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Periodontal effect of 8% Hyaluronan as an Adjunct to Scaling and Root Planning in the Treatment of Chronic Periodontitis(Comparative Study).

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Abstract:
Background:*Hyaluronic acid showed the anti-inflammatory, anti- edematous, anti-oxidant, and bacteriostatic effect on periodontal disease; the potential benefits of the periodontal effect of a single dose of hyaluronic acid on the clinical parameters were evaluated in this study.*
Methods: *Thirty patients with chronic periodontitis were recruited to participate in this study. They were divided into three groups; the first group (10 patients) was treated with SRP and 8% hyaluronic acid gel was additionally applied, the second group (10 patients) was treated with SRP, and the third group (10 patients) was treated with 8% hyaluronic acid gel only. The clinical parameters which include Plaque index (PLI),Gingival index (GI), Bleeding on probing(BOP) and Relative Attachment level wereassessed at base line , one week and four weeks post treatment. Hyaluronic gel was administered subgingivally in the test sites at baseline*
Results:*The main results of the three treated groups was summarized as follows: 1. A significant reduction in bleeding on probing and RAL was observed in all groups after four weeks (P <0.05), 2. Significantly lower bleeding on probing were observed in the hyaluronan groups, group 1and 3 compared to group 2 after 4 weeks (P <0.05), 3. RAL reductions between the groups were statistically significant.*
Conclusion:*The local applications of hyaluronan gel (0.8%) in conjunction with scaling and root planning or used it only have a beneficial effect on clinical periodontal parameter in patients with chronic periodontitis.*
Key word:*Hyaluronic acid; root planning; periodontitis*

I. Introduction

Periodontitis is defined as "an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both[1]. Chronic periodontitis is generally a slowly progressing of periodontal disease that at any stage may undergo an exacerbation resulting in additional loss of attachment apparatus[2]. The severity of periodontal diseases varies over time, depending on the quantity and quality of the biofilm and the presence of factors modifying the permanence of plaque[3].

Chronic periodontitis may affect one or several periodontal sites within the mouth, leading to different levels of tissue destruction[3].

Poor oral hygiene is an important risk factor in highly susceptible individuals and is of less importance in individuals with strong host resistance[4]. Periodontal diseases are caused by local inflammatory irritation caused by overgrowth and differentiation of dental plaque bacteria[5].

When examining a patient who has plaque and calculus deposits on several teeth, the dentist must decide whether prophylaxis, root planning, or a combination of the two is the appropriate first step in therapy.

Root planning is defined as the removal of calculus, most plaque, essentially all cementum, and some dentin from the roots of teeth while they are planned to clinical smoothness.Root planning, however, is one of the most difficult and demanding procedures in dentistry. In planning treatment, the dentist has an obligation to differentiate fairly and correctly between the procedures to charge the patient or a third party properly for the work done. Whenever loss of attachment has occurred and no root restorations are present, root planning must be performed.

The beneficial effects of scaling and root planning are based on a reduced mass of bacteria in the periodontal pockets (6) and a shift towards a less pathogenic micro flora.

Occasionally, a subset of bacterial species is either introduced, overgrow or exhibit new properties that lead to the destruction of the periodontium. In either instance, microbial species continue to colonize above and below the gingival margin, hopefully in a new and "peaceful" equilibrium[2].

Hyaluronic acid (HA) is a natural occurring linear polysaccharide of the extracellular matrix of connective tissue, synovial fluid, and other tissues. HA structure consists of polyanionic disaccharide

unitsglucuronic acid and N-acetyl-glucosamine connected by alternating β 1-3 and β1-4 bonds[7]. There is no anti-genic specificity for species or tissues; and thus, these agents have a low potential for allergic or immunogenic reaction[6]. HA have specific physical and biochemical properties in normal tissue that make them ideal structural compounds[2]. HA is the ground substance of the synovial fluid, as well as the skin, different organs and tissues[8, 9].

