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RESEARCH ARTICLE

EVALUATION OF TOPICAL SUBGINGIVAL APPLICATION OF HYALURONIC ACID (HA) GEL ADJUNCTIVE TO SCALING AND ROOT PLANING (SRP) IN THE TREATMENT OF CHRONIC PERIODONTITIS.

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Abstract

Objective: The aim of this study is to assess effect of sub-gingival application of hyaluronic acid (HA) gel .8% adjunctive to non-mechanical debridement in treatment of chronic periodontitis based on clinical scoring and microbiological study.

Subjects and Methods: The present study was carried out on 20 patients with chronic periodontitis with at least two quadrants with pocket depth ≥5 mm within the same arch. Split-mouth study design was used where control side received (SRP) only and study side received both (SRP) and subgingival application of high molecular weight 0.8% hyaluronic acid gel in a 3 months follow-up study. After clinical assessment and plaque sample collection all patients received full mouth SRP in both study and control sites at the baseline, 2nd, 4th and 6th weeks. Study sites additionally received 0.8% HMW HA gel sub-gingivally till the depth of pocket, this done once a week starting at the baseline then repeated at weeks 1, 2, 3, 4, 5 and 6 for a total seven applications.

Subgingival plaque samples were collected prior to clinical measurements from both study and control sites at the baseline, 6th and 12th weeks for PCR examination. Evaluation included clinical and microbiological parameters that recorded at the base line, th 6th and then in 12th week. Clinical parameters included: Plaque index (PI), Gingival index(GI), Periodontal probing depths (PPD), Clinical attachment loss (CAL) and clinical attachment regain where, micriobiological assessment included qualitative polymerase chain reaction (PCR) for detection of both provotella intermedia and porphyromonus gingivalis in both sides .

Results: In the present study, there were highly significant clinical improvements in all measured clinical

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parameters (Plaque index (PI), Gingival index (GI), Periodontal probing depths (PPD), Clinical attachment loss (CAL) and clinical attachment regain) in study sites which received SRP and HA more than that achieved within controls at all interval points of recording after starting the treatments: 6th and 12th weeks. In addition, PCR results showed high significant bacteriostatic effects of HA on the two periodontal pathogens under study: provotella intermedia and porphyromonus gingivalis have been found in study sites at 6th and 12th weeks more than that achieved among controls.

Conclusions: Scaling and root planning (SRP) can result in the reduction of many clinical signs of the chronic periodontitis but applications of (0.8%) high molecular weight hyaluronic acid gel form (HMW HA) as an adjunctive to scaling and root planning (SRP) found to have significant clinical effects among study defects more than that achieved by (SRP) alone in controls with high power of repair attachment regain with long time prolonged action. (HMW HA) gel form has significant bacteriostatic effects on p.intermedia and p.gingivalis.

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Introduction:-

Chronic periodontitis is considered to be a cumulative oral inflammatory disease that leads to destruction of extracellular matrix within underlying periodontal tissues including: proteo-glycans, collagen fibers, and glycosaminoglycans leading to destruction in supporting tissues of the teeth, alveolar bone loss and periodontal pocket formation [(1)]. It is the most popular type of periodontal disease that occurs between 80% of the Americans and about 51% of the United Kingdom population at life [(2)]. The amount of destruction is proportional with local factors particularily dental plaque and calculus. In most cases, rate of progression of the disease is slow to moderate with periods of rapid progression may also be observed [(2)].

Chronic periodontitis as a disease is initiated by bacterial accumulation and invasion of periodontium mainly gram -ve anaerobic or facultative ones that found in dental plaque where long term accumulation of dental plaque induces chronic inflammation leading to attachment destruction of periodontal ligament and alveolar bone loss [(3)]. It had been accepted that chronic periodontitis is initiated by special infectious bacteria which are able to modulate the inflammatory and immune system to prove that the quality not the quantity of pathogenic local factor that controls the progression of the disease [(4)].

Ideal therapy of the disease for the patients with chronic periodontitis differs with the pattern and severity of the loss of attachment, anatomical variations, and therapeutic goals. The main goal of therapy of chronic periodontitis is to stop progression of disease and to reduce amount of inflammation to restore periodontal health and thereby to satisfy the patient's functional and esthetic needs and to restore destructed tissues. Therapy is aimed to reduce and remove all causitive factors under threshold that can produce damage and to allow the repair of the affected area by decreasing the probing depths and to improve the levels of attachment which increase the tooth retention and decrease the amount of bone loss and tooth mobility [(5, 6).]

