DOI: 10.7860/JCDR/2017/22778.9599

Original Article

Dentistry Section

To Evaluate and Compare Periodontal Disease and Smoking as a Parallel Risk Factor for Systemic Health by Gauging the Serum C-Reactive Protein Levels

RUCHI DINESH RAVAL[1], PAYAL SHARMA[2], SARATH CHANDRAN[3], DHARMESH VASAVADA[4], PRIYADARSHINI NADIG[5], GAURAV BAKUTRA[6]

ABSTRACT

Introduction: Physiologic and metabolic changes that occur immediately after a damage or disease are known as Acute Phase Reaction (APR). Acute Phase Proteins (APP) are blood proteins secreted by hepatocytes during APR C-Reactive Protein (CRP) being the important one.

Aim: Present study was designed to estimate and compare the levels of the serum CRP in current smokers, former smokers and non-smokers, with and without periodontitis.

Materials and Methods: An experimental study was planned on 165 subjects who were divided into four groups. Group 1- nonsmokers with periodontitis. Group 2- smokers without periodontitis. Group 3- smokers with periodontitis. Group 4- former smokers without periodontitis. Healthy controls were not included in the study as the normal range of CRP in health is already established. Periodontal examination was done and serum CRP was measured. After getting the acceptance to be a part of the study, written informed consent was taken from each participant. Data analysis was done by ANOVA and post-hoc tests.

Results: Highest level of CRP was found in smokers with periodontitis followed by non-smokers with periodontitis and smokers without periodontitis. Former smokers had minimum CRP compared to the other groups (p-value=0.03).

Conclusion: Periodontitis alone and in combination with smoking increases the systemic inflammatory burden and associated cardiovascular risk. This fact should be communicated thoroughly to the general population, general dentist, physicians and cardiovascular specialist to enhance early screening and multidisciplinary treatment.

Keywords: Acute Phase proteins, Chronic periodontitis, Inflammation, Tobacco

INTRODUCTION

Keen interest in relation between oral and systemic health has been developed since many years. In 17th century for the first time the repercussions of oral health on human body was found. In the late 20th century, the term "Periodontal Medicine" was introduced to display inter-relationship between oral and systemic health [1]. Serum concentration of the APP changes by minimum of 25% as a reaction to inflammation and includes proteins of the complement, coagulation and fibrinolytic system, antiproteases, transport proteins, inflammatory mediators and others [2]. APR refers "to physiological and metabolic alterations that ensue immediately after the onset of infection or tissue injury". CRP, an acute-phase marker in inflammation is produced as a reaction to various types of injury apart from cell death or surface pathogens, it is non specific in nature and distressed by slight changes in the body [3,4]. These results into several systemic manifestations like release of plasma proteins from liver, stimulation of plasma proteins and metabolic modifications [5]. Association has also been found between CRP with smoking, obesity, triglycerides, diabetes and periodontal disease [6].

An elevated level of CRP can result in increased risk for cardiovascular diseases, cancers, neurodegenerative conditions, etc. Substantial amount of work has been done to validate smoking and periodontitis as an established risk factor for systemic diseases [7]. This study was designed to estimate the levels of the serum CRP in current and former smokers with or without periodontitis and to postulate periodontal disease as an equivalent risk factor to smoking for systemic health.

MATERIALS AND METHODS

An experimental study was planned on a population of smokers with or without periodontitis. A total of 165 patients, 86 males and 79 females (matched), within age range of 30 to 50 years, with Indian nationality and belonging of high, middle and low socioeconomic status were selected from the Outpatient Department of Periodontics and Implantology, Manubhai Patel Dental College and Hospital, Vadodara, Gujarat, India, after fulfilling the inclusion andexclusion criteria. The study protocol was approved by Institutional Research and Ethical Committee (IEC), Bhavnagar University, India. Informed consent was provided by all the participants. Sample size of 165 patients was chosen, to estimate Standard Deviation (SD) of CRP level by 0.05 with 0.01 absolute precision at 1% risk. A pilot study was conducted and based on the finding of the pilot study, statistical software NMASTER2 was used for sample size determination. Patients who underwent periodontal treatment, who were prescribed drugs such as antibiotics, non steroidal anti inflammatory or immune-modulators in last six months, lactating females, obese patients, one having recent history of trauma and on nicotine replacement therapy were excluded. Patients with systemic diseases or infection were also excluded from the study. Based on smoking and periodontal status, the patients were divided into four groups.

