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Original article

Evaluation of Tumor Necrosis Factor α (TNF-α) in Gingival Crevicular Fluid and Serum Before and After Phase I Therapy with Subgingival delivery of Hyaluronic Acid in Chronic Periodontitis Patients

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Abstract
The aim of this study was to estimate the levels of TNF-a in serum and gingival crevicular fluid, in patients with chronic periodontitis (CP), before and after phase I therapy with subgingival delivery of Hyaluronic acid (Gengigel Gel). A total of thirty subjects were included in this study; 20 CP patients as a study group (group I) and 10 systemically healthy subjects with clinically healthy gingiva as a control group (group II). The nonsurgical periodontal therapy (SRP) + HA gel were done in group I. Periodontal parameters, including PI, GI, BOP, PD and the CAL, were recorded. GCF and serum were collected from all individuals included in the study; the first sample was collected from all groups (study and control) before treatment. The second sample was collected 4 weeks after subgingival delivery of Hyaluronic acid & phase I therapy from group I. Levels of TNF-a in GCF and serum were quantified using ELISA. The results showed a statistically significant reduction in total levels of TNF-a in serum and TNF-a in GCF in study group after application of Hyaluronic acid with phase I therapy. Results also showed all clinical parameters were significantly improved after application of Hyaluronic acid with phase I therapy in groups I ($p<0.001$). Scaling and root planing (SRP) is the mainstay of treatment of periodontal diseases, furthermore subgingival application of Hyaluronic acid gel with SRP were effective in improving clinical parameters in CP patients. TNF-a level in serum and GCF are reduced after phase I therapy with application of Hyaluronic acid.
***Keywords**: Tumor Necrosis Factor, Gingival Crevicular Fluid, Hyaluronic Acid, Periodontitis.*

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Introduction

Chronic periodontitis (CP) is recently defined as an infectious disease caused by inflammatory processes in supportive tooth structures that will lead to tissue disjunction and destruction of the alveolar bone and tooth loss [1,2]. It is the most prevalent form of periodontitis, which affects a majority of the adult population. Pathology of these inflammatory lesions have been attributed not only to bacterial products, but also to chemical mediators released by the host cells, as a result of inflammatory and immune reactions [3]. TNF-α "major inflammatory cytokines", mainly produced at the sites of inflammation by infiltrating monocytes and/or macrophages. Among the hallmarks of TNF-α is the extremely pleiotropic nature of its action, which could be ascribed to the presence of TNF receptors virtually on all cells. TNF-α once produced and secreted, will bind to TNF receptor present in all plasma membrane of most of the cells throughout the body [4].
TNF-α is pleiotropic cytokine that can improve the host defense mechanism by mediating inflammation and increasing immune cell function, at the same time, it can also induce disease through TNF-α toxicity by causing tissue injury, catabolic illness and mediating shock [5]. TNF-α play a great role in PD where it induces collagenase production by fibroblasts, resorption of cartilage and bone, and it effects in destruction of periodontal tissues during PD [6]. TNF-α prompts synthesis of IL1 and PGE2, also triggers osteoclasts and thus prompts bone resorption [7]. Hyaluronic acid exhibit antibacterial, antifungal, and anti-inflammatory effects, furthermore HA has angiogenesis and osteoinductive properties that enhance wound healing and limits the damage that can occur during the inflammatory process in a variety of tissues of the human body, including the periodontal tissues [8].

Methods

20 CP patients received thorough scaling and root planing (SRP) with subgingival application of Hyaluronic acid (Gengigel) as basic therapeutic modality (study group) and another 10 subjects with healthy

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periodontium were enrolled in the study to act as a control group. The selected groups were free from any systemic disease, and with normal Body-Mass-Index. Furthermore, none of them had previous periodontal treatment, including scaling, root planing, or periodontal surgery in the last 6 months. Smokers, Pregnancy and lactating mothers were also excluded from the present study**.** Periodontal parameters, including plaque index, gingival index, bleeding on probing, probing depth and the clinical attachment level were recorded. GCF and serum were collected from all individuals included in the study; the first samples were collected from all groups (study and control) before SRP + HA gel. The second sample was collected 4 weeks after phase I therapy + HA gel from group I. Levels of TNF-α in GCF and serum were quantified using ELISA.

