, such as skin and cartilage, and under physiologic conditions. it binds to water and swells when in gel form, resulting in smoother/fuller tissue contours. Hyaluronic acid is a high molecular weight (≥ 105 Da) polymer consisting of

disaccharide repeats of N-acetylglucosamine and glucuronic acid, with several thousand sugar molecules in the backbone. The viscosity of hyaluronic acid solutions increases with increasing concentrations, and its unique rheological properties make it an ideal lubricant in the biomedical realm. Chemical modification (cross-linking) of hyaluronic acid preparations results in a material that degrades more slowly (because of decreased water solubility). Hyaluronic acid preparations used as fillers have been manufactured from bacterial or animal sources, and their clinical effects typically last 6–12 months. The purpose of this prospective clinical trial was to examine the clinical and patient outcomes following non-surgical reconstruction of interdental papillae in the anterior teeth, using a non-animal-based hyaluronic acid gel.⁽⁴⁾

Chitosan is commonly obtained from the deacetylation of chitin, the second most abundant natural polymer on earth after cellulose.¹ The primary sources of chitin are crustaceans such as crabs, shrimps, and lobsters. The challenge of preparing chitosan-based hydrogels lies in the fact that a significant amount of water needs to be retained in the system. Chitosan hydrogels are generally prepared by physical or chemical cross-linking of the polymer chains, keeping enough charges and/or hydrophilic moieties to guarantee sufficient hydration in the network.

To provide mechanical stability to these hydrogels, a covalent cross-linking between the two polysaccharides can be obtained via a Schiff base reaction. Chemical prefunctionalisation of chitosan with an N-succinyl group and of hyaluronic acid with an aldehyde one allows the reaction to occur in situ without the need for additional chemicals.⁽⁵⁾

Patients and Methods

This study was conducted on A total of 42 sites that were divided randomly by using toss into two equal groups each having 21 sites for injection The patients were selected from the outpatient,s clinic of the oral medicine, Oral diagnosis, and Periodontology Department. Faculty of Dentistry, Minia University.

Ethical regulations: The complete treatment plan was explained to all patients including detailed steps, risks, and expected results, and their full signed consent will be obtained before entry into the study. The study complied with the rules set by the International Conference on Harmonization Good Clinical Practice Guidelines, and the Declaration of Helsinki. The study was approved by the research ethics committee of the Faculty of Dentistry, Minia University.

Patient selection: Selected patients of both sexes were from 20-45 years. Patients have at least one deficient papilla in inter-bicuspid region interdental space exhibiting class 1 or 2. according to the criteria set by the classification system (Tarnow 1998). Patients were free from any systemic diseases that might influence their periodontal condition and had not undergone any type of regenerative periodontal therapy six months before the initial examination. on the other hand Pregnant, lactating females and smokers were excluded from the study.

Treatment protocol: All patients underwent phase I therapy comprising of full mouth mechanical debridement including supra and sub-gingival scaling and root planning using a universal curette and ultrasonic instrument in two sessions, All patients were instructed for routine oral hygiene measures and adjunctive chemical plaque control in the form of hexitol mouth wash (Chlorhexidine HCL 0.12% mouth-wash, The Arab Drug Company for pharmaceutical & CHEM IND. CO. Egypt) twice daily for 1 week. All patients were re-evaluated 2 weeks after initial treatment, 42 sites of the black triangle were divided randomly into two equal groups each having 21 sites for injection Group (A) using hyaluronic acid gel only.

Group (B) using carboxymethyl chitosan hydrogel with hyaluronic acid gel. The procedure started with the administration of local anesthesia mepacrine-L using the infiltr-ration technique. The deficient papilla was injected with 0.2 ml of hyaluronic acid gel using a 30-gauge disposable plastic insulin syringe. The needle was inserted 2-3 mm apical to the tip of the

interdental papilla and directed coronally with an angulation of 45° to the long axis of the tooth and the bevel directed apically. Then, the papilla was light molded in an incisal direction for 1 minute using gauze. The same steps were performed for group B. the same amount of gel was injected using the same injection method 0.2ml (50% of hyaluronic acid + 50% of chitosan gel).

