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EVALUATION OF SALIVARY HEPATOCYTE GROWTH FACTOR LEVELS IN CHRONIC PERIODONTITIS PATIENTS BEFORE AND AFTER NON-SURGICAL PERIODONTAL TREATMENT

Periodontology

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ABSTRACT

Hepatocyte Growth Factor (HGF) or Scatter Factor (SF) is a growth factor which restrains the reconstruction of profound periodontal tissues. The study estimated the salivary HGF levels in unstimulated whole saliva in chronic periodontitis patients prior to treatment and also at 2nd and 6th week after non-surgical periodontal treatment and also aimed to determine the relationship of salivary HGF levels with the clinical periodontal parameters. This was a prospective-analytical study which included 45 subjects with chronic periodontitis. The highest mean concentration of HGF was obtained at the baseline and the lowest mean HGF concentration was obtained at the 6th week. There was significant decrease in the salivary HGF levels among the patients subsequent to scaling and root planing. Moreover the salivary HGF levels indicated a positive correlation with the clinical parameters at the sixth week. The above results propose that salivary HGF levels could be utilized as a biomarker for checking the response to periodontal treatment in chronic periodontitis patients.

KEYWORDS

Hepatocyte Growth Factor, chronic periodontitis, biomarker

INTRODUCTION

Hepatocyte Growth Factor (HGF) is considered to be one of the most important endogenous substances that control tissue interactions. HGF is a paracrine cell growth factor that may be of critical significance in the development and progression of oral and periodontal diseases.

Periodontitis is an inflammatory disorder of the supporting tissues of the teeth, resulting in pocket formation or recession[1].The diagnosis of the stage of periodontal disease relied on the relationship between the clinical appearance and the presence of some specific cell population [2].

Traditional clinical measurements (probing pocket depth, bleeding on probing, clinical attachment loss, plaque index) used for the diagnosis of periodontal health are often of limited use because they are not sufficiently accurate to discern between previous periodontal disease and present disease activity [2].

Advances in periodontal diagnostic research have contributed to the advancement of methods by which the periodontal risk can be detected and quantified by objective measures such as biomarkers. Over the last decade, scientists have been using signaling molecules such as growth factors in their quest to restore damaged tooth support and to be used as biomarkers [3].

Gingival crevicular fluid (GCF) and salivary levels of several growth factors, cytokines and enzymes of host origin appear to have the greatest potential as valuable biomarkers in assessing development of periodontal disease [3].

In an attempt to find these biomarkers associated with periodontal disease and progression, detailed studies have been performed on gingival crevicular fluid, glandular saliva and whole saliva. Of these fluids, unstimulated whole saliva is one of the most easily obtained fluids and it also contains the components from gingival crevicular fluid. Several studies have documented that the HGF levels in unstimulated whole mixed saliva were directly associated with probing depth and the percentage of sites positive for bleeding on probing in the general population. These findings provided evidence to corroborate previous in vivo investigations suggesting a novel connexion between HGF and periodontal disease [4]-[6]. The present study was therefore aimed at evaluating the association between salivary HGF levels and periodontal disease activity.

METHODOLOGY

Data Collection Method

The study was a prospective-analytical study which included 45 chronic periodontitis patients (24 males and 21 females) who reported to the Department of Periodontics, Sri Sankara Dental College. The subjects were enrolled for the study after obtaining an informed consent. The study was conducted in agreement with the principles embodied in the 1964 Declaration of Helsinki, as revised in 2000, and was approved by the Institutional Ethical Board (IEC/IRB No: IEC/004/2017).

At the baseline, after the selection of the subjects, full mouth periodontal examination was done. The data was collected using self-designed data proforma which contained information regarding the socio-economic and demographic characteristics of the subjects.

Saliva Sample Collection

Samples of unstimulated whole saliva (2 ml) were obtained from all subjects using the spit-out procedure in the morning hours (10 a.m.-12 p.m.). After rinsing the mouth, saliva was taken, and the subjects spit the saliva into a sterile vessel. Collected saliva was immediately put in a cryobox and held in a deep freezer at-200 Celsius.