The biological functions of HA include maintenance of the elastoviscosity of liquid connective tissues such as joint synovial and eye vitreous fluid, control of tissue hydration and water transport, supramolecular assembly of proteoglycans in the extracellular matrix, and numerous receptor-me-diated roles in cell detachment, mitosis, migration, tumor development and metastasis, and inflammation[10]. Its function in the body is, amongst other things, to bind water and to lubricate movable parts of the body, such as joints and muscles.

Its consistency and tissue-friendliness allows it to be used in skin-care products as an excellent moisturizer. Healing of periodontal wound includes a series of highly reproducible and rigidly controlled biologic events (inflammation, granulation tissue formation, epithelium formation and tissue remodeling) which begin with chemo attraction of cells that accumulate and debride the injured tissue, foreign material, and microbial cells. These events end wither formation and maturation of new extracellular matrix that restore resistance of tissue to functional stress[11].

The high molecular weight hyaluronan present in the periodontal tissues is synthesized by hyaluronan synthase (HAS) enzymes (HAS1, HAS2 and HAS3) in various cells from the periodontal tissues, including fibroblasts and keratinocytes in gingiva and periodontal ligament, cement oblasts in cementum and osteoblasts in alveolar bone[12].

Hyaluronan may also indirectly act to moderate inflammation and stabilize the granulation tissue by preventing degradation of the extracellular matrix proteins by serine proteinases derived from inflammatory cells as healing progresses[13]. As a part of non-surgical therapy, local drug therapy is a common mode of therapy .

Therefore, a positive influence on the sub gingival biofilm may be accomplished with a local delivery of the antimicrobial drug.In dental practice, the prescription of anti-inflammatory treatment is generally limited to mouth washes, which contain no high-risk active constituents. But these preparations do not guarantee lengthy contact between the active constituent and the site of inflammation. If viscous preparations (gels, pastes etc.), which adhere to the gingiva and therefore guarantee that the active constituent will perform its effect in situ for a lengthy period, are used instead of fluid mouthwash solutions, the amount ingested is likely to be greater[14].

To fulfill the above criteria, 0.8% hyaluronic acid gel (HA), a new commercially available gel that has an anti-inflammatory effect is used for topical application in periodontal therapy. The endogenous hyaluronic (HA) is a high-molecular weight (10,000-10,000,000 Da), non-sulfated polysaccharide component of the glycosaminoglycan family, which is present in the extracellular matrices of many tissues such as skin, synovial joints and periodontal tissues. Hyaluronic has been identified in all periodontal tissues, being particularly prominent in the non-mineralized tissues such as gingival and periodontal ligament. It is present in only low quantities in mineralized tissues such as cementum and alveolar bone[15]. Role of hyaluronic acid in the treatment of periodontitis HA is an essential component of periodontal ligament matrix and plays various important roles in cell adhesion, migration and differentiation mediated by various HA binding proteins and cell-surface receptors such as CD44[16, 17, 18].

During recent years, the effects of combining scaling and root planning with non-mechanical therapy HA plays an important role in post inflammatory tissue regeneration, facilitating cell migration and differentiation during tissue formation and repair. Local application of exogenous hyaluronic was found to produce beneficial wound healing outcomes in experimental animals[19, 20, 21]. It has been reported that HA has osteon inductive properties as well. Recombinant HA exerted varied bacteriostatic effects on all the bacterial strains tested, depending on its molecular Weight and concentration .The high concentrations of weight HA had the greatest bacteriostatic effect, so we used 0.8% HA in periodontal pocket

The present investigation is to determine the effect HA (0.8%) on clinical variables, and sub gingival periodontopathogenic by adding 0.8% HA gel as adjunct with SRP in the treatment of chronic periodontitis.

II. Material and Method

Thirty patients (aged 35 to 55 years) were recruited to participate in this design study. All patients with chronic periodontitis with ≥ 5 sites of PD ≥ 5mm and a minimum of 20 teeth were included in the study.

