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COMPARATIVE EVALUATION OF INTERLEUKIN -6 LEVEL IN SALIVA OF HEALTHY INDIVIDUALS WITH CHRONIC PERIODONTITIS PATIENTS BEFORE & AFTER SCALING AND ROOT PLANING: A CASE CONTROL STUDY.

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Conflicts of Interest: Nil

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Abstract:

The aim of this study was to assess and compare the levels of IL-6 in saliva of healthy individuals and chronic periodontitis patients before and after scaling & root planning. 40 subjects were selected on basis of inclusion criteria were categorized into two groups. . After selection of subjects, 20 patients were included under control group and and 20 patients were included under test group randomly. Plaque index, gingival index, bleeding on probing, probing depth, and clinical attachment level were measured at baseline and at 1 month after treatment. Interleukin-6 (IL-6) in saliva were analyzed by enzyme-linked immunosorbent assay at baseline and 1 month after the phase I therapy. In the control group ,mean value of IL-6 was 11.663±0.880 ng/l whereas in the chronic generalized periodontitis group it was 114.362±11.557 ng/l which reduced to 60.420±6.105 ng/l ,after one month of completion of non-surgical therapy. On comparison of these two groups, it was observed that the mean levels of IL-6 were significantly different.

Keywords Chronic periodontitis, Saliva, Interleukin-6.

Introduction

Periodontitis is an inflammatory disorder affecting supporting tissues of the teeth, primarily initiated by a small group of gram-negative anaerobic bacteria within periodontal pockets. The stimulation of host defense system against bacterial pathogens result in connective tissue breakdown and alveolar bone destruction.[1]It is well documented and clinically proven that inflammatory response is the contributing factor for the development of chronic periodontitis. Proinflammatory cytokine levels gets elevated in chronic periodontitis causing destruction of the periodontium.

It has been seen that the saliva, gingival crevicular fluid and ginvival tissue of a periodontitis patient contains tissue destructive molecules and contributing inflammatory molecules. There could be diagnostic and therapeutic significance in the composition of these biomarkers associated with various changes. Cytokines are glycoprotein which is soluble in water secreted by hematopoietic and nonhematopoietic cells to fight against infection. Their primary function is transmitting signals from one cell to another which are central to the cause of diseases including periodontal disease. Cytokines have a major role in ,development, proliferation, regeneration, repair and inflammation of periodontal tissues.

IL-6 is a type of cytokine which has multiple effect from a single gene. It acts as both pro-inflammatory and anti-inflammatory activity. It exerts anti-inflammatory properties through enhancement of tissue inhibitor of metalloproteinase (TIMP) production and suppression of pro-inflammatory cytokines IL-1β and TNF-α. In addition, down-regulation of proinflammatory cytokines and up-regulation of antiinflammatory molecules (e.g. IL-1 receptor antagonist, TNF soluble receptor) in acute inflammatory processes .[2,3] T and B cells, macrophages, endothelial cells, epithelial cells and fibroblasts produces IL-6 to fight against

infection,stress and neoplasia. IL-6 shows and explains many functions like acute phase protein induction. It has a major role in generation and activation of osteoclast.

Saliva is a fluid found in the body and is of great importance. Proteins and peptides are found in abundant quantity in saliva. It has an important relation with inflammation ,destruction of connective tissue and remodeling phases of bone in periodontal disease. Later on, saliva has been used to identify the nature and type of periodontal disease and to look after the treatment response. Saliva has been used as a diagnostic fluid because its easy to collect and doesn't require any complicated apparatus or any specially trained expert. The samples of saliva can also be collected in the home by patient without any inconvenience nor it requires any complicated apparatus and procedure. It is also used in the epidemiological studies. Protein synthesis derives the organic component of saliva associated with gland. The serum component of saliva is in link with the carotid arteries. By considering these all things, we can conclude that saliva is a fluid of great value and can be used in the diagnosis of various systemic diseases.