Periodontal Examination

At the baseline, clinical parameters including bleeding on probing (BOP), pocket depth (PD), clinical attachment loss (CAL) were recorded. Routine phase I periodontal treatment (scaling and root planing) was done and oral hygiene instructions were given. The patients were then recalled on the 2nd and 6th week for salivary HGF collection. The assessment of clinical parameters including bleeding on probing (BOP), pocket depth (PD), clinical attachment loss (CAL) were done and oral hygiene instructions were given.

HGF Level Estimation

Samples were analysed using the human HGF Elisa kit (Human HGF ELISA Kit, Catalog#ELH-HGF, RayBio). At the time of study, the samples were defrosted, centrifuged at 12,000 rpm for 10 minutes and the supernatant was removed. 100 µL of each sample was applied to the corresponding wells and incubated at room temperature for 2.5 hours.

After incubation, wells were washed and 100µL of prepared Biotin antibody was added to each well and incubated at room temperature for one hour. After incubation, washed and 100µL of prepared Streptavidin solution was applied to each well and incubated at room temperature for 45 minutes.

After incubation, 100µL of TMB One Step Substrate was included and incubated at room temperature for 30 minutes. The reaction was halted by adding 50 µL of stop solution. The absorbance was read at 450 nm and the HGF concentration was obtained from the standard graph.

Statistical Analysis

All the statistical analysis was carried out using statistical software (SPSS software 17.0). The outcome variable was the salivary HGF level and the independent variables include age, sex, occupation, pocket depth, bleeding on probing, clinical attachment loss.

Descriptive Part: The patient characteristics and clinical parameters are expressed as mean value and standard deviation. The correlation between the periodontal clinical parameters and the levels of HGF was determined by the parametric Pearson correlation analysis.

Inferential Part: Since HGF is a laboratory value, the significance of the difference between pre and post treatment comparisons are done using a paired t test, confidence interval is set at 95%.

RESULTS

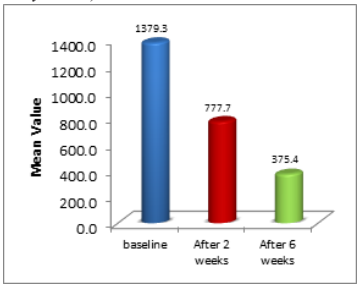
The percentage distribution of the sample according to age includes 40% of patients between 46- 50 years of age, 33.3% between 35- 40 years of age and 26.7% of patients between 41-50 years of age. The mean age range of the sample was 43.4 ± 5.1. The percentage distribution of the sample according to sex includes 53.3% of males and 46.7% of females.

All the samples tested were positive for HGF assay. The highest mean concentration of HGF was obtained at baseline (1379.3 pg/µl) and the lowest mean HGF concentration was seen at the 6th week (375.4 pg/µl). It was noted that there is statistically significant reduction in salivary HGF levels among chronic periodontitis patients at 2nd and 6th week after SRP compared to baseline salivary HGF levels. (Table.1 and Graph.1)

Table.1: Comparison of salivary HGF levels in chronic periodontitis patients at baseline and different time intervals after SRP (paired t test), * Statistically significant at p<0.01.

Stage	Mean HGF	SD	N	Group	Mean difference	Paired 't'	p
Baseline	1379.3	335.2	45	Baseline Vs 2 weeks	601.6	10.08	p<0.01*
After 2 weeks	777.7	380.0	45	Baseline Vs 6 weeks	1003.9	17.76	p<0.01*
After 6 weeks	375.4	177.2	45	2 weeks Vs 6 weeks	402.3	8.62	p<0.01*

(HGF: Hepatocyte Growth Factor, SD: standard deviation, N:sample size, P: probability value)



Graph.1, Comparison of salivary HGF levels in chronic periodontitis patients at baseline and different time intervals after SRP

There was a statistically significant reduction in bleeding on probing, probing pocket depth and clinical attachment loss at 2nd week and 6th week after SRP among patients compared to baseline. There was a statistically significant correlation between the levels of HGF and the clinical parameters.