The patients were nonsmokers, systemically healthy and exhibited no known allergies. Furthermore, they did not receive antibiotic therapy within the last 4 months or receive previous periodontal treatment.
The patients were divided into three groups according to the treatment that they received as following

- Group 1 (G1): ten patients were treated with 8% Hyaluronan as an adjunct to scaling and root planning by applying of 0.5 ml of HA to the base of the pocket.
- Group 2 (G2): ten patients were treated with SRP only, and
- Group 3 (G3): ten patients were treated with 8% hyaluronic acid gel by applying of 0.5 ml of HA to the base of the pocket only .

Four clinical periodontal parameters is included, these indices are plaque index (PLI) (Silness and Loe 1964)[22,23], Gingival index (GI)(Loe and Silness 1964)[22,23], bleeding on probing (BOP) (Carranza et al., 2008)[24]and relative attachment level (RAL) were recorded before periodontal treatment at base line data and at one and four week post-treatment.

Scaling and RP treatment consisted of sub gingival curettage of periodontal pockets with ultrasonic scalar and curettes.

Oral hygiene instructions were given, compromising instruction of brushing and the use of appropriate interdental cleaning aids. Plaque control was reinforced and the patients were encouraged to use their routine oral hygiene habits.

III. Result

The mean PLI was reduced from 1.08 ± 0.16 in the 1st to 0.56 ± 0.24 in the 3rd visited in the group 1, while for group 2, the mean in the1st visit was 0.77 ± 0.08 and 3rd visit was 0.58 ±0.06 but the HA only 1st visit was 0.75 ± 0.08 and 3rd visit was 0.53 ± 0.05. There was decreased in the mean of GI to reach 0.52 ± 0.18, 0.92 ± 0.04 and 0.55 ±0.08 in the 3rd visit for G1, G2 and G3 respectively.

Also the result showed that there was decreased in the mean RAL and mean percentage of BOP for all groups for all time intervals as shown in table (1), table (2), and table (3).

Table 1 Descriptive analysis of clinical parameters of Group 1 (HARP)

Variables	Visits	Mean	S.D.	Min.	Max.
PLI	1st visit	1.08	0.16	0.81	1.44
	2nd visit	0.64	0.23	0.36	1.12
	3rd visit	0.56	0.24	0.27	0.92
GI	1st visit	1.02	0.21	0.75	1.43
	2nd visit	0.53	0.33	0.15	1.15
	3rd visit	0.52	0.18	0.22	0.71
BOP	1st visit	76.38	7.60	67	88.60
	2nd visit	22.54	6.16	18.40	37.20
	3rd visit	21.48	5.59	17.30	34.30
RAL	1st visit	5.69	0.32	5	6
	2nd visit	5.02	0.33	4.5	5.5
	3rd visit	4.31	0.21	4	4.5

Table 2 Descriptive analysis of clinical parameters of Group 2 (RP)

Variables	Visits	Mean	S.D.	Min.	Max.
PLI	1st visit	0.77	0.08	0.71	0.95
	2nd visit	0.65	0.09	0.56	0.84
	3rd visit	0.58	0.06	0.5	0.65
GI	1st visit	0.81	0.10	0.66	0.97
	2nd visit	0.67	0.09	0.61	0.88
	3rd visit	0.59	0.04	0.53	0.67
BOP	1st visit	74.95	7.42	62.5	82.9
	2nd visit	30.47	6.24	22.1	37.5
	3rd visit	24.79	4.76	15.5	30.2
RAL	1st visit	6.12	0.43	5.6	7
	2nd visit	5.42	0.33	5	6
	3rd visit	5.10	0.21	5	5.5