There is a large amount of written content or literature which explains and reflects about the inflammatory nature of periodontal disease and relation between infection and inflammation as well as on host and how it responds to infection. It has been seen previously in so many studies regarding the variation on the levels of IL6 in relation to all clinical parameters in chronic periodontitis patient before and after scaling and root planning.

Hence in the present study IL6 was used as a diagnostic biomarker for periodontal disease to assess its level in relation with chronic periodontitis patient and healthy individuals.

Material and method

PARTICIPANTS AND STUDY DESIGN

40 Patients of both the sexes with in age range of 20-55 years were selected from out patient department, D J college of dental sciences and research, Modinagar after the approval of the ethical committee. Each patient was given a detailed verbal and written description of the study. They were required to sign an informed consent form prior to commencement of the study. 40 subjects were selected on basis of inclusion criteria were categorized into two groups. After subject selection 20 patients were randomly assigned to first group that is the contol group, and remaining 20 patients were assigned as second group. The patients were categorized into 2 groups-

Group I: Healthy individual with probing depth <5mm

Group II: Chronic generalized periodontitis patients with probing depth >5mm (SRP done).

Patients were included both male and female from the range of 18-55 years with presence of minimum of 20 teeths. Exclusion criteria were patients with habits of Smoking and alcoholism, with coexisting systemic diseases or lactating females and with history of medication in previous 5 months.

CLINICAL PARAMETERS

This would be followed by evaluation on the basis of periodontal parameters and case control study. Clinical parameters include gingival index, probing depth and Clinical attachment level. Sample was taken at baseline for both the groups. Scaling and root planning was done in group II and sample was taken 1 month after the initiation of Phase-1 therapy.Clinical parameters were assessed in both the groups at baseline and in group II one month after the initiation of phase -1 therapy.

CLINICAL PROCEDURE

In the department of Periodontology and Implantology, saliva samples were collected from both the group at baseline. Scaling and root planning was done in group II and sample was taken 1 month after the initiation of Phase-1 therapy. All samples were immediately centrifuged at 3000 rpm for 20 minutes and clear supernatant was stored at-20ºC pending analysis. The IL-6 levels in saliva samples were measured with a Human Interleukin-6 (IL-6) ELISA Kit.

CLINICAL MEASUREMENTS

On the first visit, detailed case history including clinical parameters [gingival index, probing pocket depth, clinical attachment level (with the help of UNC-15 probe to the nearest millimeter), saliva samples were taken in both the groups. Scaling and root planing were performed in group II and samples were taken after one month. No antibiotics or anti-plaque and anti-inflammatory agents were prescribed after treatment. This was followed by a comprehensive phase I therapy which included

patient education and motivation, plaque control, scaling and root planing. One month later these measurements (GI, PD, CAL) and saliva sampling were repeated and Interleukin-6 was assessed by means of commercial Enzyme linked immunosorbent assay.

COLLECTION OF SALIVA

Unstimulated whole saliva was collected from all participants in a given time between (9:00a.m. and 11:00 a.m.). The subjects (cases and controls) were refrained from eating, drinking, and practicing oral hygiene habits (flossing, brushing, and mouth rinses) within at least 2 hours prior to saliva collection. Subjects were asked to rinse their mouth with distilled water, following which they expectorated at least 3mL of un-stimulated whole saliva into a 5mL sterile tubes. All samples were immediately centrifuged at 3000 rpm for 20 minutes and clear supernatant were stored at-20ºC pending analysis. Sterile tubes contaminated with blood and saliva were excluded from the sampled group. Saliva samples were collected again after one month.

SALIVA ANALYSIS

Biochemical analysis of Saliva samples were done to estimate the level of Interleukin-6.

IL-6 concentration was measured according to manufacturer's instructions using commercially available sensitive ELISA kit (Boster Biological Technology co.LTD).

PRINCIPLE OF THE ASSAY:

Boster's human IL-6 ELISA Kit is based on standard sandwich enzyme-linked immune-sorbent assay technology. Human IL-6 specific-specific polyclonal antibodies has been precoated onto 96-well plates. The human specific detection monoclonal antibodies are biotinylated. The test samples and biotinylated detection antibodies are added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with PBS or TBS buffer. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue colour product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human IL-6 amount of sample captured in plate.

