Evaluation of the Effect of Non-Surgical Periodontal Treatment on Circulating Serum High Sensitivity Capsule Reactive Protein, Interleukin 6 and Homocysteine Levels in Otherwise Healthy Subjects - A Case-Control Study

Shivani Sharma[1], Rahul Nagar[2], Mukesh Kumar[3], Sumit Tyagi[4], Avani Nagar[5]

ABSTRACT

Background: Periodontal disease has been associated with many chronic inflammatory systemic diseases and high levels of inflammatory markers in the blood. This study was conducted to compare the chronic inflammatory markers; serum high sensitivity-capsule reactive protein, interleukin 6 and homocysteine in control individuals and in patients with periodontal disease. Also, the effect of periodontal treatment was observed on these inflammatory disease markers 3 months post therapy.

Methods: This case-control study involved 25 (age- and sex-matched) subjects with chronic periodontitis and healthy controls each. Patients were systemically healthy and did not use any medication. Periodontal and systemic parameter assessments included Oral Hygiene Index, Plaque index, Gingival index, probing depth, clinical attachment level, hematologic parameters as well as the following inflammatory markers: interleukin (IL)-6, high-sensitivity C-reactive protein (hs-CRP) and homocysteine (Hcy).

Results: Significant reduction in Oral hygiene Index, Plaque Index, Gingival Index, probing depth, clinical attachment level were found 3 months post scaling. While comparing IL-6 (pg/ml), Capsule Reactive Protein (mg/dL), Serum homocysteine (µmol/L) levels among subjects of chronic periodontitis group pre and post treatment, significant results were obtained.

Conclusion: Increase in periodontal inflammation is accompanied by an increase in the levels of inflammatory markers i.e C Reactive protein, IL-6 and Hcy levels. Increase in these markers have been associated with risk of systemic diseases. Non-surgical periodontal therapy causes significant reduction in periodontal inflammation as well as the levels of these inflammatory markers.

Keywords: C reactive Protein, Interleukin 6, Homocysteine, Inflammation, Periodontitis

INTRODUCTION

In periodontal disease an inflammatory process affects the supporting structures around the tooth. Accumulation of bacterial biofilm on the tooth causes inflammation of the marginal tissue called as gingivitis. In cases where gingival inflammation is not treated it may progress to periodontitis, which results in loss of attachment, loss of alveolar bone, leading to tooth mobility and tooth loss.[1] High concentrations of inflammatory markers, i.e C-reactive protein[2] (CRP), fibrinogen[3], and cytokines[4] are seen in these patients. Studies have shown a decrease in level of IL-6 after periodontal therapy.[5]

IL-6 is a procoagulant cytokine[6] and CRP induces the expression of molecules responsible for cellular adhesion, which mediates the attachment of leukocytes to the vascular endothelium[7]. Homocysteine (Hcy) is a sulfur containing amino acid produced during the metabolism of methionine. Increased levels of homocysteine results in multiple vascular effects attributing to atherothrombosis and peripheral arterial disease.[8] Also, a positive relation exists between the levels of homocysteine and parameters of inflammation, such as levels of cytokines,[9] adhesion molecule (s Icam-1)[9] and C-reactive protein.[10]

In chronic inflammation, several immunologic and enzymatic factors which have been recognized as biomarkers, have been and are currently being studied to understand the disease activity along with establishing their diagnostic and prognostic importance. Biomarkers can be utilized for monitoring the response to treatment.[11] A novel link between periodontal disease and other systemic inflammatory diseases can be explored by determining the effects of non-surgical periodontal therapy on the levels of these markers.[12] Hence, the purpose of this study is to evaluate the effect of non-surgical periodontal treatment on circulating serum high sensitivity capsule reactive protein, IL-6 and homocysteine levels in otherwise healthy subjects.

MATERIALS & METHODS

Of the total number of patients reporting to the OPD, Department of Periodontology and Implantology, 75 patients were examined for eligibility based on the inclusion and exclusion criteria., 50 patients were selected (using

[1]Professor, Department of Periodontology, Kalka Dental College, Meerut, Uttar Pradesh, [2]Private Practitioner, Senior Dentist, Jain Surgical and Maternity Centre, Pitampura. Delhi, [3]Medical Officer (Dental), Community Health Centre, Kurawali, Mainpuri, Uttar Pradesh, [4]Private Practitioner, Dental Care Centre, Shahdara. Delhi, [5]Private Practitioner, Jain Surgical And Maternity Centre, Pitampura. Delhi

Corresponding author: Dr. Shivani Sharma, D-10, Mansarovar Park, Shahdara. Delhi-32