Results

Our results were obtained from a study carried on 20 patients with CP, and 10 healthy individuals as controls. The age of patients ranged from 35-65 years with mean age 45.4 ± 9.3 years. Ten of them were males and ten were females. The mean age of the subjects in control group were 43.6 ± 10.47 years. Five of them were males and five were females. There were no significant differences between groups regarding age and sex (Table 1).

Table 1. Demographic data of study and control groups

Demographic data	**Study Group**		**Control Group**		**P**
Age	45.4	9.3	43.6	10.47	0.6
Sex n(%) Male Female	10 10	50 % 50 %	5 5	50 % 50%	1.00

Table 2 summerized the significant difference of clinical periodontal parameters in study and control groups. It was observed that, the clinical parameters of study group were improved after application of Gengigel with phase I therapy, in the form of reduced values of PD, CAL, PI, GI and BI. However, the noticed decrease in clinical parameters readings did not reach that of control group.

Table 2. Clinical periodontal parameters of a study group (before and after SRP with Gengigel) compared to control group

	Study group				**P1**	**Control group**		**P2**	**P3**
	Baseline		**After phase I therapy + HA gel**			**Baseline**			
	Mean	±SD	Mean	±SD		**Mean**	**±SD**		
PD	2.678	.455	1.33	.48	<0.001**	.93	.10	<0.001**	0.015*
CAL	3.196	.259	2.575	.296	<0.001**	0	0	<0.001**	<0.001**
PI	1.25	.21	.35	.16	<0.001**	.21	.09	<0.001**	0.018*
GI	1.77	.49	.31	.17	<0.001**	.19	.08	<0.001**	0.04*
BI	.99	.02	.27	.11	<0.001**	.26	.06	<0.001**	0.67

Table 3 showed statistically high significant difference between the serum mean values of TNF-α concentration before vs after application of Gengigel with phase I therapy (5.06±0.9 Pg/ml vs 3.63±0.86 Pg/ml), among the study group (P1<0.001). In addition, after four weeks of SRP+HA gel, there was significant difference between the mean values of TNF-α in serum in study group after treatment and control groups (P3<0.05).

Table 3. TNF-a levels in serum (Pg/ml) in the study group (before and after SRP with Gengigel) compared to control group

Serum TNF-α	**Study group**				**P1**	**Control group**		**P2**	**P3**
	Baseline		After phase I therapy + HA gel			Baseline			
	Mean	±SD	Mean	±SD		Mean	±SD		
	5.06	.90	3.63	.86	<0.001**	2.62	.63	<0.001**	0.003*

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The mean values of TNF-α in GCF decreased, from (4.10±1.08 Pg/30 s) at base line, to (2.87±1.02 Pg/30 s) after 4 weeks of phase I therapy with Gengigel application. This reduction in TNF-α mean values was found to be statistically high significant at (P1 < 0. 001). As previously, the mean values, of TNF-α levels in GCF of study group decreased after treatment, but still significant to that of control group (P3<0.05).

Table 4. TNF-a levels in GCF (Pg/30sec) in study group (before and after SRP with Gengigel) compared to control group

GCF TNF-α	Study group				P1	Control group		P2	P3
	Baseline		After phase I therapy + HA gel			Baseline			
	Mean	±SD	Mean	±SD		Mean	±SD		
	4.10	1.08	2.87	1.02	<0.001**	1.99	.81	<0.001**	0.02*

Discussion

The present study focused on the assessment of altered levels of TNF-α in GCF and serum among CP patients, and to gauge the effectiveness of non-surgical periodontal therapy (NSPT) with sub gingival delivery of Gengigel in adjusting the TNF-α concentration towards health along with the improvement in the periodontal parameters.