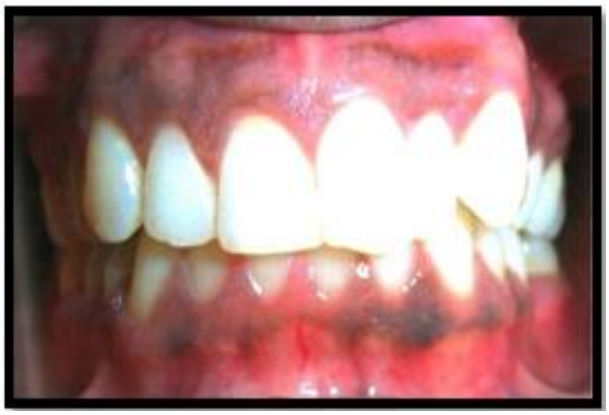


Figure 1: GROUP I(CONTROL GROUP)

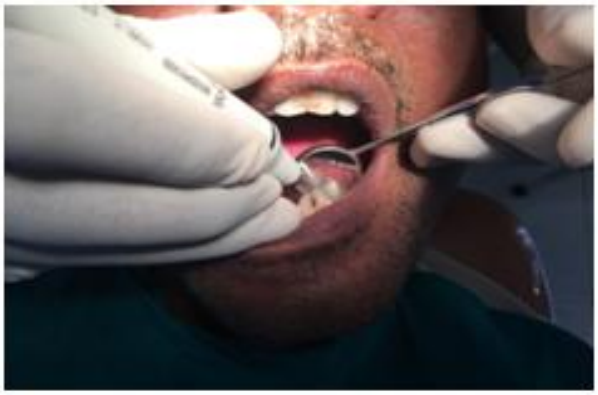


Figure 2: GROUPII(S.R.P DONE)