Group 1 (45 participants)- non-smokers with chronic periodontitis. The non-smoker participants consisted of patients who never smoked. Patients with moderate generalized chronic periodontitis (i.e., 3-4 mm attachment loss, ≥ 30% sites involved of the mouth) were recruited for the study [8].

Group 2 (45 participants)- current smokers without chronic periodontitis. Patients who have smoked ≥ 5 cigarettes per day for five years or more were considered as current smokers.

Group 3 (45 participants)- current smokers with chronic periodontitis.

Group 4 (30 participants)- former smokers without chronic periodontitis. Patients who quit smoking for ≥ 6 months were considered as former smokers.

Clinical parameters including Plaque Index (PI) [9] (on selected index teeth using mouth mirror and explorer), Bleeding on Probing (BOP) [10] and Clinical Attachment Level (CAL) [11,12] were evaluated.

History of smoking was taken including the number of years of smoking, number of cigarettes per day, type of cigarette and type of smoking habit.

To assess CRP, 5 ml blood was drawn by venipuncture from the median cubital vein which lies within the cubital fossa anterior to the elbow using needle of 23 guage in plain bulb. The blood was subsequently centrifuged for 15 minutes and serum obtained was used for the estimation of CRP. A 5 µl of serum sample was mixed with the working reagent (1 ml of latex reagent and 9 ml of diluent) and the sample was run on a Mindray Semiautomatic Biochemical Analyser. Quantitative measurement of serum CRP is done by turbidimetric test known as turnilatex [13].

STATISTICAL ANALYSIS

Data analysis was done by Post-hoc test and ANOVA.

RESULTS

Mean values, SD and p-value for metabolic parameter of CRP are presented in [Table/Fig-1] and results for clinical parameters CAL, BOP and PI are displayed in [Table/Fig-2]. Multiple intergroup comparisons with $p<0.05$ using post-hoc test (Tukey HSD) depending on CRP levels showed a statistical significant difference between Group 3 and Group 4 [Table/Fig-3]. An association between cigarette and bidi smoking to CRP level was evaluated using Chi-square test. The test results indicated a non significant relationship (p= 0.367). Corelation of CRP with CAL, BOP and PI evinced CRP having significant corelation with BOP, unlike with PI and CAL [Table/Fig-4]. There was a significant difference observed between mean CRP values of each group (p=0.36). [Table/Fig-5] shows group wise age and gender distribution of 165 participants with mean being 36.80±8.4 years.

Groups	N	Mean	Std. Deviation	Minimum	Maximum	p-value
Group I	45	3.53	2.22	1.00	15.00	0.03*
Group II	45	3.41	1.15	2.00	6.00	
Group III	45	3.70	2.48	1.00	17.00	
Group IV	30	2.44	0.80	1.00	5.00	
Total	165	3.35	1.90	1.00	17.00	

[Table/Fig-1]: Distribution of subjects in each group on the basis of C-Reactive Protein (mg/l).
*- Statistically significant difference between the four groups using ANOVA (p<0.05)

Parameter		N	Mean	Std. Deviation	Minimum	Maximum
CAL	Group 1	45	3.12	1.64	2.75	7.23
	Group 2	45	1.11	0.28	0.05	1.87
	Group 3	45	3.29	1.59	1.41	9.10
	Group 4	30	1.23	1.60	1.50	4.12
BOP	Group 1	45	2.71	1.54	1.41	5.00
	Group 2	45	1.27	1.21	0.00	3.08
	Group 3	45	3.37	2.51	2.12	5.37
	Group 4	30	1.76	1.70	0.17	4.29
Plaque Index	Group 1	45	2.13	1.57	0.91	3.00
	Group 2	45	1.65	0.76	0.66	2.79
	Group 3	45	2.78	1.76	0.83	5.40
	Group 4	30	1.32	0.54	1.08	4.80

[Table/Fig-2]: Distribution of subjects in each group on the basis of Clinical Attachment Level (CAL), Bleeding on Probing (BOP) and Plaque Index (PI).
*- Statistically significant difference between the four groups using ANOVA (p<0.05)

Groups	p-value
Group 1 and Group 2	0.99
Group 1 and Group 3	0.97
Group 1 and Group 4	0.06
Group 2 and Group 3	0.88
Group 2 and Group 4	0.12
Group 3 and Group 4	0.02(*)

[Table/Fig-3]: Multiple inter-group comparisons using post-hoc test depending on CRP levels.
Multiple inter-group comparisons using post-hoc test (Tukey HSD) depending on CRP levels showed a statistical significant difference between Group 3 and Group 4
*-The mean difference is significant at the 0.05 level