Assessment method: both groups were evaluated clinically regularly at baseline (before surgery), 3-and 6-months post-operative. Every patient was assessed by the following clinical parameters include PI, GI, PD, CAL, the height of the black triangle, the surface area of the black triangle.

Statistical analysis

Categorical data were presented as frequency and percentage values and were analyzed using Fisher's exact test. Numerical data were presented as mean and standard deviation values. Parametric data were analyzed using an independent t-test for intergroup comparisons and repeated measures ANOVA followed by Bonferroni post hoc test for intragroup comparisons. Non-parametric data were analyzed using the Mann-Whitney U test for intergroup comparisons and Friedman's test followed by the Nemenyi post hoc test for intragroup comparisons. Correlations were analyzed using Spearman's rank-order correlation coefficient. The significance level was set at $p \leq 0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.1.2 for Windows.



injection of hyaluronic acid
fig 1



injection of hyaluronic and chitosan gel
fig 2

Results

when comparing (hyaluronic acid) group with (hyaluronic & chitosan gel) trials, There was an improvement in clinical parameters by time from baseline to 6 months.

Table 1

Clinical Parameters	Hyaluronic acid & Chitosan	Hyaluronic acid	p-value
Plaque index			
PI 0	1.43±1.27A	1.43±1.27A	1ns
PI 3	0.29±0.49B	0.57±0.79AB	0.545ns
PI 6	0.00±0.00B	0.14±0.38B	0.391ns
Gingival index			
GI 0	1.14±1.07A	1.14±1.21A	1ns
GI 3	0.00±0.00B	0.29±0.49B	0.172ns
GI 6	0.00±0.00B	0.00±0.00B	1ns
Probing depth			
PD 0	3.79±0.76A	3.86±0.90A	0.875ns
PD3	3.07±0.19B	3.29±0.49B	0.300ns
PD 6	2.79±0.27B	3.14±0.48B	0.109ns
Height of black triangle (mm)			
Baseline			
3 months	1.98±0.45A	2.23±0.40A	0.280ns
6 months	1.71±0.45B	2.03±0.40B	0.185ns
	1.33±0.47C	1.79±0.34C	0.056ns
The surface area of a black triangle			
Baseline	0.66±0.20A	0.68±0.15A	0.776ns
3 months	0.34±0.11B	0.42±0.08B	0.145ns
6 months	0.19±0.07C	0.24±0.05C	0.150ns

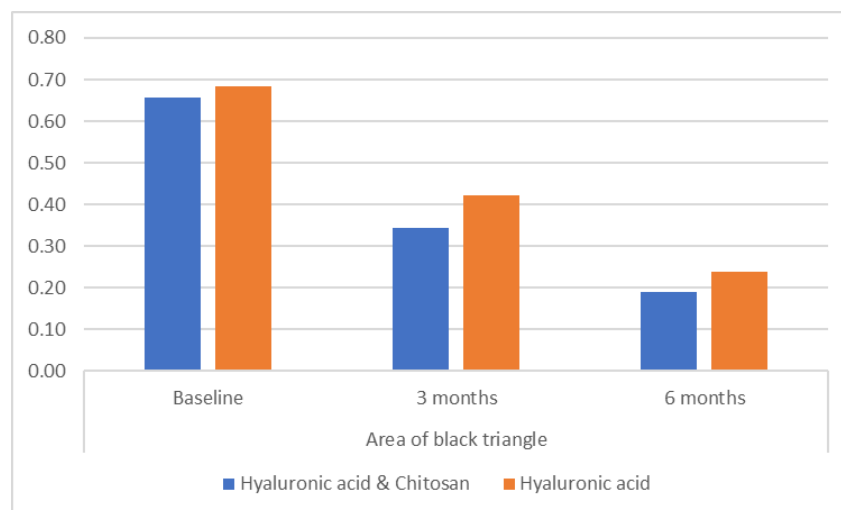


Fig. (3): Bar chart showing the average surface area of a black triangle (mm²) for different groups.

Discussion

Interdental “black triangles” were considered as the third less attractive esthetic problem after caries and crown margins. The presence or absence of the interdental papilla was and still is a great concern to periodontists and patients⁽⁶⁾.

A black triangle is technically defined as a cosmetic deformity due to the loss of interdental papilla. However, a black triangle appearance is not simply a matter of gingival recession and may be caused by many other factors, including bone level, tooth morphology, and contact points⁽⁷⁾.