Figure 3: COLLECTION OF SALIVA

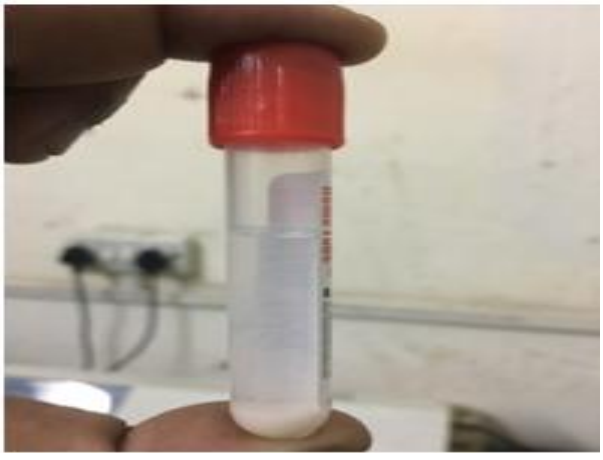


Figure 4: SALIVA SAMPLES CENTRIFUGED

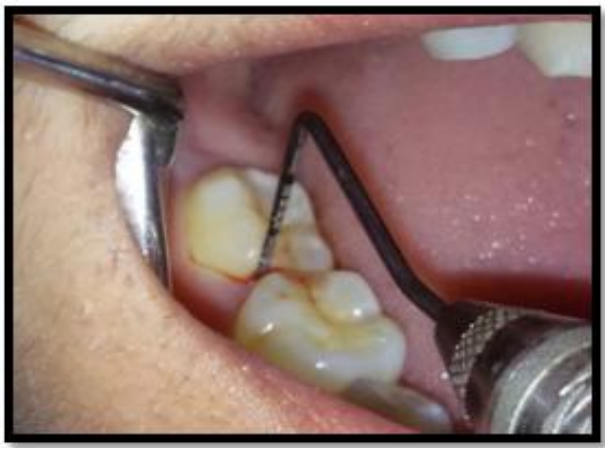


Figure 5: MEASUREMENT OF PROBING DEPTH BEFORE S.R.P

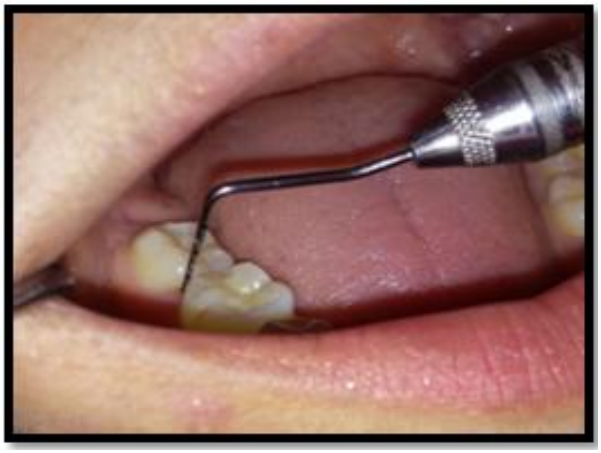


Figure 6: MEASUREMENT OF PROBING DEPTH AFTER S.R.P

Results

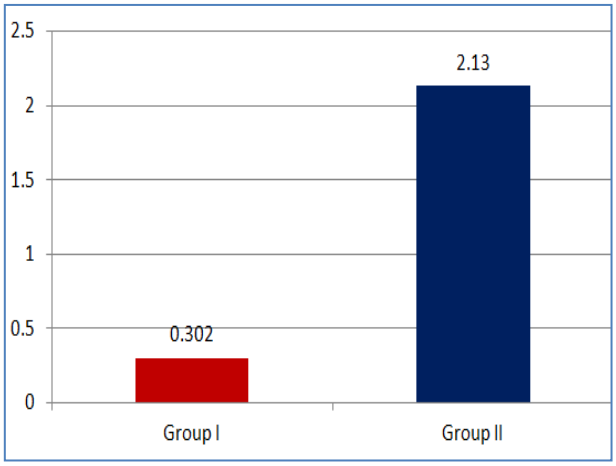
All subjects completed the entire study. Healing was uneventful in all cases. No adverse effects, such as discomfort and pain were reported by any of the subjects.

Clinical assessments

The results of the whole-mouth clinical measurements (mean±SD) between baseline and time points in test and control groups are displayed in graphs below

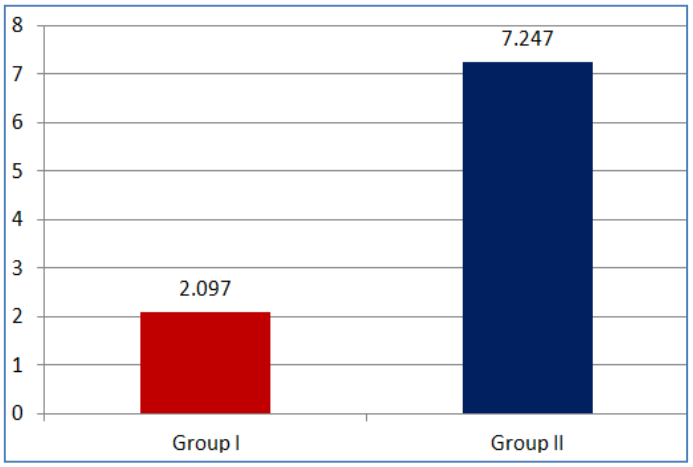
In test group, all clinical parameters showed statistically significant reductions after one month compared to baseline. The mean PD at baseline was 7.247±0.488 in the chronic periodontitis group and 2.097±0.391 in the control group. After treatment, these values became 4.086±0.794 at one month. The mean CAL at baseline was 7.916±0.530 in the chronic periodontitis group and 0.598±0.114 in the control group. After treatment, these values decreased to 5.071±0.869 at 1 month . The mean GI at baseline was 2.130±0.275 in the chronic periodontitis group and 0.302±0.088 in the control group. After treatment, these values decreased to 0.793±0.282 at 1 month. . The mean IL-6 value at baseline was 114.632 ±11.557 in the chronic periodontitis group and 11.663±0.880 in the control group. After treatment, these values decreased to 60.420 ±6.105 at 1 month.