How to cite this article: Shivani Sharma, Rahul Nagar, Mukesh Kumar, Sumit Tyagi, Avani Nagar. Evaluation of the effect of non-surgical periodontal treatment on circulating serum high sensitivity capsule reactive protein, interleukin 6 and homocysteine levels in otherwise healthy subjects - a case-control study. International Journal of Contemporary Medical Research 2024;11(9):I1-I5.

power:0.8, CI:95, α:0.05, Odd ratio:1; n=22, considering the unknown error, the sample size was increased to 25). 25 chronic periodontitis patients (cases; mean age 35.6 years) and 25 patients without periodontitis (controls; 36.7 years) were selected; by coin toss method for each group by an independent examiner not participating in this case-control study. The study protocol followed the ethical outlines given in 1964, declaration of Helsinki as revised in 2013 and was approved by the Ethical Committee of the institute. (KDC/IEC/2016/02)

Inclusion Criteria included: Subjects with age range 35 - 55 years, minimum 20 natural teeth, probing depth (PD) ≥5 mm, attachment loss ≥6 mm in at least 2 teeth, and radiographic evidence of alveolar bone loss, patients who signed the consent form.

Exclusion Criteria included patients of periodontal surgery, or antimicrobial therapy within last six months, those who were regularly taking any form of drug therapy in previous 6 months. Pregnancy, lactation & smokers in the previous 10 years, presence of any chronic inflammatory or immunologic disease, and <20 permanent teeth remaining.

The case group comprised of 25 subjects with untreated chronic periodontitis. The control group included systemically and periodontally healthy subjects selected from the volunteer's accompanying patients, staff and students of dental college. Patients were assessed using a questionnaire which included recording of characteristics, such as age, gender, diet, medical history, body mass index. Full-mouth IOPA were also taken.

Turesky-Gilmore-Glickman modification of the Quigley Hein plaque index[13], Simplified Oral Hygiene Index (calculus component),[14] and Modified Gingival Index[15] were used for the assessment of plaque, oral hygiene and gingival status. Pocket depth and clinical attachment level were measured using a graduated periodontal probe with William's markings on all six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual). All measurements were done by a single examiner. These parameters were evaluated at the baseline and after 3 months of completion of initial phase of periodontal treatment for case group and for control group at baseline only.

Both case and control groups were given instructions on plaque control techniques including demonstration of brushing and use of dental floss. Case group received non-surgical periodontal therapy over a period of four weeks. Subjects were put up on follow up of 15 days for 3 months and oral hygiene instructions were reinforced in every visit. The case group were re-examined after completion of non-surgical periodontal treatment (scaling and root planing) 3 months post-operatively and the clinical parameters were recorded.

Blood samples were collected at baseline for control group, at baseline and at three months post-operatively for the case group. The blood collection was done in plain vials and biochemical analysis for the evaluation of systemic parameters was done in the laboratory for both the case and control group.

Serum homocysteine and Interleukin 6 were assessed by means of Enzyme linked Immunoassay.

Serum CRP was determined using a latex agglutination test.

STATISTICAL ANALYSIS

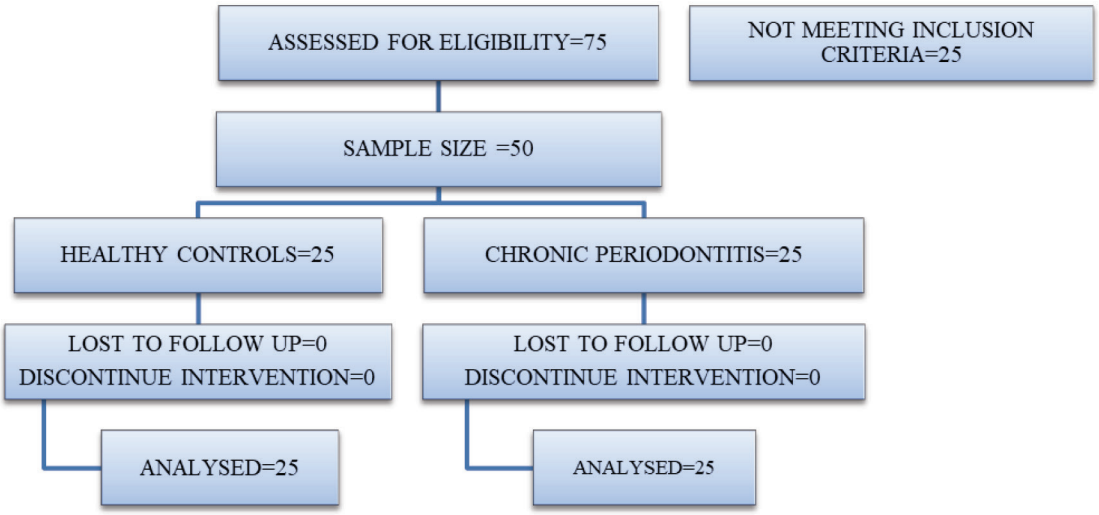
All the results were analysed by SPSS version 27.0 software.