Scaling and root planing (SRP) still represent the first step in treatment of chronic periodontitis and represent an effective therapy for reducing both gingival inflammation and probing depths (PDs) among most of patients with chronic periodontitis[(7,8)]. The actual effect of (SRP) is based mainly on reducing the mass of bacteria whatever supra-gingival and sub-gingival within periodontal pockets [(9)] that is to shift to less pathogenic micro-flora [(10)].

Recently, combination of both (SRP) and topical sub-gingival pharmacological therapies has been evaluated to provide effective treatment of chronic periodontitis and healing of periodontal pockets [(11, 12)]. However, systemic

antibiotics should not be used in treatment of all forms of chronic periodontitis [13], the development of resistance as well as drug interactions as a side effects are important reasons to limit the usage of systemic chemotherapeutic agents and to begin a new era in usage of topical sub-gingival delivery of therapeutic agents[14].The main advantage of the topical delivery system therapy is to bring high concentrations and slowly sustained release of chemotherapeutic agents within the periodontal pocket without exposing the whole body to avoid their systemic side effects [15]. For example, the local delivery systems of chlorhexidine in a bio-absorbable chips for sustained release, this added benefits to SRP [16, 17].Another similar improvements were observed with adjunctive use of locally administrated microencapsulated tetracycline as minocycline [18, 19].

One of the most recently used topical chemotherapeutic agents is Hyaluronic Acid (HA) which is considered to be a naturally linear poly-saccharide of extracellular matrix that form the main ground substance within connective tissues, joints, and other tissues within human body. Structural and physiological functions of HA within periodontium include many extracellular and even cellular interactions, regulation of the osmosis within periodontal tissues, interactions with variable growth factors, and lubrication of tissues that maintain the hemostatic and structural integrity of the tissues. All these physiological and chemical properties made from HA as an ideal bio-material for medical and pharmaceutical uses [20]. Hyaluronic acid is polysaccharide present in vertebrates connective tissue, glucoronic acid and N-acetyglucosylamine polymer, and act as a member of glycosamine family with a high molecular weight [21].This chemical structure gives HA a unique physiochemical and biological properties. HA is considered to be between most hygroscopic chemicals in nature giving it ideal function in lubrication, space filling and shock absorption, Also it is visco-elastic substance that assists in maintaining spaces and protecting surfaces to modify extra-cellular and cellular macro and micro-environments, this illustrates slow penetration of viruses and bacteria which provide special interest in treatment of periodontitis [22].

It was found that high concentration of HA have a great bio-compatible and non‑immunogenic molecule and these properties led multiple uses and many applications such as joint fluid supplements as in arthritis, in ophthalmic surgery, and to help in the regeneration of bone and periodontal tissues [23, 24]. HA has an anti‑inflammatory effect acting as a scavenger which drain, metalloproteinases, prostaglandins and others which promote inflammatory activities [24]. The anti‑edematous property of HA also may be related to its osmotic properties [25]. HA regulates the inflammatory and immune response acting as an anti-oxidant so, HA may stabilize the granulation tissue matrix [26].

Patients and Methods:-

The present study was carried out on 20 patients seeking periodontal treatment attending the department of Oral medicine and Periodontology, Faculty of Dentistry, University of Mansoura. These patients were diagnosed to have chronic periodontitis with at least two posterior quadrants with pocket depth $\geq$ 5 mm within the same arch where incisors and canines were excluded from study to avoid carry out effect of HA gel between study and control sites. No history of any preceding oral infections or periodontal treatment for at least three months before starting the study. Smokers and alcoholic patients have been excluded also with pregnant, post-menopausal and lactating women with age range of patients is between 35 to 55 years old. Patients with poor systemic health like uncontrolled diabetes, hypertension, osteoporosis, collagen disorders are excluded also with patients who were on or expected to take antibiotics or anti-inflammatory drugs within duration of the study.

Split-mouth study design was used where the control side received SRP only and study side received both SRP and sub-gingival application of 0.8% hyaluronic acid gel using flexible tips within the (Gengigel®) kit. This HA (Gengigel®) gel preparation contains non-animal derived, biotechnologically manufactured high molecular weight of HA dissolved in xylitol as a carrier.