INTERGROUP COMPARISON OF GINGIVAL INDEX SCORES BETWEEN TWO GROUPS



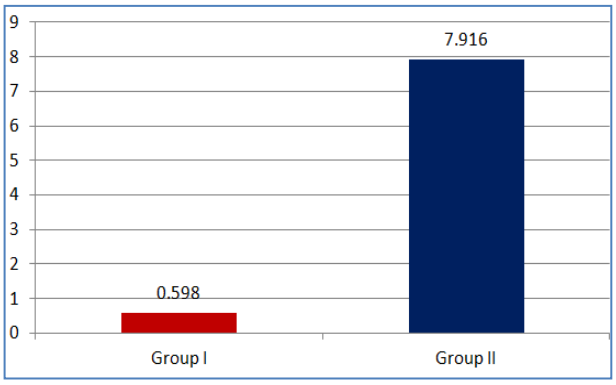
shows the intergroup comparisons of gingival index scores between two groups.The mean score for gingival index in group I was 0.302±0.088 where as in group II was 2.130±0.275

INTERGROUP COMPARISON OF PROBING DEPTH SCORES BETWEEN TWO GROUPS



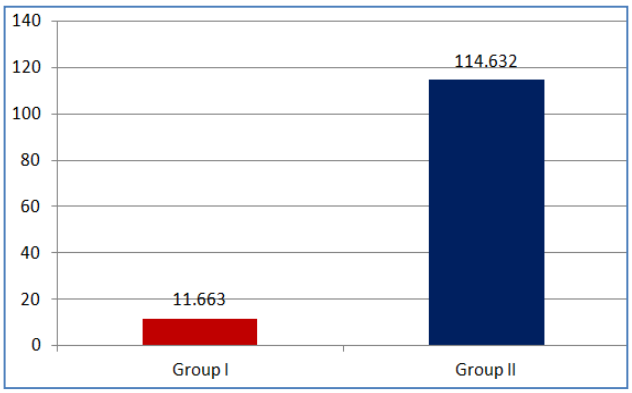
shows the intergroup comparison of probing depth scores between two groups.The mean score of probing depth in group I was 2.097±0.391 where as in group II was 7.247±0.489.

INTERGROUP COMPARISON OF CAL SCORES BETWEEN TWO GROUPS



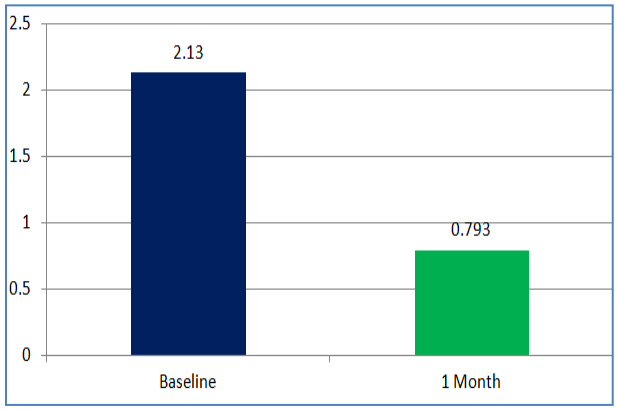
shows the intergroup comparison of C.A.L scores between two groups.The mean score for CAL in group I was 0.598±0.114 where as in group II was 7.916±0.531.

INTERGROUP COMPARISON OF SALIVARY ITERLEUKINS BETWEEN TWO GROUPS



shows the intergroup comparison of salivary IL6 scores between two groups.The mean score of IL6 in group I was 11.663±0.880 ng/l where as in group II was 114.632±11.557 ng/l.