Table 3 Descriptive analysis of clinical parameters of Group 3 (HA) only

Variables	Visits	Mean	S.D.	Min.	Max.
PLI	1st visit	0.75	0.08	0.61	0.85
	2nd visit	0.61	0.07	0.5	0.69
	3rd visit	0.53	0.05	0.44	0.6
GI	1st visit	0.88	0.04	0.84	0.92
	2nd visit	0.63	0.09	0.5	0.76
	3rd visit	0.55	0.08	0.41	0.69
BOP	1st visit	75.36	5.75	65.9	84
	2nd visit	23.83	7.15	18.4	37.5
	3rd visit	22.34	5.10	17.4	32.2
RAL	1st visit	5.88	0.45	5	6.5
	2nd visit	5.09	0.35	4.3	5.5
	3rd visit	4.82	0.33	4	5

Intra group comparison showed that there was significant deference in all periodontal parameters on deferent time intervals for all groups as shown in table 4, 5 and 6

Table 4 Comparison between the visits using Wilcoxon signed ranks test for G1 (HARP)

Variables		1st visit vs. 2nd visit	1st visit vs. 3rd visit	2nd visit vs. 3rd visit
PLI	Z-test	-2.507	-2.814	-1.586
	p-value	0.012 (S)	0.005 (HS)	0.113 (NS)
GI	Z-test	-2.405	-2.814	-0.256
	p-value	0.016 (S)	0.005 (HS)	0.798 (NS)
BOP	Z-test	-2.803	-2.803	-2.803
	p-value	0.005 (HS)	0.005 (HS)	0.005 (HS)
RAL	Z-test	-2.877	-2.831	-2.831
	p-value	0.004 (HS)	0.005 (HS)	0.005 (HS)

Table 5 Comparison between the visits using Wilcoxon signed ranks test for G 2(RP)

Variables		1st visit vs. 2nd visit	1st visit vs. 3rd visit	2nd visit vs. 3rd visit
PLI	Z-test	-2.809	-2.814	-1.992
	p-value	0.005 (HS)	0.005 (HS)	0.046 (S)
GI	Z-test	-2.609	-2.809	-2.814
	p-value	0.009 (HS)	0.005 (HS)	0.005 (HS)
BOP	Z-test	-2.803	-2.803	-2.803
	p-value	0.005 (HS)	0.005 (HS)	0.005 (HS)
RAL	Z-test	-2.640	-2.816	-2.041
	p-value	0.008 (HS)	0.005 (HS)	0.041 (S)

Table 6 Comparison between the visits using Wilcoxon signed ranks test for G3 (HA)

Variables		1st visit vs. 2nd visit	1st visit vs. 3rd visit	2nd visit vs. 3rd visit
PLI	Z-test	-2.844	-2.816	-2.844
	p-value	0.004 (HS)	0.005 (HS)	0.004 (HS)
GI	Z-test	-2.821	-2.816	-2.406
	p-value	0.005 (HS)	0.005 (HS)	0.016 (S)
BOP	Z-test	-2.803	-2.803	-2.429
	p-value	0.005 (HS)	0.005 (HS)	0.005 (HS)
RAL	Z-test	-2.821	-2.821	-2.041
	p-value	0.005 (HS)	0.005 (HS)	0.041 (S)

Inter group comparison showed that there was significant deference in RAL and BOP in 2nd visit and highly significant deference in RAL in 3rd visit, also the result showed highly significant deference in PLI in the 1st visit as shown in table (7).