Figure:- Showing HA (Gengigel®) sub-gingival application using flexible tips within periodontal pockets of study site.

All defects within study and control sites in patients were assessed in clinical level at the baseline before any type of treatment, 6th week after treatment and finally after 12 weeks of treatment. These clinical parameters included: Gingival index (GI), Periodontal probing depths (PPD), Plaque index (PI), Clinical attachment loss (CAL) and Clinical attachment regain. Both of periodontal pathogens Porphyromonous gingivalis (P.gingivalis) and Provotella intermedia (P.intermedia) were detected within subgingival dental plaque samples at the base line before treatment, 6th and 12th weeks by qualitative polymerase chain reaction (PCR) after plaque sample collection using a sterile manual curette or even with s sterile paper point that is placed within the periodontal pocket in cases where subgingival plaque is previously removed where saliva is removed with a cotton pad, swab or high suction to minimize collection of any transient bacteria from any exogenous source. Plaque samples were pooled in a sterile 1.5 ml micro-centrifuge tube (Eppendorf tubes) that contain normal sterile saline, then transported to Microbial Diagnostic and Infection Control Unit (MDICU) to be stored at – 20 °C.

At the first visit all patients received full mouth SRP and repeated in both study and control sites twice weekly starting from the first visit of treatment, 2nd, 4th and 6th week time interval. The patients were instructed to establish good oral hygiene measurements in the form of regular tooth brushing & dental flossing where chlorhexidine toothpastes were intstructed to be avoided that is to prevent destruction of organic material within HA gel after application and to prevent secondary effect of other therapeutic antimicrobial agent. No antibiotics were prescribed during the course of treatment.

The microbiological evaluation was divided into two steps: DNA template preparation (DNA extraction) and qualitative polymerase chain reaction (PCR).

Preparation of DNA template:Quick-DNA™ Miniprep Plus Kit (ZYMO RESEARCH, USA) was used for DNA extraction to obtain DNA extract that was stored at -20 ^{o}C till PCR reaction was done while Qualitative Polymerase Chain Reaction (PCR): Amplifications were performed using specific primer pairs .PCR was performed with a final volume of 50 µl in PCR tubes. Each reaction contained 20 mM Tris-HCl (pH 8.4); 50 mM KCl; 0.2 mM each deoxy-nucleoside triphosphate; 1.5 mM MgCl2; 0.6 µl of each ACC primer, and 1.25 µl of Taq DNA polymerase. Template DNA (2 µl) was added to 48 µl of the master mixture and then overlaid with mineral oil .The mixture is then transferred to programmable thermal controller to apply PCR programme .The PCR program consisted of an initial denaturation step at 94°C for 3 min.This is followed by 25 cycles of DNA denaturation at 94°C for 30 seconds.Primer annealing at 64°C for 30s, and primer extension at 72°C for 1 min. After the last cycle, a final extension step at 72°C for 7 min was added. Agarose gel electrophoresis using ultraviolet (UV) transilluminator of

the amplified DNA with DNA standard marker: Φ X 174 (HaeIII) digest marker (Promega) to detect the expected 346 and 404 bp bands respectively for both p.gingivalis and p.intermedia to detect their presence or absence according to band thickness and intensity.

Figure:- Showing Agarose gel electrophoresis, from left to right: electric supplier, agarose gel and (UV) transilluminator.

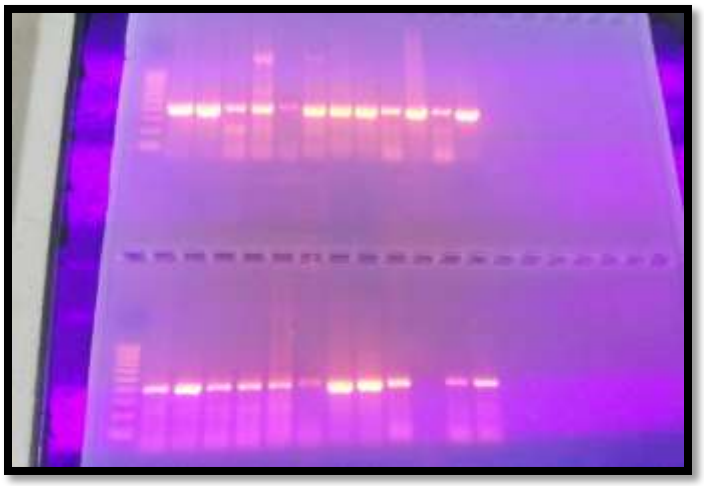

Figure:- Showing UV bands for P.intermedia.