Groups N= 25 each)	Control group (Baseline)	Case group (Baseline)	Case Group (3 months)	Control Vs Case Group (Baseline)	Case Group (Baseline) Vs Case Group (3Months)
parameters	**Mean ±SD**	**Mean ±SD**	**Mean ±SD**	**P- value**	**P- value**
CI-OHIS	0.07±0.15	2.53 ±0.38	0.03 ±0.04	0.01*	0.00*
PI	0.17±0.09	2.49 ±0.39	0.17 ±0.11	0.03*	0.01*
GI	0.05±0.08	2.49 ±0.75	0.04±0.03	0.00*	0.00*
PD (mm)	0.89±0.32	3.23 ±0.66	2.53±0.71	0.00*	0.00*
CAL (mm)	0.94±0.41	3.76±0.99	2.61±0.79	0.01*	0.02*
P value *Significant					

Table-1: Comparison of the periodontal parameters between control group (baseline) and case group at baseline & case group at baseline and after 3 months of non-surgical therapy.

Groups N= 25 each)	Control group (Baseline)	Case Group (Baseline)	Case Group (3 months)	Control Vs Case Group (Baseline)	Case Group (Baseline) Vs Case Group (3Months)
parameters	**Mean ±SD**	**Mean ±SD**	**Mean ±SD**	**P- value**	**P- value**
IL-6	7.36±1.16	14.66±2.08	7.63±1.56	0.01*	0.00*
Hs-CRP	0.26±0.04	0.685±0.10	0.59±0.06	0.03*	0.04*
Homocysteine	14.17±2.36	20.22±3.41	13.11±2.12	0.0*	0.03*
P value *Significant **Non-significant					

Table-2: Comparison of serum levels of IL-6, Hs-CRP, Homocysteine between control and case group at baseline & case group at baseline and after 3 months of non-surgical therapy



Flow chart

Wilcoxon signed-ranks test was used for comparison of the parameters within the group at baseline and 3 months post-treatment. Mann Whitney U test was used for inter-group comparison of assessed parameters. For assessment of level of significance, p- value < 0.05 was considered significant.

RESULTS

All the participants completed the study, the mean age of subjects for the case and control group was 35.6 years and 36.7 years respectively. Intergroup comparison of the periodontal parameters i.e CI-OHIS, PI and GI showed a statistically significant difference between the control and case group at baseline. Significant reduction in these parameters with p values (<0.05) was seen within the case group after 3 months of non-surgical therapy. (Table 1)

Probing depth between control and case group at baseline was reported to be 0.89± 0.32 for the control group and 3.23 ±0.66 for the case group, which was found to be significant with the p value of 0.00 (< 0.05). Further, intra group comparison of probing depth within the case group showed statistically significant reduction with p value of 0.00 (< 0.05) after 3 months of non-surgical therapy. Similarly, for intergroup comparison of CAL between the control and case group at baseline, a statistically significant difference was found with mean values, 0.94±0.41 and 3.76±0.99 with p value of 0.01(<0.05), respectively. Also, three months intra-group comparison of CAL showed statistically significant reduction with p value of 0.02 (<0.05) (Table 1)

On comparing levels of IL- 6, C Reactive Protein, Serum homocysteine, significant difference was found, with the mean values of 7.36±1.16 and 14.66±2.08, p value 0.01(<0.05) for IL-6, 0.26±0.04 and 0.6± 0.1, p value 0.03 (<0.05) for CRP, 14.17±2.36 and 20.22±3.41, p value 0.0 (<0.05) for homocysteine among subjects of control and case group respectively at baseline. Further, on comparing IL- 6 (pg/ml), Capsule Reactive Protein (mg/dL), Serum homocysteine (µmol/L) levels among subjects of chronic periodontitis group pre-treatment and 3 months post treatment, statistically significant reduction was found with p values 0.0, 0.04, 0.03 respectively (<0.05). (Table 2)

DISCUSSION

Periodontitis has been identified as a risk factor for many systemic illnesses including CVD. In periodontitis, the released bacterial products result in the production of cytokines like interleukin 1, and tumour necrosis factor α. These cytokines cause atherogenesis which subsequently lead to CVD.