		CRP r value (p-value)	CAL r value (p-value)	BOP r value (p-value)	PI r value (p-value)
CRP	Pearson Correlation	1	-0.005	0.199*	0.085
	Sig. (2-tailed)		0.949	0.013	0.294

[Table/Fig-4]: Correlation of CRP with CAL, BOP and PI.
No correlation of CRP with CAL and PI. Significant correlation between CRP and BOP

Groups	Number of individuals	Age		Gender	
		Mean	Std. dev	Males	Female
I	45	36.9	8.4	25	20
II	45	36.7	8.6	18	27
III	45	36.5	8.9	24	21
IV	30	36.3	9.05	19	11
TOTAL	165	36.8	8.4	86	79

[Table/Fig-5]: Group wise age and gender distribution.
No correlation of CRP with CAL and PI. Significant correlation between CRP and BOP

DISCUSSION

Pathologic changes occurring in tissues of peridontium- gingiva, periodontal ligament, cementum and alveolar bone is known as periodontal disease. The branch of "Periodontal Medicine" was introduced to show the relationship between oral infection and systemic disease. APP levels elevates (positive APR) or lessens (negative APR) by minimum 25% in inflammatory reactions, with CRP displaying the quantification of the acute phase response. [14]. The purpose of these responses is to restore homeostasis and to remove the cause of its disturbance [15]. Being the most sensitive APP, CRP describes systemic inflammation by series of cytokines like IL-6 [16]. Old age, smoking, chronic bacterial infections, and chronic bronchial inflammation are the established risk factors for "high-normal" values of CRP [17].

Rise in APR have been found in localized infections due to systemic inflammation [18]. When bacteria, bacterial products and cytokines enter blood circulation, increase in value of CRP, IL-6 and neutrophils in patients with periodontal disease have been recorded [19]. Due to elevation in polymorphonuclear neutrophils released from bone marrow, smokers have higher levels of white blood cells [20]. CRP gene expression is induced during lung inflammation due to the stimulation of bone marrow cells and release of IL-β and IL-6 [21]. A research has shown that, following nine days of abstinence, when levels of inflammatory markers are measured in blood of intermittent smokers 24 hours post two cigarettes, TNF-α, IL-10 and IL-1β were same but IL-8 was elevated after three hours [22].

In the present study, subjects were divided into four groups, and the levels of serum CRP were evaluated and compared following

periodontal examination. Analysis of the results showed that highest level of CRP was found in smokers with periodontitis followed by non-smoker periodontitis patients and smoker non periodontitis patients. Former smokers had minimum CRP compared to the other groups. Former smokers who had quit smoking for the longer duration had CRP levels much lower than the ones who had quit recently. Nevertheless in Group 3, it was difficult to substantiate whether the change in CRP is due to smoking or periodontitis. The many fold increase in its proportion cannot be precisely predicted and none of the studies had been able to explain the same.

Difference between Group 1 and Group 2 was not statistically significant evincing the analogous contribution put by smoking and periodontal infection on inflammatory load by elevation of CRP. This finding is strengthened by Vigushin DM et al., depicting oral disease as a risk factor for cardiovascular disease by channel of systemic inflammatory reaction and thus putting "apparently healthy" patients to cardiovascular risk [23].

Additionally, CRP levels were also found to increase with increase in frequency, number of cigarettes smoked per day and duration of time of smoking. Yet, type of smoking did not have much of out-turn. No statistical difference was found in the levels of CRP between bidi and cigarette smokers. This was supported by the Das I. in his study, which did not show any disparity in nicotine concentration of filtered and unfiltered cigarettes ($p>0.05$) [24]. None of the patient had the habit of reverse smoking. Similar results were found in one of the earlier studies. Male and female smokers had higher CRP compared with non-smokers (median values of 1.0 mg/l and 11.2 mg/ l for male non-smokers and smokers, respectively, and for females 2.0 mg/ l and 11.6 mg/ l, respectively) [25]. Furthermore, in our study CRP had a significant correlation with BOP, establishing the fact that active disease multiplies the inflammatory burden compared to inactive state. Besides this, elevation in CRP was noticed with the increase in extent and depth of periodontal pocket.

'Speedwell' survey showed that those who had stopped smoking within a year, CRP levels were 2.10 mg/l, but eventually reduced to 1.34 mg/l 15-9 years and 1.36 mg/l > 10 years post quitting, though this CRP measure was still more than never smokers [26]. Present study showed indistinguishable results with the Speedwell study, in terms of inverse inter-relationship between CRP levels and the stretch of time left for the smoking.