The interdental papilla as a structure with a minor blood supply was left more or less untouched by clinicians. Reconstruction of the lost interdental papilla is one of the most challenging and least predictable problems and hence, it is very important to respect papillary integrity during all dental procedures and to minimize its disappearance as far as possible⁽⁸⁾.

Several surgical approaches using traditional periodontal plastic and augmentation procedures have been proposed to overcome this problem. However, these techniques were found to be invasive with increased patient morbidity, limited success, and long-term stability. Non-surgical attempts to treat papillary deficiencies include orthodontic, restorative approaches, or a combination of both. However, these methods are invasive but time-consuming⁽⁹⁾.

Hyaluronic acid has more structural and physiological functions in tissues, including extracellular and cellular interactions, interactions with “growth” factors, and regulation of osmotic pressure and tissue lubrication, which helps in maintaining the structural and homeostatic integrity of tissues⁽¹⁰⁾.

HA possessed no tissue or species specificity and induced minimal immunologic response and therefore has been considered as one of the safest substances available. Thus it has been recommended as a dermal filler in treating some of the signs of aging. In the field of dentistry, HA has shown anti-inflammatory, anti-edematous, and anti-bacterial effects, hence; it was used in the treatment of periodontal disease. Besides, different studies have demonstrated HA as an effective method to enhance deficient papillae⁽¹¹⁾.

Chitosan is one of the new biomaterials, One of the most important properties of chitosan is high bioactivity which makes this material very interesting to develop new biomaterials for application in the dentistry area⁽¹²⁾.

Chitosan and its derivatives have excellent biocompatibility, nontoxicity to human beings, biodegradability, the reactivity of the deacetylated amino groups, selective permeability, polyelectrolyte action, antimicrobial activity, ability to form gel, film, and sponge, absorptive capacity, anti-inflammatory and wound healing.⁽¹³⁾

Miranda et al.,⁽¹⁴⁾ investigated hydrogel acquired from chitosan-hyaluronic and its applications in periodontal tissue engineering. The freeze-drying technique was used to collect the chitosan and hyaluronic acid hybrid. Miranda and others argue that developing periodontium for treating periodontitis is a very crucial challenge. In the study, a modified hyaluronic acid together with chitosan developed the chitosan-hyaluronic acid hybrid (CS-HA) hybrid. The CS-HA hybrid generated a perfect scaffold material for periodontal tissue regeneration. The study concluded that hydrogel acquired from chitosan-hyaluronic can be used to create scaffolds that are used to support osteoblasts, gingival, cementoblasts and support the periodontal fibroblast cells, as well as used for periodontal tissue engineering.

The chitosan-hyaluronic acid hybrid hydrogel can be used as a scaffold material for dental tissue engineering. The chitosan-hyaluronic acid hybrid hydrogel can also be used to address osteoblasts, gingival, cementoblasts and supports the periodontal fibroblast cells⁽¹⁵⁾. Natural polymers are utilized in the synthesis of injectable hydrogel for tissue regeneration such as in dental tissue engineering. The use of the chitosan-hyaluronic acid (CS/HA) hydro-gel in periodontal tissue engineering prevents any complications and promotes effective dental tissue regeneration.⁽¹⁶⁾ Hydrogel acquired from chitosan-hyaluronic and its applications in periodontal tissue engineering and hyaluronic acid-based scaffolds are used in controlling cell response and constructing ultimate tissue engineering products, for example, in dental tissue or other tissue engineering applications⁽¹⁷⁾

On the other hand, hyaluronic acid is a commonly used natural material used to fabricate scaffolds as hydrogels Silva, ⁽¹⁸⁾

The hydrogel scaffolds are used to regulate inflammation responses, cell angiogenesis, migration, as well as differentiation of cells, and thus they are ideal scaffold materials for tissue engineering due to their non-toxic degradation and excellent biocompatibility features. ⁽¹⁹⁾

The technique of injection of hyaluronic acid and chitosan was suggested by ⁽²⁰⁾. The patients were evaluated at 3 and 6 months from the first injection since the longevity of Restylane in the tissue lasts for 6 months as stated by the manufacturer.

