# Hyaluronic Acid Gel Effectiveness As An Adjunctive Treatment in Patients with Periodontal Disease

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# فعالية جل حمض الهيالورونيك كعلاج إضافي في المرضى الذين يعانون من أمراض ما حول الأسنان

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## Abstract

Background: Both hard and soft periodontal components require hyaluronic acid (HA) for extracellular matrices to function properly. HA is crucial to understanding how inflammation and wound healing work. Different amounts of HA are found in periodontal tissues, including non-mineralized tissues like gingiva and periodontal ligament, and lesser levels in mineralized tissues like cementum and alveolar bone. Preliminary research indicates that HA can control periodontal tissue regeneration and treat periodontal disease. In both deeper periodontal tissues and marginal gingiva, HA helps to relieve symptoms.

Aims: This research aimed to detect the influence of hyaluronic acid 1% gel as an adjunctive therapy on gingival crevicular fluid flow rate in patients with plaque-induced gingivitis.

Materials and methods: Throughout the study, fifty male patients with (PIG) were enrolled (25 per group) with an (18 to 30 years old) median age. They were split into two groups: (G1) scaling and receiving treatment with chlorhexidine, and (G2) scaling and receiving treatment with hyaluronic acid. 0.1 with 0.20 percent gel of chlorhexidine. For a week, patients were directed to use the Hyaluronic acid gel to massage their gingiva twice daily.

Results: At zero time and day seven, GCF was obtained using paper strips (PS) from four sampling tooth locations. The median GCF volume (µl) values at 0 time demonstrated that statistically speaking, between the two groups. The hyaluronic acid gel group, however, displayed significantly reduced GCF volume at day 7 post-treatment. Comparing the Hyaluronic acid gel group to the Chlorhexidine group, Hyaluronic acid gel treatment significantly reduced GCF volume (µl).

Conclusion: Comparing the Hyaluronic acid (G2) group to the Chlorhexidine group, the Hyaluronic acid (G2) treatment was significantly more effective at reducing GCF volume (µl) (G1).


**Keywords: Hyaluronic acid, periodontal disease, treatment, gingival crevicular fluid.**

## المستخلص

الخلفية العلمية: تتطلب كل من مكونات دواعم الأسنان الصلبة واللينة حمض الهيالورونيك (HA) لكي تعمل المصفوفات خارج الخلية بشكل صحيح. HA أمر بالغ الأهمية لفهم كيفية عمل الالتهاب والتئام الجروح. تم العثور على كميات مختلفة من HA في أنسجة اللثة، بما في ذلك الأنسجة غير المعدنية مثل اللثة وأربطة اللثة، ومستويات أقل في الأنسجة المعدنية مثل الملاط والعظم السنخي. تشير الأبحاث الأولية إلى أن HA يمكن أن يتحكم في تجديد أنسجة اللثة وعلاج أمراض اللثة. في كل من أنسجة اللثة العميقة واللثة الحدية، يساعد HA في تخفيف الأعراض.

الأهداف: يهدف هذا البحث إلى الكشف عن تأثير حمض الهيالورونيك بنسبة ٪1 كعلاج مساعد على معدل تدفق السوائل اللثوية في المرضى الذين يعانون من التهاب اللثة الناجم عن البلاك.

المواد و طرائق العمل: تم تسجيل خمسين مريضاً مصاباً بـ (PIG) طوال التقرير (25 لكل مجموعة) بمتوسط عمر (18–30) سنة. تم تقسيمهم إلى مجموعتين، (G1) ازاله تكلسات وعولجت بماده الكلورهيكسيدين و (G2) ازاله تكلسات وعولجت بحمض الهيالورونيك ٪1 والكلورهيكسيدين ديجلوكونات ٪0,20 جل. لمدة أسبوع، تم توجيه المرضى لاستخدام هلام حمض الهيالورونيك لتدليك اللثة مرتين يوميًا.