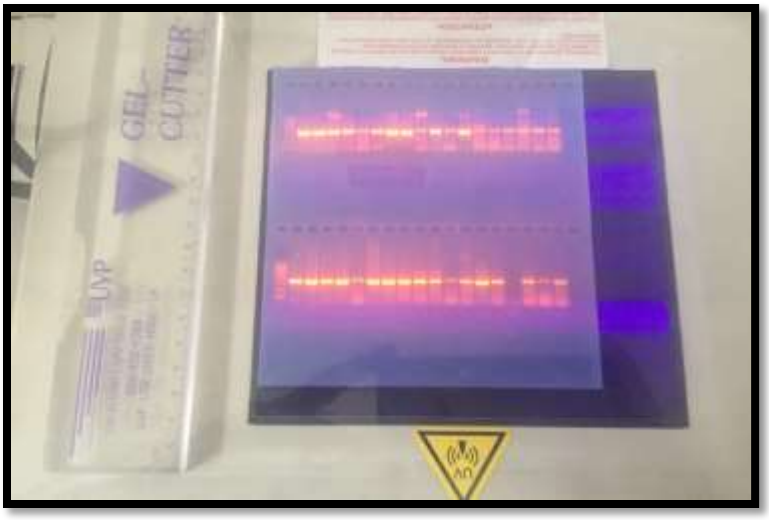

Figure:- Showing UV bands for P.gingivalis.

All previous DNA amplification steps were repeated for both p.gingivalis and p.intermedia. All collected data from our clinical and microbiological measurements are grouped and then they were entered and statistically analyzed using the version 16 of the Statistical Package for Social Sciences (SPSS) software.

Results:-

Clinical and Microbiological parameters of groups under study at the Baseline.

Characteristics	**study sites Number=20**	**Control sites Number=20**	**Test of significance**
Probing depth (PD) Mean ± SD	**5.5 ± 0.7**	**3.5± 0.7**	**t =0.9 p=0.4**
Clinical attachment loss (CAL) Mean ± SD	**3.5± 0.7**	**3.3± 0.7**	**t=0.9 p=0.3**
Gingival index (GI) Mean ± SD	**2.1± 0.3**	**2**	**t=1.4 p = 0.2**
Plaque index (PI) Mean ± SD	**1.9 ± 0.6**	**1.6 ± 0.5**	**t = 1.7 p = 0.1**
Porphyromonus Gingivalis (PG):	**No. (%)**	**No. (%)**	**_____**
+VE	**20 (100.0)**	**20 (100.0)**	
-VE	**0 (0.0)**	**0 (0.0)**	
Provotella Intermedia (PI) :	**No. (%)**	**No. (%)**	**_____**
+VE	**20 (100.0)**	**20 (100.0)**	
-VE	**0 (0.0)**	**0 (0.0)**	

Clinical and Microbiological parameters of groups under study at 6th week.

Characteristics	**study sites Number=20**	**Control sites Number=20**	**Test of significance**
Probing depth (PD) Mean ± SD	**2.4 ± 0.8**	**3.8 ± 0.6**	**t=6.1 p <0.0001**
Clinical attachment loss (CAL) Median Min-Max	**0 (0-2)**	**2 (1-3)**	**z =4.5 p < 0.0001** Mann Whitney test
Clinical attachment regain Median Min-Max	**3 (1 - 5)**	**1 (1-3)**	**z =4.3 p < 0.0001** Mann Whitney
Gingival index (GI) Median Min-Max	**0 (0 - 1)**	**1 (0-2)**	**z =3.2 p = 0.001** Mann Whitney

Plaque index (PI) Median Min-Max	**0 (0 -1)**	**1 (0-1)**	**z =2.5** **p = 0.011** Mann Whitney
Porphyromonus Gingivalis (PG):	**No. (%)**	**No. (%)**	**χ^2 = 14.4** **p < 0.0001** Chi square test
+VE	**4 (20.0)**	**16 (80.0)**	
-VE	**16 (80.0)**	**4 (20.0)**	
Provotella Intermedia (PI) :	**No. (%)**	**No. (%)**	**P = 0.02** Fisher's Exact test
+VE	**0 (0.0)**	**6 (30.0)**	
-VE	**20 (100.0)**	**14 (70.0)**	

Clinical and Microbiological parameters of the groups under study at 12th week.