To avoid biasing the estimation of TNF-α concentration, factors that may modify TNF-α levels such as obese subjects, smokers, pregnant and lactating mothers were excluded. The clinical finding of the present study reflected significant reduction in the periodontal parameters (PI, GI, BOP, PD, and CAL index) after using Gengigel with phase I therapy (Table 2). This may be related to the fact that non-surgical periodontal therapy with Gengigel is effective in reducing the bacterial load leading to improvement of clinical parameters and oral health.

Surface root debridement is the most effective approach for treating cases of periodontitis [9], which involves removing the biofilm and other bacterial products on the surfaces of the involved roots to minimize the inflammation of gingival tissue and the depth of the periodontal pockets [10]. However, Gontiya and Galgali reported that scaling and root planing (SRP) was technically demanding and was not always efficient in eliminating all periodontal pathogens and in reducing the level of inflammation in periodontitis. .[11] This is due to the presence of pathogens within the gingival tissue and in areas anatomically inaccessible to mechanical instrumentation. Therefore, several studies applied systemic and local antimicrobial therapy as an adjunct to mechanical surface root debridement to improve clinical outcomes [12,13].

The antibacterial and anti-inflammatory activities of hyaluronic acid led to its introduction as a local chemotherapeutic agent with several clinical therapeutic properties for the treatment of periodontitis. .[11]

The mean values of TNF-α in the serum of CP group, at baseline, was significantly higher than the control group (5.06±0.9 Pg/ml > 2.62±0.63 Pg/ml), respectively (Table 3). Similarly, TNF-α in GCF of CP group, at baseline, was significantly higher than the control group (4.10±1.08 Pg/30s > 1.99±0.81 Pg/30s), respectively (Table 4). This indicates that TNF-α in serum and GCF increase as periodontal disease progress. These findings were consistent with *H. Passoja et al.* [14] *Noh et al* [15] and *Monea et al,* [16] who reported that study group at baseline presented higher TNF-α levels in serum, and with *K Gokul et al,* [17] and *Heralgi et al* [18] who reported that study group at baseline presented higher TNF-α levels in GCF.

It was not surprising that small amount of TNF-α could also be detected in healthy gingiva, as the presence of a low number of macrophages and neutrophils normal gingival tissues could be responsible for the presence of TNF-α in GCF in control group. The increased levels of TNF-α, in CP, could be attributed to the stimulatory action of the bacterial *LPS* on resident immune cells, provoking much broader systemic inflammatory response, including release of TNF-α [19]. Consequently, after 4 weeks of subgingival delivery of Gengi gel with phase I therapy in study group regarding the level of TNF-α in serum and GCF, significant reduction was found (Table 3,4). Concomitantly, these findings were consistent with the results obtained by *Yun et al*, [20] and *Erciyas et al.* [21] who reported a significant improvement in circulating levels of TNF-α, after non-surgical periodontal therapy, and with *CA Mohammad et al,* [22] who reported that HA gel has a positive effect on improvements of inflammatory mediators including TNF-α, Therefore, HA gel can be used as an adjuvant to SRD in the treatment of periodontitis. Finally, regarding TNF-α in CP, there were only significant correlations between serum TNF-α with GI four weeks after SRP+HA gel. This may be due to the direct action of TNF-α to increase vascular endothelial cell permeability which cause redness in periodontal disease. TNF-α also induce fibroblasts apoptosis and stimulates collagenase production, which in turn breaks down periodontal CT. Nevertheless, there are no significant correlations between TNF-α in serum & GCF and other clinical parameters. Our observation is in line with *Ozgoren et al,* [23] who found no

significant correlation between TNF-α and all clinical parameters (PI, GI, PD, GBTI, and CAL). Contrarily, *Zahraa et al* [24], revealed significant positive association of TNF-α with each of gingival index, probing pocket depth and clinical attachment level. Although TNF-α was associated to the degree of inflammation, they were not correlated to the changes in the clinical parameters. This may be due to differences in disease stage between patients, discrepancies of periodontal pocket activities and differences in sample collection methods.