النتائج: في وقت الصفر واليوم السابع، تم الحصول على GCF باستخدام شرائط ورقية (PS) من أربعة مواقع لأخذ العينات. أظهرت قيم متوسط حجم l) GCF) عند 0 ذلك من الناحية الإحصائية، بين المجموعتين. ومع ذلك، أظهرت مجموعة هلام حمض الهيالورونيك انخفاضًا كبيرًا في حجم GCF في اليوم السابع بعد العلاج. بمقارنة مجموعة هلام حمض الهيالورونيك بمجموعة الكلورهيكسيدين، أدى العلاج بالجيل الهيلارونيك إلى خفض كبير في حجم GCF (ميكرولتر)

الخلاصة: بمقارنة مجموعة حمض الهيالورونيك (G2) بمجموعة الكلورهيكسيدين، كان العلاج بحمض الهيالورونيك (G2) أكثر فاعلية بشكل ملحوظ في تقليل حجم G1) (µl) GCF).


**الكلمات المفتاحية: حمض الهيالورونيك، أمراض اللثة، العلاج، السائل اللثوي المفصلي**

## Introduction

One of the most common glycosaminoglycans in the extracellular matrix is hyaluronic acid (HA), also known as hyaluronan(Amorim *et al*., 2021). Inflammatory illnesses with a long course are treated with HA. An important distinction is that periodontal disease affects the periodontium and is an inflammatory condition. Very little HA is present in the mineralized periodontal tissues of the cementum and alveolar bone, but the extracellular matrix of the gingiva and the periodontal ligament are is essential for its formation(Aydinyurt *et al*., 2020). Following HA therapy, wound healing quickens because HA receptors are affected, which affects cellular migration, angiogenesis, and inflammation. In the deeper periodontal tissues as well as the marginal gingiva, HA reduces symptoms(Abatangelo *et al*., 2020). Additionally, several periodontal treatments that involve non-surgical and surgical therapy, as well as soft and hard tissue regeneration, have made advantage of this wound-healing property of HA. Additionally, HA controls the cell-matrix and cell-cell exchanges and is essential for cell signaling, hemostasis, and these processes. The influx and outflow of nutrients and waste materials are also influenced by HA(Vigetti *et al*.,2014). The body uses HA for several functions by utilizing its physical, chemical, and biological properties. These biological activities range from fundamental structural responsibilities in the extracellular matrix to control over tissue macro- and microenvironments, effects on cell behaviour, and developmental regulation. Additionally, many HA activities have direct, receptor-mediated impacts on gene expression(Abatangelo *et al*., 2020). A reversible inflammatory condition called gingivitis can develop into periodontitis, which results in the loss of the tooth's supporting bone and soft tissue(Shrivastava *et al*., 2021).

Gingival crevicular fluid (GCF) is an inflammatory exudate that includes elements from a vascular, such as serum, connective tissue, and the epithelium through which it passes on its approach to the gingival crevice, as well as inflammatory cells and bacteria found in the tissues and crevice (Lamster & Ahlo, 2007). The GCF study is a non-invasive way to assess the pathophysiological state of the periodontium in a specific location. Wang and his colleagues (2016) highlighted the importance of GCF flow as an evaluation tool, stating that it is proportional to the degree of inflammation(Wang *et al*., 2016).

Aerobes and anaerobes of both gram-positive and gram-negative bacteria, as well as fungi-like yeasts, can be eliminated by chlorhexidine digluconate (CHX). It was initially proven to be effective in preventing plaque and gingivitis in people without practicing good dental care more than 40 years ago(Puig & Montiel, 2008). The use of chlorhexidine in several formulations for a range of oral conditions as well as its usage as an effective antibacterial agent and plaque management agent. Typically, chlorhexidine is administered as a gel or mouthwash. Chlorhexidine can be used as a paste at home or in trays in a dental office when it is in gel form. Use may occur up to three times daily for two days or once daily for ten to fourteen days. (Palwankar *et al.*, 2021).

This study aimed to detect the influence of Hyaluronic acid 1% with Chlorhexidine digluconate 0,20% gel as an adjunctive therapy on gingival crevicular fluid flow rate in patients with plaque-induced gingivitis.

## Materials and Methods

This research was done at a privet clinic, in Baghdad, Al-Karkh. Throughout the trial, 50 male patients with gingivitis, (25/group), were enrolled. Group 1 (G1) scaling was applied using chlorhexidine gel, while Group 2 (G2) scaling was applied using hyaluronic acid 1% with Chlorhexidine digluconate 0,20% gel.