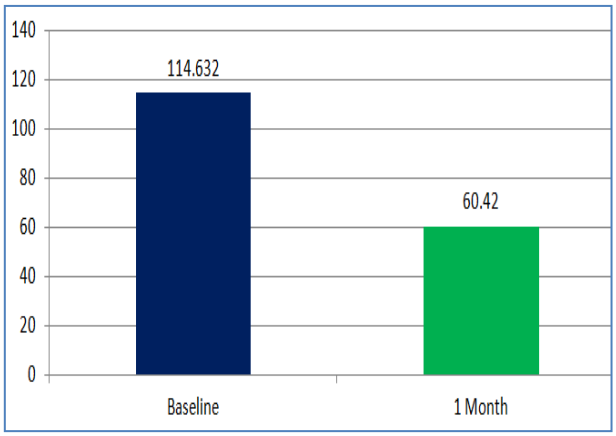
INTRAGROUP COMPARISON OF GINGIVAL INDEX SCORES BETWEEN BASELINE AND 1 MONTH TIME PERIOD



shows the intragroup comparison of gingival index scores between baseline and one month time period.

.The mean score for gingival index at baseline was 2.130±0.275 and after one month was 0.793±0.282

INTRAGROUP COMPARISON OF INTERLEUKIN-6 LEVELS BETWEEN BASELINE AND 1 MONTH TIME PERIOD



Shows the intragroup comparison of interleukin-6 levels between baseline and one month time period.The mean score for IL6 at baseline is 114.632±11.557 and after one month is 60.420±6.105.

DISCUSSION

Periodontal diseases are bacterial infections characterized by inflammation and destruction of the attachment apparatus, often leading to tooth level. 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.

Several clinical studies have clearly indicated that scaling and root planing, in combination with optimal oral hygiene, result in an alteration of the subgingival plaque which is sufficient to stop periodontal destruction in most cases.

According to **Zhang L et al**[4], traditional diagnostic measures, such as periodontal pocket depth, attachment level, plaque index, bleeding on probing and radiographic assessment of alveolar bone level, are informative to evaluate disease severity but provide few useful determinants of disease activity **.**

Saliva is one of the most important body fluids, which contains a large number of proteins and peptides that are easily accessible and may serve as a potential source to measure biomarkers released during disease initiation and progression, it has significant

association with inflammatory, connective tissue destruction and bone remodeling phases of periodontal disease.[5]

In the present study, unstimulated whole saliva was collected from all participants The subjects (cases and controls) were refrained from eating, drinking, and practicing oral hygiene habits (flossing, brushing, and mouth rinses) within at least 2 hours prior to saliva collection. Subjects were asked to rinse their mouth with distilled water, following which they expectorated at least 3mL of un-stimulated whole saliva into a 5mL sterile tubes. All samples were immediately centrifuged at 3000 rpm for 20 minutes and clear supernatant were stored at-20ºC pending analysis.The mean concentrations of salivary IL-6 was significantly elevated in chronic periodontitis subjects compared to controls which was similar to a study done by **Costa et al and Ebersole et al**

In our present study, an attempt was made to analyze the value of salivary IL6 as an indicator of the severity of periodontal disease. Therefore, in our study a total of 40 patients were selected and were allocated in two groups. –

Group I (control group) n=20 and

Group II (Chronic periodontitis patients) (n=20) (S.R.P done).

Salivary IL6 levels were assessed in control group and in test groups at baseline and 1 month after non-surgical periodontal therapy by means of a commercial Enzyme linked immunosorbent assay.

It was seen that the level of IL-6 in chronic periodontitis group was reduced to 60.420±6.105 ng/l at one month after phase-1 therapy compared to 114.362±11.557 ng/l at baseline.The level of IL6 in control group was 11.663±0.880 ng/l. The levels of IL6 were significantly different when compared between two groups.

The baseline values of salivary IL6 were in accordance to the study conducted by **Haween T. Nanakaly (2016)**[6] who evaluated that the salivary level of IL-6 was directly proportional with the extent of probing pocket depth, suggesting that IL-6 in saliva can be considered as one of inflammatory biomarkers of severity of periodontitis.

Alwan A.A et al(2015)[7] also conducted a study and found that the median level of IL-6 in the chronic periodontitis group (13.4 pg/mL) was higher than in the gingivitis, (7.5pg/mL) and control (5.27 pg/mL) groups this difference was statistically high significant. These findings were in agreement with results of (**Javed et al 2014)**[8], who reported that salivary IL-6 levels are elevated in patients with chronic periodontitis (CP).

