Effect of Non- Surgical Periodontal Therapy on Plasma Homocysteine Levels in Patients with Chronic Periodontitis- A Prospective Study

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Abstract

Background: The aim of the study was to evaluate and correlate the effect of non surgical periodontal therapy on homocysteine levels in patients with chronic periodontitis.

Methods: Fifty participants were enrolled in the study *(n=50)* in the age range of 20-45 yrs. Each patient was examined using a mouth mirror and UNC-15 graduated periodontal probe. After recording the clinical parameters & indices (CAL, OHI-S, GI), venous blood was drawn from the antecubital vein and transferred to a vial and centrifuged to isolate the plasma, which was then sent for evaluation of plasma homocysteine level. Non-surgical therapy was performed which consisted of scaling and root planing (SRP). After 90 days, the patient was re-evaluated for clinical parameters and the readings were recorded again. Blood samples were sent for analysis of post treatment plasma homocysteine levels.

Results: The plasma Hcy level in periodontitis subjects during pre treatment was 20.7± 3.4 µmol/L which was significantly higher. Post therapy levels reduced to 14.7± 2.2 µmol/L. The OHI's and GI were also significantly reduced after post therapy.

Conclusion: An inflammatory condition like chronic periodontitis is significantly associated with elevated plasma homocysteine levels. However, no significant change was seen in the plasma homocysteine levels between males and females. Periodontal intervention shows statistically significant improvement in plasma homocysteine values.

***Keywords**: Chronic Periodontitis, Root planning, Scaling, Homocysteine, Plasma*

Introduction

Homocysteine (Hcy) is an amino acid and a breakdown product of protein metabolism. Homocysteine present in high concentrations has been linked to increased risk of cardiovascular diseases and strokes.[1,2] A positive correlation exists between the concentration of Hcy and bio-humoral parameters of inflammation, indicating an increase in Hcy levels during inflammatory conditions. [3]

Periodontal disease is an inflammatory condition and is the major cause of tooth loss in adults. Periodontitis is characterized by inflammation of gingiva, destruction of periodontal ligament, and alveolar bone resorption. Chronic periodontitis is also associated with increased circulating levels of CRP and IL-6.[4] Therefore, a similar association could exist between chronic periodontitis and plasma Hcy. Recently, a study showed a highly significant relationship between elevated plasma Hcy and periodontal disease.[5] Furthermore, both chronic periodontitis and raised plasma Hcy levels could act as independent risk factors for cardiovascular disease/ atherosclerosis. This new association could help in part by explaining how a chronic infection like periodontitis could be linked to cardiovascular disease through elevated plasma Hcy levels.

Several studies have shown that levels of various inflammatory biomarkers commonly associated with cardiovascular disease and periodontal disease are reduced following periodontal treatment.[6] With this background the current study was undertaken to assess the levels of plasma Hcy in periodontitis patients and to evaluate the effect of non surgical periodontal therapy on plasma Hcy levels among them.

Materials and Method

A total of 55 subjects suffering from moderate to severe periodontitis were selected for the study from outpatient section of Department of Periodontology, School of Dental Sciences, Karad. All the selected subjects were explained about the objective of the study and the nature and benefits of the clinical investigation and associated procedures. An informed consent was obtained from all participants before enrolling them. Institutional ethical clearance was obtained from Krishna Institute of Medical Sciences, Deemed University, Karad (KIMSDU/IEC/04/2014) before commencing the study. The study was conducted during the period from December 2015- September 2016.

Sample size estimation: The optimum sample size to ensure adequate power for this clinical study was calculated considering standard deviation of 8.27 and a precision of 0.5 i.e. 50% of mean. Based on the above values, it was found that 43 subjects should be studied. As the present study was a follow up study, the final sample size selected was 55 subjects.

Criteria for selection: Screening was conducted among systemically healthy, non- smoker subjects, age 20-45 years, who wilfully consented to attend follow- up visits. Subjects with less than 20 permanent teeth remaining, who had received either antibiotic, anti-inflammatory or vitamin supplementation therapy within 3 months or had undergone any periodontal therapy (surgical or non- surgical) within 6 months of baseline examination were excluded from the study. Systemic diseases and conditions such as cardiovascular disease (CVD), renal disease, rheumatoid arthritis, diabetes mellitus, nutritional deficiencies, pregnancy and lactation have the potential to influence systemic Hcy concentrations and hence, were excluded from the study. A structured proforma was designed to collect patient's personal and demographic data which also included periodontal parameters.