CRP is an important marker for an inflammatory state and an has also been established as an independent predictor of CVD disease. Thus, to assess the risk of CVD, the American Heart Association and Centre for Disease Control and Prevention (CDC) has recommended its clinical use.[16] Serum CRP levels can also be used to illustrate active disease and can also differentiate active from an inactive one. Recently, CRP has been proved to be the strongest and most significant predictor of future cardiovascular events.[17]

In chronic periodontitis the host in response to inflammation and tissue injury releases one of the key cytokines such as IL-6 which induces bone resorption by itself along with other bone-resorbing agents.[18] Hcy is a product of the methionine metabolic pathway. In patients with chronic periodontitis elevated levels of plasma Hcy has been detected.[19] Elevated plasma Hcy levels increase the risk for CVD, stroke and Alzheimer's disease. Periodontal inflammation has shown to affect the methionine metabolism leading to increased plasma Hcy levels.[19] Thus, the present study was conducted to evaluate the effects of non-surgical periodontal treatment on circulating serum high sensitivity capsule reactive protein, IL-6 and homocysteine levels in otherwise healthy subjects.

In our study the baseline value of serum IL-6 for control and case group at baseline and at 3 months post-operatively, correlate with the finding of Loos et al[20] and Pitiphat et al.[21] who have found elevated levels of CRP and IL-6 in patients with chronic periodontitis who had much higher levels for IL-6 than the controls.[20] Thus, the elevated values of IL-6 and hsCRP in the case group subjects showed that periodontitis resulted in an increase in systemic inflammatory burden.

However, these results could not be applied to patients with less severe and or more localized periodontitis. Matilla et al[22] in their study reported reduction of hsCRP after therapy in some individuals and suggested that this may thus decrease their risk of coronary heart disease. Results of our study were also in accordance with the study of Marcaccini et al[12] who showed increased concentrations of IL-6 and hsCRP, which decreased 3 months post treatment in patients with periodontal disease.

The results of our study were in contrast to the study of George & Janam[23] and Yamazaki et al[24] who showed statically insignificant difference in IL-6 and hsCRP levels before and after treatment. They reported that this insignificant difference could be due to relatively small numbers of patients and varied effects of periodontal disease to the total inflammatory burden in different patients.

In our study, on comparing serum homocysteine levels within the subjects of chronic periodontitis pre and post treatment, significant results were obtained. The results of our study were also in accordance with the results obtained by Mallapragada et al[19], Bhardwaj et al[25], Joseph et al[26] who showed that chronic periodontitis resulted in an increase in hsCRP and Hcy levels in plasma and periodontal treatment decreased periodontal inflammation, which further reduces systemic inflammation causing a decrease in their levels.

The host response to bacteria in any form of systemic challenge can thus provide explanation for the mechanisms of the interaction between periodontal infection and a variety of systemic disorders. Any therapeutic oral changes or the inappropriate intervention or absence of intervention of periodontitis can have its influence on the levels of systemic inflammation.[5] The results presented in this study suggest that elevated IL-6, Hcy and CRP values in periodontitis patients could contribute to the increased risk for cardiovascular disease.

Further, certain limitations of this study can be overcome by increasing the sample size with the assay of vitamin B6 to provide a panoramic view of the role of C Reactive proteins, IL-6 and Hcy in periodontal disease. Also, role of antimicrobial periodontal treatment on these systemic inflammatory mediators should be determined and their association with the risk for various systemic diseases should be studied thoroughly.

CONCLUSION

Increase in periodontal inflammation is associated with a marked increase in the concentration of C Reactive proteins, IL-6 and Hcy levels. Scaling and root planing reduces C Reactive proteins, IL-6 and Hcy levels, resulting in decrease in periodontal inflammation which may result in reduction of the risk for cardiovascular disease development.