### Inclusion criteria:

- People who have mild to severe plaque-induced gingivitis.
- Ages between 18 and 30 years old
- The patients showed no signs of an actual loss of attachment.
- Willingness to provide permission.

### Exclusion criteria:

- Recent use of antibiotics (within the last 3–4 weeks).
- Mouthwash usage and dental care background
- Patients who have known systemic diseases.
- 4-Smokers.

## Collection of Gingival Crevicular Fluid (GCF) samples:

Four paper strips were inserted in a 1.5 mL Eppendorf tube and weighted (weight, W1) on an electronic scale before sample collection (PerioPaper Strips; Oraflow Inc., New York, USA). The primary GCF's rest calculation used the weight of the four (PS) as a reference weight. At four distinct locations on the anterior teeth, the GCF was repeatedly collected. After that, the (PS) were put into an Eppendorf tube and weighed right away.

## Samples collection:

Samples were taken from 50 patients in the morning, 2–3 hours after breakfast. All patients consented to ultrasonic scaling, and they were urged to practice proper dental hygiene. Chlorhexidine (group 1) and Hyaluronic acid 1% with Chlorhexidine digluconate 0,20% gel was administered to patients twice daily (morning and night) for one week Fig (1).


**Figure (1): Application of the Hyaluronic acid 1% with Chlorhexidine digluconate 0,20% gel**

Additionally, patients were instructed to wait 30 minutes following gel application before eating, drinking, or washing. Using paper strips, GCF samples were taken from each patient's four sampling tooth locations at baseline (zero-time) and day 7 (PS). Pre-secreted saliva was removed by washing the mouth cavity with water. The four incisors' four sites were then cleaned of any remaining

salivary contamination by gently airdrying the tooth surface for 10 seconds while utilizing cotton rolls to do so. After two minutes, the freshly produced crevice fluid was collected using a 2 6-mm (PS), 0.22 m pore size filter. Each (PS) was slowly introduced until there was no resistance, at which point it was left in place for 30 seconds. The four (PS) were removed after 30 seconds and moved to an Eppendorf tube, where they were immediately weighed and sealed.

## Extraction of the GCF (elution and centrifugal procedure from PS):

300 µl of phosphate-buffered saline was used to incubate the four (PS) in each Eppendorf tube. Overnight at 4°C, samples were eluted (Refrigerator). At 400 g (2000 RPM) for 4 min, samples were centrifuged. Before being examined, the supernatants were kept frozen at -80°C (Schwendicke, 2017).

## Determination of the GCF volume:

To calculate GCF volumes, all sites from each patient were combined. By using differential weighing, the main weight of GCF absorbed on the four PS was calculated using the formula: W2-W1 = MGCF (Emilson, 1977). M stands for Main GCF Weight (g), W2 for GCF Weight (after GCF collection), and W1 for GCF Weight (before GCF collection). The mean value per patient was then calculated by dividing the value by four. Using the formula Volume = Mass/Density, the acquired value, represented in g, was converted to volume (L) (Griffiths, 2003).

## Data processing and statistical analysis

The statistical software SPSS ver. 11.5 (SPSS Inc., Chicago, USA) and the computer program by Walonick, 2010, Stat Pac Inc., were used to analyse the data. Non-parametric data analysis.(Walonick, 2010)

## Results

In G1 and G2, the effects of the gels on gingival crevicular fluid volume (l) at 0 time (baseline measurement) and day 7 were noted. The median values of the (GCF) for each group (G1 and G2) measured at 0 time are (0.54 and 0.56), respectively, according to the data in Table 1. H-statistics = 1.0688 and P-value = 0.5896 when comparing the GCF median values between the two groups. On the other hand, the results at day 7 post-treatments (Table2) revealed that there were statistically significant (p0.05) differences between the two groups (0.44 and 0.36 in G1 and G2, respectively). (H-statistics: 12.8279; P-value: 0.002) Additionally, the results (Table 3) demonstrated that the median values in G1 and G2 were (0.54) died and (0.36) respectively from the pre-treatment (0-time) values of (0.56) and (0.44), respectively. In G1, this reduction had a W-value of 46.5 and a critical value of 48, however in G2, the W-value was 35.5 and the critical value was 67, making this reduction statistically significant (P 0.05).