The case reported a sensation of pressure in the injected region for 1 day. After the second injection session, a pronounced mucosal tissue blanching was observed in the region. The blanching of the mucosal tissue persisted for more than 30 min, but no pain or obvious swelling was noted at that moment. ⁽²¹⁾

Therefore, the aim of our study (randomized, comparative study clinical trial) was to assess patient satisfaction after the injection of cross-linked hyaluronic acid gel for the reconstruction of deficient interdental papilla compared to the injection of hyaluronic and chitosan gel. The secondary outcome included the change in surface area and height of the black triangle, clinical parameters as probing depth, plaque index, and gingival index at the deficient papilla site, and pain level 4 hours after injections.

All participants included in the study had at least one deficient papilla exhibiting class I or class II interdental papillary loss according to the classification proposed by Nordland, and Tarnow ⁽²²⁾. Complete removal of bacterial plaque from the dental-gingival region is the most effective method of preventing gingivitis and periodontitis. ⁽²³⁾ The amount of plaque and debris present on the teeth. This index was designed to assess the sequelae of longstanding oral hygiene deficiencies and current oral hygiene status, respectively.

indices such as plaque index (PI) and the gingival index (GI). ⁽²⁴⁾

Exclusion criteria excluded subjects with medical conditions that may affect periodontal healing or regeneration. Subjects with a history of allergic reactions, pregnant or breastfeeding females, smokers, and alcoholics were not included. Patients with current or previous drugs intake that may predispose to gingival enlargement were not allowed to participate. Patients under orthodontic treatment or who had orthodontic treatment in the past six months were not selected. Patients with a history of traumatic oral hygiene measures or periodontal surgeries over the last six months at the area of interest were not endorsed. ⁽⁹⁾.

Before inclusion, the distance between the contact point and interproximal bone crest (CP – BC) was measured for each deficient papilla. Deficient papilla with CP-BC distance exceeding 7 mm were excluded from this study. Tarnow et al 1992 stated that when the measurement from the contact point to the crest of bone was 5 mm or less, the papilla was present almost 100% of the time. When the distance was 6mm, the papilla was present 56% of the time, and when the distance was 7 mm or more, the papilla was present 27% of the time or less.

The hyaluronic acid filler used in this study was Restylane lidocaine. was FDA-approved. Nonanimal-based cross-linked HA gel with HA concentration of 20 mg/mL. It is biocompatible and does not induce any immunogenic reactions. ⁽²⁵⁾

Clinical and photographic measurements were recorded at baseline, 1, 3, and 6 months ⁽⁸⁾. Alginate impressions were taken and poured into study casts to fabricate a customized stent for reproducible positioning of the periodontal probe for measuring the PT-CP distance at each time interval. In the present study, pain level after injection was evaluated using a visual analog scale since Awartani and Tatakis, 2016 ⁽²⁰⁾ reported patients expressed dissatisfaction with the procedure in terms of pain/discomfort during

the first postoperative week, while many of the patients reported post-operative discomfort.

A visual analog scale was used. Reliable and accurate pain assessment is a prerequisite for successful pain management. Reliable and valid measures of pain are also essential for conducting clinical trials of pain treatments⁽²⁶⁾.

The present study compares the effectiveness of HA only (group A) and a mix of hyaluronic and chitosan gel (group B) materials in the treatment of deficient papillae. There was no significant difference between both groups regarding sex distribution ($p=0.592$) and age ($p=0.830$).

Regarding PI, at baseline, both groups had the same mean value ($p=1$). At other intervals (3 and 6 months follow up period), the Hyaluronic acid group had a higher mean value yet the difference between both groups was not statistically significant ($p>0.05$).

Regarding GI, At baseline and after 6 months, both groups had the same mean value ($p=1$). After 3 months, Hyaluronic acid had a higher mean value yet the difference between both groups was not statistically significant ($p=0.172$).

Regarding PD, For both groups, the value measured at baseline was significantly higher than values measured at other intervals ($p<0.05$). For all intervals, Hyaluronic acid had a higher mean value yet the difference between both groups was not statistically significant ($p>0.05$).