However, a study performed by (**Teles R.P et al 2009)**[9] demonstrated that there was no difference in the levels of, interleukin-6 in saliva, between periodontal health and periodontal diseases. There were weak, statistically significant positive associations between salivary interleukin-8 and pocket depth ($rs = 0.2$, $p < 0.05$) and bleeding on probing ($rs = 0.2$, $p < 0.05$), and weak negative correlations between salivary interleukin-10 and attachment level ($rs =)0.2$, $p < 0.05$) and bleeding on probing ($rs =)0.3$, $p < 0.001$).He concluded that Mean salivary levels of granulocyte–macrophage colony-stimulating factor, interleukin-1b, interleukin-2, interleukin-4, interleukin-5, interleukin-6, interleukin-8, interleukin-10, interferon-c and tumor necrosis factor-a could not discriminate between periodontal health and disease.

Thus, it was seen that a positive association exists between the presence of chronic periodontitis and high salivary IL6 levels. So, in periodontal diseases, IL6 play key roles in the degradation of the extracellular matrix, basement membrane and protective serpins as well as in the modification of cytokine action and activation of osteoclasts. **(Haween T. Nanakaly,2016)**[6]

Although the clinical results in test groups were variable as the outcome of periodontal therapy depends on several factors such as the initial probing depth, type of tooth being treated, the type of site being treated, assessibility etc, still mechanical therapy reduced the IL6 levels effectively in the test groups.

One of the factors that could have influenced the results of this study was the age factor of the participants. Also,hidden factors such as genetics and undiagnosed conditions were not taken into cognizance in this study.

Moreover, whole saliva contains contributions from the GCF, oral bacteria, cells and other sources that make identification of the exact site of disease activity limited, and flow rate of saliva varies within and between subjects. Therefore, work needs to be done to confirm the usefulness of these salivary biomarkers.

REFERENCES

1. Tatakis D.N, Kumar P.S. Etiology and Pathogenesis of Periodontal Diseases. Dent Clin North Am 2005; 49:491-516.
2. Irwin C.R, Myrillas T.T. The role of IL-6 in the Pathogenesis of Periodontal Disease. Oral Dis 1998; 4(1): 43–7.
3. Xing Z, Gauldie J, Cox G, Baumann H, Jordana M, Lei X.F et al. IL-6 is an Anti-Inflammatory Cytokine Required for Controlling Local or Systemic Acute Inflaammatory Responses. J Clin Invest 1998; 101: 311-20.
4. Zhang L, Henson B.S, Camargo P.M & Wong D.T. The clinical value of salivary biomarkers for periodontal disease. 2009; Periodontology 2000; 51: 25-37
5. Thomas M.V, Branscum A, Miller C.S, Ebersole J, AlSabbagh M, Schuster J.L. Within-Subject Variability in Repeated Measures of Salivary Analytics in Healthy Adults. J Periodontol 2009;80 (7):1146–53
6. Haween T. Nanakaly. Interleukine-6 Level in Saliva of Patients with Chronic Periodontitis: A Case-Control Study.J Bagh college of dentistry .volume 28(1) march 2016.
7. Alwan A. H et al. Estimation of the level of Salivary Interleukin 6 (IL-6) and its' correlation with the clinical parameters in patients with periodontal diseases. Journal of Dental and Medical Sciences (IOSR-JDMS) e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 14, Issue 9 Ver. VI (Sep. 2015), PP 82-88.
8. Javed F et al . Whole Salivary Interleukin-6 and Matrix Metalloproteinase-8 Levels in Patients With Chronic Periodontitis With and Without Prediabetes. J Periodontol • May 2014 Volume 85 • Number 5
9. Teles R. P et al . Salivary cytokine levels in subjects with chronic periodontitis and in periodontally healthy individuals: a cross-sectional study. J Periodont Res 2009; 44: 411–417. 2009.