**Table 1: Gingival crevicular fluid (GCF) volume measurements at 0 time in G1 and G2 are compared.**

| Groups (25/group) | Median | GCF(µl) at 0-time | | |
|---|---|---|---|---|
| | | Kruskal-Willis Test | | |
| | | *H-statistics* | P-value | Result at P<0.05 |
| G1 | 0.54 | 1.0688 | 0.5896 | NS |
| G2 | 0.56 | | | |

**G1 = CHX group, G2 = Hyaluronic acid 1% with Chlorhexidine digluconate 0,20% gel group. NS = non-significant (P > 0.05).**

**Table 2: Comparing the volume of gingival crevicular fluid (GCF) at day 7 as measured in G1 and G2**

| Groups (25/group) | Median | GCF(µl) at day 7 | | |
|---|---|---|---|---|
| | | Kruskal-Willis Test | | |
| | | *H-statistics* | P-value | Result at PÂ0.05 |
| G1 | 0.44 | 12.8279 | 0.002 | S |
| G2 | 0.36 | | | |

**G1 = CHX group; G2 = Hyaluronic acid 1% with Chlorhexidine digluconate 0,20% gel group; S = significant (P > 0.05).**

**Table 3: Effect of various treatments (pre and after) on gingival crevicular fluid (GCF, l) measurements taken at 0 time and day 7 in G1 and G2.**

| Groups | Medi | An | Wilcoxon Signed-Rank Test | | P≤0.05 |
|---|---|---|---|---|---|
| | Before (0-time) | After (day -7) | W-value* | The critical value of W | |
| G1 | 0.56 | 0.44 | 46.5 | 48 | S |
| G2 | 0.54 | 0.36 | 35.5 | 67 | S |

**G1 = CHX group, G2 = Hyaluronic acid 1% with Chlorhexidine digluconate 0,20% group, S = significant (P ≤ 0.05).**

## Discussion

GCF is a biological exudate, and analysing its components is a novel method for specifically identifying biomarkers with the necessary specificity. Both periodontology and orthodontics can benefit greatly from the examination of GCF as a diagnostic tool (Eden, 2017). Several researchers have hypothesized that gingivitis and periodontitis cause a rise in GCF levels (Huth *et al*, 2006). The findings of this investigation supported those of Gupta and Mansi (2012), who showed that when gingiva is extensively inflamed, both clinically and historically (Gupta & Mansi, 2012), GCF production increases significantly (Tables 1 & 2)(Albrecht *et al.*, 2011)"type":"article-journal","volume":"6"},"uris":["http://www.mendeley.com/documents/?uuid=1ca18a45-1cc1-4e9b-bda9-a071436672a1"]}], "mendeley": {"formattedCitation":

"(Albrecht et al., 2011. Griffiths (2003) concluded that a low GCF flow suggests healthy tissue, whereas a high GCF flow denotes inflammatory tissues. Perhaps the most preferred sample method for GCF is filter paper strip selection, and this technique can be used to determine the concentration of different cytokines and other biomarkers in GCF (Gamonal, 2001). According to a study done by researchers' Hyaluronic acid gel has been demonstrated to have a similar effect to chlorhexidine in treating plaque-induced gingivitis in patients using fixed orthodontic appliances. It is advised that this gel be used as the initial treatment for plaque-induced gingivitis. (Albrecht *et al*., 2011) "type": "article-journal", "volume": "6"},"uris": ["http://www.mendeley.com/documents/?uuid=1ca18a45-1cc1-4e9b-bda9-a071436672a1"]}], "mendeley":{"formattedCitation":"(Albrecht et al., 2011. Also in another study that evaluates the therapeutic efficacy of 0.2% hyaluronic acid, they found that in addition to scale, a gel containing 0.2% hyaluronic acid is more effective than scaling alone for treating plaque-induced gingivitis(Jain, 2013).

## Conclusion

When compared to the Chlorhexidine group, treatment for 7 days with Hyaluronic acid 1% with Chlorhexidine digluconate 0,20%gel (G2) was significantly more effective at reducing GCF volume (l) (G1). The use of gingival crevicular fluid in the diagnosis and treatment of plaque-induced gingivitis can be crucial.

## Conflict of interest: None.

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