Periodontal parameters: Gingival Index (Loe and Sillness 1963), Oral Hygiene Index (Loe and Sillness) and clinical attachment level were recorded for each patient. Subjects included in the study group (n=50) suffered from moderate to severe chronic periodontitis and were classified based on the AAP classification as, moderate periodontitis = 3 or 4 mm CAL, severe periodontitis ≥ 5mm CAL.[7] Each subject underwent an initial full-mouth periodontal examination. The CAL was measured on six sites (mesio-buccal, midbuccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual) per tooth using a UNC-15 periodontal probe (UNC-15, Hu-Friedy, Chicago, IL, USA).

Sample collection and storage: Pre treatment venous blood samples about 5 ml were collected from median cubital vein. The collected blood samples were centrifuged immediately to extract plasma. The plasma was pipetted out and immediately transferred to a vial and stored till the time of Homocysteine estimation.

Homocysteine analysis: The collected plasma samples were then used for homocysteine analysis using Autopure® (Accurex Homocysteine kit) in EM 360 auto analyzer. The Siemens Diagnostics enzymatic test for the quantitative Homocysteine determination (HCY) is based on a series of enzymatic reactions, causing a decrease in absorbance value due to NADH oxidation to NAD+. HCY concentration in the sample is directly proportional to the quantity of NADH converted to NAD+ (ΔA_{340nm}).

Periodontal treatment: The subjects in the study received nonsurgical periodontal therapy, which included Scaling and root planing (SRP) along with oral hygiene instructions. The periodontal treatment was performed by the same clinician (A.P).

Recall visits: Each patient was recalled after 90 days for follow up examination. Out of 55 patients, 5 failed to follow up, therefore only 50 patients reported. The follow up visits included examination of periodontal parameters and evaluation of homocysteine levels.

Statistical Analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS)-20 for windows (SPSS Inc., Chicago, IL, USA). The 'Wilcoxon' matched pairs test was used to test the hypothesis and significant difference in the level of Hcy, GI and OHI-s scores between pre-treatment and post-treatment. P-values

<0.05 were considered statistically significant.

Results

The demographic data of the study subjects is illustrated in Table 1. The degree of severity of gingival inflammation was evaluated by Gingival Index at baseline and 90 days after treatment. The baseline Gingival Index was 2.0 ± 0.27 which indicates severe gingival inflammation. Following treatment the Gingival Index value was 1.1 ± 0.22 resulting in decrease of 0.9 ± 0.05 post treatment. There was a significant improvement in the gingival condition following periodontal treatment with a p-value of <0.0001*. Similarly the oral hygiene index improved significantly after treatment with a p-value of <0.0001* **(**Table 2).

The baseline mean plasma Hcy value was 20.7± 3.4 µmol/L while the mean Hcy values after 90 days of follow up were 14.7± 2.2 µmol/L. Thus the treatment resulted in a significant reduction of 6.0±1.2µmol/L with a p-value of <0.01* (Table 3).

Hcy levels when compared based on gender revealed that there was no statistical significant difference found between Hcy levels of both males and females pre operatively and post operatively (Table 4). A correlation analysis was carried to confirm any relationship between Hcy and age which was suggestive of, no correlation between Hcy and age (p>0.05) (Table 5).

Table 1: General characteristics of the study population

Characteristic	**N**
Number of participants	50
Males	28
Females	22
Age in years	
≤ 30 years	06
31-39 years	25
40-45 years	19
Mean ± SD	37.6 ± 5.7

Table 2: Periodontal parameters at baseline and after 90 days of follow-up

Periodontal parameters	Baseline	After 90 days of follow up	Mean Difference	P-value
GI	2.0 ± 0.27	1.1 ± 0.22	0.9 ± 0.05	<0.0001*
OHI's	2.6 ± 0.5	1.6 ± 0.9	1.0 ± 0.5	<0.0001*

Table 3: Comparison of Hcy scores at baseline and after 90 days of follow-up

Biochemical Parameter	Mean	Mean Diff.	p-value
Baseline	20.7± 3.4		
After 90 days of follow up	14.7 ± 2.2	6.0± 1.2	<0.0001*

Table 4: Gender wise mean score of Hcy

HYC	**Pre**	**Post**	**Age (in years)**
Males	20.9	14.8	37.3
Females	20.5	14.6	38.04

Table 5: Age wise correlation scores of HYC

Variables	Hcy	Age
Hcy	1.0	-0.196
Age	-0.196	1.0

*p<0.05

Discussion

Hcy is a sulphur containing amino acid obtained during the metabolism of methionine. Dietary protein, methionine is the only known source of Hcy in the human body.[5] The average daily intake of methionine is about 2 grams. Methionine is liberated from dietary protein in the digestive system after meal ingestion. Blood acts as a transport media for free methionine in the body which is ultimately taken up by the cells. Animal dietary proteins contain twice as much as methionine unlike cereals, fruits and vegetables.[8,9]