Table. 2, Correlation Of HGF at 6th Week With CAL And PD at 6th week

Clinical Variable	Mean HGF	SD	N	r	P
CAL	375.4	177.2	45	0.696	p<0.01*
PPD	375.4	177.2	45	0.542	p<0.01*

(HGF: Hepatocyte Growth Factor, SD: standard deviation, N:sample size, CAL: clinical attachment loss, PPD: probing pocket depth, r: Pearson's correlation coefficient, P: probability value, Statistically significant at p<0.01).*

DISCUSSION

In the present study 45 chronic periodontitis patients were included with the mean age of 43.4 ± 5.1 years and had 24 males and 21 females. These demographic parameters were comparable with previous studies [7],[8]. All the samples tested were positive for HGF assay. The highest mean concentration of HGF was obtained at baseline (1379.3 pg/µl) and this may be due to inflammation in the periodontal tissues or be produced systemically due to dissemination of inflammatory mediators and the periodontal bacteria. The salivary HGF level was reduced to 777.7 pg/µl at the 2nd week and the lowest mean HGF concentration was obtained at the 6th week (375.4 pg/µl).

HGF causes a continuum of biological activity in epithelial cells. HGF is a potent endogenous growth factor for epithelial cells and activates the spread and migration of gingival epithelial cells deep into the periodontal pocket. The regeneration of the collagen fibres of the connective tissue is slowed by the growth of this epithelium. This activity of HGF prevents the regeneration of deep periodontal tissue. HGF also affects matrix remodelling by stimulating the development of matrix metalloproteinase (MMP) during tissue healing. Stimulation of HGF output and secretion occurs in response to local inflammatory conditions and degradation of periodontal tissues. Thus, HGF can be viewed as a periodontal factor in disease progression [8].

It was noted that there was statistically significant reduction in salivary HGF levels among chronic periodontitis patients at the 2nd and 6th week after SRP compared to baseline. There were few studies available that examined salivary HGF levels in the second week. A research by Nagaraja and Pradeep (2007) [9] tested the concentration of HGF in GCF in healthy and chronic periodontitis patients following SRP. The findings of the above analysis showed a substantial reduction in the mean concentration of HGF in GCF accompanied by SRP. The baseline values of HGF were equivalent to those of HGF after 6 to 8 weeks. Centered on the findings of the report referred to above, the present study assessed salivary HGF level at 6th week.

While comparing the mean BOP%, the present study showed a significant reduction at the 2nd and 6th week after SRP, compared to baseline values. The mean pocket depth at the baseline, 6.4mm was reduced to 3.8mm at the 6th week of evaluation. The mean values of clinical attachment loss also showed a significant reduction at the 2nd and 6th week after SRP compared to the baseline measurements (p<0.05). The present study also showed statistically significant correlation between the salivary HGF levels and the mean pocket depth and clinical attachment loss (p<0.05) at the 6th week.

The study was conducted only among chronic periodontitis patients with a small sample size. Further studies with shorter and longer evaluation period for the assessment of HGF levels in saliva, GCF and perhaps serum in a larger sample size are required to better evaluate the correlation between HGF levels and periodontal disease extent and severity. Based on the results of the present study, the salivary HGF level can be used as a biomarker to measure periodontal disease activity.

CONCLUSIONS

The study was a prospective-analytical study which included 45 subjects with chronic periodontitis. It was concluded that the salivary HGF levels was high among chronic periodontitis patients and there was a significant reduction in the salivary HGF levels among patients at the 2nd and 6th week after SRP. The above findings suggest that

salivary levels of HGF could be used as a biomarker for monitoring the response to periodontal therapy in chronic periodontitis patients.

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