REFERENCES

1. Shaddox LM, Walker CB. Treating chronic periodontitis: current status, challenges, and future directions Clin Cosmet. Investig Dent. 2010;2:79–91.
2. Loos BG, Craandijk J, Hoek FJ, Wertheim-van Dillen PM, Van Der Velden U. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. J. Periodontol 2000;71:1528-1534.
3. Kweider M, Lowe GD, Murray GD, Kinane DF, McGowan DA. Dental disease, fibrinogen and white cell count; links with myocardial infarction? Scott Med J 1993;38:73-74.
4. Gorska R, Gregorek H, Kowalski J, Laskus-Perendyk A, Syczewska M, Madalinski K. Relationship between clinical parameters and cytokine profiles in inflamed gingival tissue and serum samples from patients with chronic periodontitis. J Clin Periodontol 2003;30:1046-1052.
5. D'Aiuto F, Nibali L, Parkar M, Suvan J, Tonetti MS. Short term effects of intensive periodontal therapy on serum inflammatory markers and cholesterol. J. Dent Pes 2005;84:269-273.
6. Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. Circulation 2004; II2-10.
7. Yeh ET, Anderson HV, Pasceri V, Willerson JT. C reactive protein: linking inflammation to cardiovascular complications. Circulation 2001;104:974-975.
8. Lazzerrini PE, Capecchi PL, Selvi E. Lorenzini S, Bisogno S. Galeazzi M et al. hyperhomocysteinemia: A cardiovascular risk factor in auto-immune diseases? Lupus 2007; 16:852-862.
9. Wallberg-Jonsson S, Cvetkovic JT, Sundqvist KG, Lefvert AK, Rantapa¨a¨-Dahlqvist S. Activation of the immune system and inflammatory activity in relation to markers of atherothrombotic disease and atherosclerosis in rheumatoid arthritis. J Rheumatol 2002;29:875-882.
10. Schroecksnadel K, Frick B, Winkler C, Leblhuber F, Wirleitner B, Fuchs D. Hyperhomocysteinemia and immune activation. Clin Chem Lab Med 2003;41:1438-1443
11. Gokhale NH, Acharya AB, Patil VS, Trivedi DJ, Setty S, Thakur SL. Resistin levels in gingival crevicular fluid of patients with chronic periodontitis and type 2 diabetes mellitus. J Periodontol 2014;85:610-17.
12. Marcaccini AM, Meschiari CA et al. Circulating Interleukin-6 and High-Sensitivity C-Reactive Protein Decrease After Periodontal Therapy in Otherwise Healthy Subjects. J Periodontol 2009;80:594-602.
13. Greene G, Vermillion J. The simplified Oral Hygiene Index. J. Am Dent Asso. 1964;68:1-13
14. Loe H, Silness. The gingival Index, plaque index, and retention index system. J. Periodontol 1967;38(6):610-616
15. Silness J, Loe H. Periodontal diseases in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand. 1964;22:121-135.
16. Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontol 2000.2014;64(1):57-80.
17. Tousoulis D, Charakida M, Stefanadis C. Endothelial function and inflammation in coronary artery disease. Heart 2006;92:441-444.
18. Gorgun PE, Toker H, Korkmaz EM, Poyraz O. IL-6 and IL-10 gene polymorphisms in patients with aggressive periodontitis: effects on GCF, serum and clinic parameters. Braz.Oral Res 2017;31.

19. Mallapragada S, Kasana J, Agrawal P. Effect of nonsurgical periodontal therapy on serum highly sensitive capsule reactive protein and homocysteine levels in chronic periodontitis: A pilot study. Contemp Clin Dent 2017;8(2):279.
20. Loos BG. Systemic markers of inflammation in periodontitis. J. Periodontol 2005;76:2106-2115.
21. Pitiphat W, Savetsilp W, Wara-Aswapati N. C-reactive protein associated with periodontitis in a Thai population. J Clin Periodontol 2008;35:120-125.
22. Matilla k, Vesanen M, Valtonen V, Nieminen M, Paslosuo T, Rasi V et al. effect of treating periodontitis on C-reactive protein levels: A pilot study. BMC Infect Dis 2002;2:30
23. George AK, Janam P. The short-term effects of non-surgical periodontal therapy on the circulating levels of interleukin-6 and C-reactive protein in patients with chronic periodontitis. J Indian SocPeriodontol 2013;17:36-41.
24. Yamazaki K, Honda T, Oda T, Ueki- Maruyama K, Nakajima T, Yoshie H, et al. Effect of periodontal treatment on the C-reactive protein and proinflammatory cytokine levels in Japanese periodontitis. J. Periodontol Res 2005;40:53-58.
25. Bhardwaj S, Prabhuji MV, Kartikeyan BV. Effect of non-surgical periodontal therapy on plasma homocysteine levels in Indian population with chronic periodontitis. A pilot study. J Clin Periodontol. 2015;42(3):221-227.
26. Joseph R, Nath SG, Joseraj MG. Elevated plasma homocysteine levels in chronic periodontitis: A hospital based study. J Periodontol 2011;82:439-444.

Source of Support: Nil; **Conflict of Interest:** None

Submitted: 28-07-2024; **Accepted:** 24-08-2024; **Published:** 30-09-2024