Periodontitis affects the systemic health of an individual, and may contribute to CVD, coronary artery disease (CAD), diabetes mellitus, and preterm low-birth-weight infants as suggested by the current era of evidence-based medicine.[10-12] These novel risk factors include chronic infections and infection related biomarkers such as CRP, elevated Hcy levels etc.[13,14] Impaired Hcy metabolism has been implicated in CAD/ atherosclerosis, cerebrovascular disease, and peripheral vascular disease.[15]

Plasma homocysteine levels are increased in patients having cardiovascular disease as mentioned in numerous case-control studies.[16] Additionally various meta-analyses indicate that increased plasma homocysteine levels serves as a independent risk factor for atherosclerosis and vascular disease. A study also showed that, for every 5-µmol/L increase in serum homocysteine concentration, the risk of ischemic heart disease increased 20% to 30%.[17] Analysis of the data showed that after adjusting for known cardiovascular risk factors and regression dilution bias in the prospective studies, a 25% lower homocysteine level is associated with an 11% lower IHD risk and 19% lower stroke risk.[18]

The present study was undertaken to evaluate the effect of non-surgical periodontal therapy on plasma homocysteine levels. The study group chosen had a mean age of 37.6 yrs. Studies done by Chang et al 2002[19], Ferruci et al 2005 [20] have shown that a large percent of old (>65years) and very old persons are affected by a chronic mild pro-inflammatory state mediated through their nutritional deficiency which could be linked to HHcy, thus indicating that the total Hcy increases with age. Chronic periodontitis is also found to be age associated, and its prevalence and severity increases with age.

Framingham heart study cohorts has shown that total homocysteine concentration is higher in men and in post-menopausal women.[21] Robinson et al[22] showed that males had increased levels of homocysteine as compared to females, but in the present study it was found that there was no difference associated in homocysteine levels when compared between males and females.

The baseline plasma Hcy values in the current study were 20.7 ± 2.43 µmol/L, which is categorized as mild hyperhomocysteinemia and are similar to those obtained by Joseph et al in 2011.[5] Thus the mean values of Hcy obtained in the current study group show that chronic periodontitis could be a risk factor for CAD. Endothelial dysfunction is considered to be the first inflammatory change of the vascular endothelium leading to arteriosclerosis. The atherogenic and thrombogenic potentials of Hcy have been implicated in promoting endothelial dysfunction; however the exact mechanisms are not clearly understood.[23] Oxidation of low-density lipids, increased monocyte adhesion to the vessel wall, increased lipid uptake and retention, activation of the inflammatory pathway, stimulatory effects on smooth-muscle proliferation, thrombotic tendency mediated by activation of coagulation factors and platelet dysfunction could mediate the relationship between CAD and Hcy. Subjects with periodontitis have also been reported to have higher levels of endothelial dysfunction.[6]

The link between periodontal bacterial systemic exposure and atherosclerosis is biologically plausible. Periodontal pathogens especially P.gingivalis has been detected within inflamed atheromatous plaques and have molecular pathways to induce macrophage uptake of LDL cholesterol, leading to foam cell generation and atheroma progression.[24]

The results of the present study are in accordance with the study done by Bhardwaj S et al[25], which showed significantly higher levels of Plasma Hcy in chronic periodontitis subjects than healthy subjects. There was a significant reduction in plasma Hcy after periodontal treatment.

Thus, from the above discussion it is evident that the elevated levels of plasma homocysteine in chronic

periodontitis patients can act as a marker for systemic inflammation. Moreover in patients with chronic periodontitis, periodontal treatment can be used to augment conventional homocysteine lowering therapies.

Conclusion

In the current study, subjects showed elevated plasma homocysteine levels which were reduced following interventional non-surgical periodontal therapy, this reduction was statistically significant. Periodontal therapy can be used to augment the traditional therapies used to improve the cardiovascular status. To enumerate the limitations of the present study the cofactors involved in homocysteine metabolism namely vitamin B6, B12 and serum folate were not assessed in the current study. Hence, large scale multicenter randomized controlled trials using heterogeneous populations are required to assess the correlation between periodontitis and inflammatory pathways.

Ethical Clearance: Taken from Krishna Institute of Medical Sciences, Deemed University, Karad (KIMSDU/IEC/04/2014) committee

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Conflict of Interest: Nil

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