A positive relation between age, severity of smoking, periodontitis and pocket depth on rise of CRP was recently documented. However, BOP had negative effect on the same [27]. Yet another research proved CRP, IL-1β, IL-6, IL-8, Tumour Necrosis Factor-α (TNF-α), and homocysteine (HCY) in the serum of a Korean population were synergistically associated with periodontitis-MetS (metabolic syndrome) coexistence [28].

When measured by high sensitivity –CRP, level < 1.0 mg/l denotes low risk, 1.0 to 3.0 mg/l intermediate risk and 3.0 mg/l high risk. [29]. In current study, CRP levels above 3 mg/l was demonstrated in current smokers and participants with periodontitis, putting patients in the high risk category. Former smokers allied in class of moderate risk. In spite of that, one should always remember that CRP secreted in the body is affected by lifestyle and genetic coding of the person and it also differs between individuals [30]. Findings of our research affirm the result of the studies done by D' Aiuto F et al., and Azizi A et al., [16,27].

LIMITATION

Timely changes in the levels of CRP cannot be understood due to absence of follow up. Longitudinal studies needs to be done to know the phased alterations in CRP. Further, there was sample size disparity in Group 4 due to only sparse number of people quitting the habit, thus, making it difficult to reach the large sample size.

CONCLUSION

Periodontitis alone and in combination with smoking increases the systemic inflammatory burden and associated cardiovascular risk of patients. This fact should be made aware to the general population, general dentist, physicians and cardiovascular specialist to enhance early screening and multidisciplinary treatment.

ACKNOWLEDGEMENTS

We would like to thank technical staff of Department of Periodontics and Implantology and Department of Oral Pathology, Manubhai Patel Dental College, Hospital and ORI for assistance during the course of study.

REFERENCES

[1] Arvind K, Pradeepa R, Deepa R, Mohan V. Diabetes & coronary artery disease. Review. Indian J Med Res. 2002;116:163-76.
[2] Gruys E, Toussaint MJ, Niewold TA, Koopmans SJ. Acute phase reaction and acute phase proteins. Review. J Zhejiang Univ Sci B. 2005;6(11):1045-56.
[3] Black S, Kushner I, Samols D. C-reactive protein. J Biol Chem. 2004;279(47):48487-90.
[4] Ebersole JL, Cappelli D. Acute-phase reactants in infections and inflammatory diseases. Review. Periodontol 2000. 2000;23:19-49.
[5] Kravitz MS, Shoenfeld Y. Autoimmunity to protective molecules: is it the perpetuum mobile (vicious cycle) of autoimmune rheumatic diseases. Nat Clin Pract Rheumatol. 2006;2:481–90.
[6] Gomes-Filho IS, Freitas Coelho JM, da Cruz SS, Passos JS, Teixeira de Freitas CO, Aragão Farias NS, et al. Chronic periodontitis and C-reactive protein levels. J Periodontol. 2011;82(7):969-78.
[7] Mealey BL and Perry R. Periodontal Medicine' in Newman Editors. Clinical Periodontology 9th ed. Philadelphia; WB Saunders; 2003: pp. 229-24.
[8] Armitage GC. Development of a classification system for periodontal diseases and conditions. Review. Ann Periodontol. 1999;4(1):01-06.
[9] Mombelli A, van Oosten MA, Schurch E Jr, Land NP. The microbiota associated with successful or failing osseointegrated titanium implants. Oral Microbiol Immunol. 1987;2(4):145-51.
[10] Haffajee AD, Socransky SS. Attachment level changes in destructive periodontal diseases. Review. J Clin Periodontol. 1986;13(5):461-75.
[11] Machtei EE, Christersson LA, Grossi SG, Dunford R, Zambon JJ, Genco RJ. Clinical criteria for the definition of "established periodontitis". J Periodontol. 1992;63(3):206-14.
[12] Abbas, A, Lichtman A, Pillai S. Basic immunology functions and disorders of the immune system. 4th ed. PA. Philadelphia, Saunders/Elsevier. 2012:40.
[13] Vaishnavi C. Immunology and Infectious Diseases. 1996;6:139-44.
[14] Ockene IS, Miller NH. Cigarette smoking, cardiovascular disease, and stroke: a statement for healthcare professionals from the American Heart Association. American Heart Association Task Force On Risk Reduction. Circulation. 1997;96: 3243–47.
[15] Eikelboom JW, Lonn E, Genest J Jr, Hankey G, Yusuf S. Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. Review. Ann Intern Med. 1999;131(5):363-75.
[16] D'Aiuto F, Parkar M, Andreou G, Brett PM, Ready D, Tonetti MS. Periodontitis and atherogenesis: causal association or simple coincidence? J Clin Periodontol. 2004;31(5):402-11.
[17] Slade GD, Ghezzi EM, Heiss G, Beck JD, Riche E, Offenbacher S. Relationship between periodontal disease and C-reactive protein among adults in the Atherosclerosis Risk In Communities study. Arch Intern Med. 2003;163(10):1172-79.
[18] Mariotti A. A primer on inflammation. Compend Contin Educ Dent. 2004;25:07-15.
[19] Potempa J, Banbula A, Travis J. Role of bacterial proteinases in matrix destruction and modulation of host responses. Review. Periodontol 2000. 2000;24:153-92.
[20] van Eeden SF, Hogg JC. The response of human bone marrow to chronic cigarette smoking. Eur Respir J. 2000;15(5):915-21.
[21] van Eeden SF, Yeung A, Quinlam K, Hogg JC. Systemic response to ambient particulate matter: relevance to chronic obstructive pulmonary disease. Review. Proc Am Thorac Soc. 2005;2(1):61-67.
[22] van der Vaart H, Postma DS, Timens W, Hylkema MN, Willemse BW, Boezen HM, et al. Acute effects of cigarette smoking on inflammation in healthy intermittent smokers. Respir Res. 2005;06:22.
[23] Vigushin DM, Pepys MB, Hawkins PN. Metabolic and scintigraphic studies of radioiodinated human C-reactive protein in health and disease. J Clin Invest. 1993;91:1351-57.
[24] Das I. Raised C-reactive protein levels in serum from smokers. Clin Chim Acta. 1985;153(1):09-13.
[25] Reddy SS, Shaik HA. Estimation of nicotine content in popular Indian brands of smoking and chewing tobacco products. Indian J Dent Res. 2008;19(2):88-91.
[26] Lowe GD, Yarnell JW, Rumley A, Bainton D, Sweetnam PM. C-reactive protein, fibrin D-dimer, and incident ischemic heart disease in the Speedwell study: are inflammation and fibrin turnover linked in pathogenesis? Arterioscler Thromb Vasc Biol. 2001;21:603-10.
[27] Azizi A, Sarlati F, Bidi M, Mansouri L, Azaminejad SM, Rakhshan V. Effects of smoking severity and moderate and severe periodontitis on serum C-reactive protein levels: an age- and gender-matched retrospective cohort study. Biomarkers. 2015;20:306-12.