Conclusion

Scaling and root planing (SRP) is the mainstay of treatment of periodontal diseases; Sub gingival delivery of HA gel has a positive outcome on clinical periodontal parameters and improvements in inflammatory mediators, where levels of TNF-α were reduced in serum and GCF after using of Gengigel in adjunct to SRP.

Conflict of interest. Nil

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المستخلص

الغرض من هذه الدراسة هو تقييم معدل عامل نخر الورم الفا في المصل والسائل اللثوي قبل وبعد العلاج الغير جراحي مع استخدام حامض الهيالورونيك كدواء موضعي في الجيوب اللثوية لمرضي التهاب الأنسجة الداعمة المزمن. اشتملت هذه الدراسة على ثلاثون شخصا تم تقسيمهم الي مجموعتين: المجموعة الاولى احتوت على 20 مريض يعانون من التهاب اللأنسجة الداعمة للأسنان كمجموعة اختبار, والمجموعة الثانية احتوت على 10 اشخاص أصحاء لايعانون من أمراض اللثة كمجموعة ضابطة. وقد روعي عند اختيار الحالات ان يكونوا ضمن المعدل الطبيعي للوزن كما تمت مراعاة خلوهم من الامراض الجهازية و تم استبعاد المدخنين و الحوامل. تم تقيم كلا من عمق الجيوب اللثوية و معدل فقدان التلاصق بين اللثة والاسنان, و معامل اللثة ومعامل تراكم البلاك ومعامل الادماء اللثوي قبل البدء في العلاج وبعد الانتهاء منه. وتم جمع السائل اللثوي و المصل من جميع الأفراد الواردة في الدراسة. وقد تم جمع العينة الأولى من المجموعتين (دراسة و ضابطة) قبل كحت وازالة الرواسب الجيرية وتنعيم اسطح الجذور ,واستخدام حامض الهيالورونيك في الجيوب اللثوية. العينة الثانية جمعت بعد 4 أسابيع من كحت وازالة الرواسب الجيرية، وتنعيم اسطح الجذور وتطبيق حامض الهيالورونيك في الجيوب اللثوية للمجموعة الاولي فقط. يتم قياس تركيز عامل نخر الورم الفا في السائل اللثوي و المصل كميا باستخدام ELISA كما تم اجراء التحليلات البيانية باستخدام البرنامج الاحصائي SPSS. واظهرت نتائج الدراسة الحالية أن هناك تحسن سريري وفروق ذات دلالة إحصائية فى قياس جميع المظاهر السريرية بعد العلاج اللثوي لمجموعةالاختبار باستخدام حامض الهيالورونيك في الجيوب اللثوية. وكدلك اظهرت الدراسة انخفاض في معدلات عامل نخر الورم الفا في المصل و السائل اللثوي بعد العلاج الغير جراحي و استخدام حامض الهيالورونيك في الجيوب اللثوية في مجموعة الاختبار. تدعم هذه الدراسة ان استخدام الادوية الموضعية بالاضافة الي كحت وازالة الرواسب الجيرية, وتنعيم أسطح الجذور لمجموعة الاختبارهي الطريقة الفعالة لعلاج الامراض اللثوية وقد حققت تحسنات سريرية وفروق ذات دلالة احصائية في قياس جميع المظاهر السريرية. تشير الدراسة أيضا إلى أن تقييم مستويات عامل نخر الورم الفا في مصل الدم و السائل اللثوي قد يكون مفيد لمراقبة نشاط الالتهابات اللثوية والاستجابة للعلاجات الغير الجراحية بمساعدة الادوية الموضعية كما ينصح باجراء المزيد من الدراسات علي عدد اكبر من الحالات واستخدام طرق علاجية مختلفة.