Characteristics	**study sites N=20**	**Control sites N=20**	**Test of significance**
Probing depth (PD) Median(Min-Max)	**2 (1-4)**	**4 (3-5)**	**z =4.4** **p <0.0001** Mann-Whitney Test
Clinical attachment loss (CAL) Median(Min-Max)	**0 (0 – 2)**	**2 (1 – 3)**	**z =4.5** **p <0.0001** Mann-Whitney
Clinical attachment regain Median(Min-Max)	**3 (1 – 5)**	**1 (0 - 4)**	**z =4.0** **p <0.0001** Mann-Whitney
Gingival index (GI) Median(Min-Max)	**0 (0- 1)**	**1 (0 – 2)**	**z =4.4** **p <0.0001** Mann-Whitney
Plaque index (PI) Median(Min-Max)	**0 (0 – 1)**	**1 (0 - 1)**	**z =3.8** **p <0.0001** Mann-Whitney
Porphyromonus Gingivalis (PG):	**No. (%)**	**No. (%)**	**χ^2 = 21.5** **p < 0.0001** Chi-square Test
+VE	**0 (0.0)**	**14 (70.0)**	
-VE	**20 (100.0)**	**6 (30.0)**	
Provotella Intermedia (PI) :	**No. (%)**	**No. (%)**	**χ^2 = 17.1** **p < 0.0001** Chi-square Test
+VE	**0 (0.0)**	**12 (60.0)**	
-VE	**20 (100.0)**	**8 (40.0)**	

Discussion:-

Chronic periodontitis is usually related to poly-bacterial infection, including among others Porphyromonas gingivalis and Provotella intermedia. Destruction of the attachment apparatus and loss of epithelial attachment of the dentition is a hallmark of chronic periodontitis. The tissue destruction appears to result from a complex interaction between these bacteria and the host's immune and inflammatory responses. Proteolytic activities of these bacterial products, including collagenase and hyaluronidase may participate in collagen and connective tissue ground substance (hyaluronic acid) degradation [(27)]. HA sub-gingival application decreases the risk of resistance of pathogenic strains and also decreases drug interactions that done by systemic drug administration. The main effect of HA application is mainly represented in non-toxicity, bio-compatibility, viscoelastic properties and counter-action of bacterial pathogenic products (as hyaluronidase enzyme) in modulation of the host response when applied within inflamed tissue and restriction of bacterial spread or migration within diseased tissues by its unique viscoelasticity and also prevent newly species of bacterial re-growth which gives HA by frequent clinical applications some sort of bacteriostatic properties. The effect of HA mainly depends on molecular weight, concentration, form of HA applied and frequency of applications.. HA also, participate directly in cell-cell interaction and act as a scaffold to promote adhesion and proliferation of periodontal ligament cells. These matrix-induced effects on cells are in turn supported and directed by a wide variety of HA-binding proteins [(28, 29)]. The split-mouth study design was used in our study that is to gain the advantage of allowing paired comparisons between both study and control sites within the same patient, same conditions, environment and same immune response. Incisors and canines were excluded from study that is to avoid the carry out or spill-over effect of HA gel between study and control sites [(30)].

The main goal of HA application within the study defects is to maintain the optimal concentration of HA gel to be maintained within periodontal pocket with sustained long term release and contact with ulcerated lining epithelium so HA gel is applied weekly from the baseline to the 6th week where this intensive application of HA may overcome some problems such as the constant crevicular fluid flow rate which is responsible for a rapid clearance. In addition, the amount of HA applied is further reduced by bacterial products as hyaluronidases enzymes. Consequently, saturation of the bacterial hyaluronidases, which are needed to break through the physiological HA network, may have prevented bacterial spread [31].

Recent advances within molecular biology gave as a chance to use qualitative polymerase chain reaction (PCR) for detection of pathogenic bacteria within plaque samples instead of conventional culturing techniques. This microbiological culture method was one of the most widely used techniques for microbiological detection. However, this method has some drawbacks that limit its application in our study as it has a very low sensitivity, specifity and it is especially difficult to cultivate anaerobic species which are considered species within point of research where microorganisms related to the periodontal disease, in turn, are predominantly anaerobic and are very difficult to cultivate [32].