Table 7 Comparison among the groups using Kruskal-Wallis H test

Variables	Visits	HARP	HA	RP	Comparison		
		Mean	Mean	Mean	X^2	d.f.	p-value
PLI	1st visit	1.08	0.75	0.77	17.081	2	0.000 (HS)
	2nd visit	0.64	0.61	0.65	0.668	2	0.716 (NS)
	3rd visit	0.56	0.53	0.58	2.358	2	0.308 (NS)
GI	1st visit	1.02	0.88	0.81	4.242	2	0.120 (NS)
	2nd visit	0.53	0.63	0.67	2.908	2	0.234 (NS)
	3rd visit	0.52	0.55	0.59	1.266	2	0.531 (NS)
BOP	1st visit	76.38	75.36	74.95	0.259	2	0.879 (NS)
	2nd visit	22.54	23.83	30.47	3.188	2	0.203 (S)
	3rd visit	21.48	22.34	24.79	0.764	2	0.682 (NS)
RAL	1st visit	5.69	5.88	6.12	5.721	2	0.057 (NS)
	2nd visit	5.02	5.09	5.42	7.747	2	0.021 (S)
	3rd visit	4.31	4.82	5.10	20.535	2	0.000 (HS)

IV. Discussion

This study analyzed the possible beneficial effects of HA alone (G3) and an additional application of HA gels during SRP (G1) on clinical periodontal parameters (up to four weeks) and compare to SRP alone (G2).

There was highly significant reduction in all clinical parameters for all groups .The inter comparison groups showed that there was significant difference in Bop at deferent time interval when compare G1 and G3 with G2 .This observation is in concert with previous reports (Jentsch et al., 2003)[25],(Pistorius et al., 2005)[26] of improved gingival health after the supragingival application of various hyaluronan formulations in subjects with gingivitis. And agreement with study which was done by Johannsen et al., 2009[27] who also applied a 0.8% HA gel subgingivally, demonstrated a higher, significant improvement of BOP in the HA group in comparison with SRP only However, other investigators did not show a beneficial effect of hyaluronan periodontal health. Xu et al.,2004 [28] who did not find any difference in BOP between HA test and control groups after treatment. It may

be speculated that the use of 8% HA is of great importance for healing and clinical outcomes, especially in the first days and weeks after treatment, as considered in this study Also this study showed that there is significant reduction in RAL for all groups, but inter groups comparison showed that the reduction of RAL for G1 and G3 was significantly higher than G2 and this result was in agreement with Johannsen et al., [27]

Hyaluronate has shown anti-inflammatory, anti-edematous and anti-bacterial effects for the treatment of gingivitis and periodontitis.

The anti-inflammatory effect may be due to the action of exogenous hyaluronan as a scavenger by draining prostaglandins, metalloproteinase and other bio-active molecules. [29]

The ant edematous effect may also be related to the osmotic activity.

It could be used as an adjunct to mechanical therapy due to its acceleration in tissue healing properties[30].

Hyaluronan administration to periodontal wound sites could achieve comparable beneficial effects in periodontal tissue regeneration and periodontal disease treatment[31].

Hyaluronic acid has been studied as a metabolite or diagnostic marker of inflammation in the gingival crevicular fluid[32].

As well as a significant factor in growth, development and repair of tissues[33]. Topical application of subgingivally hyaluronic acid gel can be used as an antimicrobial agent as an adjunct to scaling and root planning[30,34,35].

HA plays an important role in post inflammatory tissue regeneration, facilitating cell migration and differentiation during tissue formation and repair. Local application of exogenous hyaluronan was found to produce beneficial wound healing outcomes in experimental animals[31, 36, 37].

It has been reported that HA has osteoinductive properties as well[38]. An investigative study that was done by Pirnazar et al.,1999[39], they found that recombinant HA exerted varied bacteriostatic effects on all the bacterial strains tested, depending on its molecular Weight and concentration.

The high concentrations of the medium molecular weight HA had the greatest bacteriostatic effect, particularly on the Actinobacillusactynomycetemcomitans, Prevotellaoris, Staphylococcus aureus, and Propionibacterium acnes strains.

High molecular-weight HA gel reduces cell proliferation in gingival epithelial cells, fibroblasts and lymphocytes, abates the inflammatory process, and improves periodontal lesions in patients with chronic periodontitis[38].

V. Conclusions

This study indicates possible improvement of clinical periodontal parameter by usingHigh-molecular-weight HA (8%) alone or as an adjunct to SRP to patient with chronic periodontitis.

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