Regarding the Height of the black triangle (mm), For all intervals, Hyaluronic acid had a higher mean value yet the difference between both groups was not statistically significant ($p>0.05$). As for the Surface area of a black triangle (mm²) in both groups, values measured at different intervals were significantly different from each other ($p<0.001$). For all intervals, Hyaluronic acid had a higher mean value yet the difference between both groups was not statistically significant ($p>0.05$).

The mean pain VAS score 4 hours after injection in the presented study was For

both intervals, Hyaluronic acid & Chitosan group had a significantly higher mean value ($p<0.05$). For both groups, the value measured at baseline was significantly higher than the value measured after 6 months ($p<0.05$).

The primary outcome of the present study was to assess the patient satisfaction about their esthetic appearance at the end of the study. the mean VAS satisfaction score at baseline (3.29 ± 1.11 , 4.29 ± 1.11) at both groups A and B respectively, after 6 months were (8.86 ± 0.69 , 9.14 ± 0.90) at both groups A and B respectively. For both intervals, group B had a higher mean value yet the difference between both groups was not statistically significant ($p>0.05$). For both groups, the value measured after 6 months was significantly higher than the baseline value ($p<0.05$).

Results of the current study showed that marked improvement occurred in both groups regarding the PPI scores and the IDP height. This improvement was significant at the three follow-up periods compared with baseline. These results agree with Mansouri et al.,⁽²⁷⁾ who demonstrated that application of HA gel was successful for the reconstruction of the interdental papilla in the maxillary anterior region at six months follow up.

Correlation between the change in height of the black triangle and different clinical parameters For different groups and intervals, there was no significant correlation between the height of the black triangle and different clinical parameters ($p>0.05$).

Similarly, the results of IDP height measured by adobe photoshop cc 2019 showed that (hyaluronic and chitosan gel) offered greater improvement after three weeks compared with HA only.

Our results are similar to the study by Bertl et al., 2017⁽²⁸⁾ which included 13 papillae adjacent to implants in twenty-one patients (twelve women and nine men) who were injected with HA. The manufacturer's recommended technique was used in the study. (Hayden Barrier Gel, Elaf Medical Supplies, Zagreb, Croatia) Thus, 0.36 ml of HA was injected, of

which 5.76 mg were crosslinked HA and 0.72 mg was non-crosslinked HA.

The average papilla defect at the beginning of the study was 2.0 +/- 1.1. At the end of the study (6 months later), the average was 1.9 +/- 0.8. This difference was not statistically significant.

Our results were also similar to Mansouri et al., 2013⁽²⁹⁾ who found a positive effect of HA on papilla reconstruction in 11 patients (three men and eight women) with a total of 21 interdental papillae in the anterior maxillary region. The result of this study was an average change of 5.04 +/- 1.5 mm in the papilla defect.

At the first follow-up, three weeks after the first injection, there was an improvement of 0.17 +/- 0.15. In the second follow-up, three months later, there was an improvement with an average of 1.48 +/- 0.94. In the third follow-up, six months after the injection, the size of the triangle was further improved, with an average size of 2.38 +/- 1.02. The total filling of the interdental papillae at six months was 2.66 mm. In short, the application of HA was successful in reconstructing the interdental papilla at 6 months. In addition, this There were no studies that evaluate the chitosan gel and hyaluronic acid for reducing black triangle, so I could not compare our results with author studies

Conclusion

From the present study, we can conclude that:

*The use of commercially available hyaluronic acid gels can be a promising minimally invasive modality for the treatment of interdental papillary deficiency.

•The reduction of the black triangles after 6 months was not statistically significant between both groups

•For pain level Hyaluronic acid & Chitosan group had a significantly higher mean value

•For patient satisfaction in both intervals, the Hyaluronic acid & Chitosan group had a higher mean value yet the difference between both groups was not statistically significant ($p>0.05$).

Recommendation:

The trial paves the way for a series of future studies to determine the appropriate protocol of injection and to identify the pre-treatment determinants of better outcomes with consideration of

- Larger sample size
- Inclusion of gingival biotype and horizontal distance between roots in the eligibility criteria.
- Trying hyaluronic acid-based gels with higher HA concentrations
- The number of hyaluronic acid injections to be used
- Chitosan need further study to evaluate it is an effect with hyaluronic acid injection in reducing dental black triangle
- The need for longer follow-up periods

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