Analysis of recent contemporary microbiological diagnostic methods suggests the PCR as the most appropriate approach for the identification of specific periodontal pathogens because of its better sensitivity than that of culturing methods where, K. Kotsilkov et al. made a study to compare between PCR and culture techniques in detection of periodontal pathogens within deep periodontal pockets ≥ 7mm about presence of nine periodontal pathogens including p.gingivalis and p.intermedia with both techniques. The comparison of both methods revealed a better diagnostic capability and sensitivity of the PCR [32].

At the baseline all clinical parameters recorded showed no difference of significance between study and control groups. There is no any test of significance in relation to PCR results of both PG and PI at the baseline as whole plaque samples were positive for both periodontal pathogens at this time. These results are suggestive for the equal distribution of microbiological and clinical parameters which is an essential component for split-mouth study design [30].

After 6 weeks of treatment both study and control sites showed improvements on clinical parameters with highly difference of significance was recorded according to study sites after HA applications. These results are attributed to adequate oral hygiene maintenance and to process of debridement itself applied within both sites which results in removal of plaque and calculus and reduction of microbial load, thereby providing conductive environment for periodontal tissues to heal. The improvement in this healing process being higher in the study sites signifies additional beneficial effect of HA in healing process. Comparison between study and control sites showed a statistically significant difference in relation to GI, PI, PPD and clinical attachment regain, this additional improvement within all aspects of clinical parameters is possibly due to the beneficial effects of HA. Prolonged close contact of HA with periodontal tissues helps sulcular epithelium to regain its structural integrity and optimal thickness. Also, the blood vessels regain normal tonus as proteolytic enzymes and inflammatory mediators diminish by intimate contact with HA. These results can be attributed to the anti-edematous and scavenger effect of HA on prostaglandins and metalloproteinases. The anti-inflammatory effect of HA is attributed to its action of deactivation bacterial hyaluronidases, normalizing the aggregation of connective tissue proteoglycans and bonding with free water, thus performing an anti-edematous effect. HA gel is additionally known to enhance formation of extracellular connective tissue matrix leading to non-inflamed and healthy periodontal tissue [29, 28, 30].

Microbiologically after 6 weeks of treatment only 20% of study sites is showing positive results of PG presence after PCR ($p < 0.0001$) and all study sites are free from PI while PG is present in 80% of control sites and PI is present in 30% of controls with highly significant difference between both groups. Additional bacteriostatic effect of HA in our study is mainly promoted by usage of HMW HA, high 0.8% concentration, its gel form and also to highly frequency of application within study sites. All these factors maintain optimal concentration of HA within defect with slowly prolonged sustained release and very low rate of clearance from the field.HMW HA acted as a physiological network that prevented bacterial migration and penetration within periodontal tissues that promoted by hyaluronidases enzymes action which are neutralized by HA applications. HA also may have immunomodulatory effect on polymorphonuclear leukocytes (PMNs) as they stimulate PMN and improve their functions in immune response playing an important role in host modulation so, HA is seemed to stabilize low counts of periodontopathogens and prevent early regrowth giving antibacterial action (bacteriostatic) to certain extent. Very

high significant difference in all parameters of study over controls that indicate the long term prolonged action of HA till 12th wee [31]. Payman Pirnazar etal (1999) found that the high concentration of HMW HA had the greatest bacteriosttic effect over lowe molecular weights and this concentrate on the fact that with increase of MW and concentration of the HA , there is higher power of bacteriostatic effect of HA [33]. Yi Xu etal studied HA applications with low concentrations (0.2%) in infra-bony pockets. They measured also both clinical parameters and microbiological effects of HA (0.2%) on p.gingivalis and p.intermedia using also PCR. But here, they found no clinical or even microbiological improvements and they attributed their results to the very low concentration of HA used within the study [34].By using real time polymerase chain reaction (PCR) , in 2013 Sigrum Eick etal could approve in their study the bacteriostatic effect of HMW HA 0.8% with sub-gingival application after HA application in 6 months follow up study specially against p.gingivalis , Trepenoma denticola and p.intermedia .This study proved the adjunctive application of HMW HA on clinical parameters and also on microbiological effect on the level of bacteriostatic effect and also in prevention of periodontal pathogens recolonization [31].

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