[28] Han DH, Shin HS, Kim MS, Paek D, Kim HD. Group of serum inflammatory markers and periodontitis-metabolic syndrome coexistence in Koreans. J Periodontol. 2012;83:612-20.
[29] Shishehbor MH, Bhatt DL, Topol EJ. Using C-reactive protein to assess cardiovascular disease risk. Review. Cleve Clin J Med. 2003;70:634-40.
[30] Ramamoorthy RD, Nallasamy V, Reddy R, Esther N, Maruthappan Y. A review of C-reactive protein: A diagnostic indicator in periodontal medicine. J Pharm Bioallied Sci. 2012;4:S422-26.

PARTICULARS OF CONTRIBUTORS:
1. Senior Lecturer, Department of Periodontics and Implantology, Manubhai Patel Dental College, Hospital and ORI, Vadodara, Gujarat, India.
2. Postgraduate Student, Department of Periodontics and Implantology, Manubhai Patel Dental College, Hospital and ORI, Vadodara, Gujarat, India.
3. Professor and Head, Department of Periodontics and Implantology, Manubhai Patel Dental College, Hospital and ORI, Vadodara, Gujarat, India.
4. Senior Lecturer, Department of Oral Pathology, Manubhai Patel Dental College, Hospital and ORI, Vadodara, Gujarat, India.
5. Reader, Department of Periodontics and Implantology, Manubhai Patel Dental College, Hospital and ORI, Vadodara, Gujarat, India.
6. Senior Lecturer, Department of Periodontics and Implantology, Manubhai Patel Dental College, Hospital and ORI, Vadodara, Gujarat, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Ruchi Dinesh Raval,
Senior Lecturer, Department of Periodontics and Implantology, Manubhai Patel Dental College,
Hospital and ORI, Vadodara-390011, Gujarat, India.
E-mail: ruchi.raval18@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jul 15, 2016**
Date of Peer Review: **Aug 31, 2016**
Date of Acceptance: **Dec 20, 2016**
Date of Publishing: **Mar 01, 2017**