

Estimation of Homocysteine Levels in Overweight Patients With and Without Chronic Periodontitis

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Abstract: Homocysteine, a sulfur containing amino acid linked to an increased risk of cardiovascular diseases, is seen to be elevated in individuals who are overweight or are suffering from chronic inflammatory conditions.

Aim: The aim of this study was to evaluate the levels of serum homocysteine in overweight patients with and without chronic periodontitis.

Materials and method: Total 20 individuals - 10 overweight individuals with healthy periodontium (Controls) and 10 overweight individuals with chronic periodontitis (Cases), with comparable Body Adiposity Index were included in the study and their Serum Homocysteine levels were estimated using Enzyme immunoassay. The data was subjected to statistical analysis using SPSS v 21.0

Results: The results showed a statistically significant difference between the means of Homocysteine serum levels, Gingival index and Russell's Periodontal index

between the cases and controls ($p < 0.01$) with values higher in the cases than controls.

Conclusion: There is a positive relationship between serum homocysteine levels and chronic periodontitis in patients with comparable body adiposity index.

Keywords: Homocysteine, chronic periodontitis, overweight

Introduction

Chronic periodontitis is one of the most common inflammatory diseases that lead to destruction of the periodontal ligaments and loss of the adjacent bone and teeth.

¹ With the progression of periodontitis, bacteria and their endotoxins (lipopolysaccharide) and other products enter the periodontal tissue and blood circulation causing, systemic or local inflammatory reactions in the host. Also, periodontal disease causes Th1 reaction and releases cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β), which can increase the risk of

coronary heart disease.² According to World Health Organization health statistics,³ cardiovascular disease (CVD) is the single leading cause of morbidity and mortality globally and 50% of patients with atherosclerosis lack currently identified risk factors, an observation indicating that unrecognized novel risk factors may be involved.⁴ These include chronic infections and inflammation and related biomarkers, such as C-reactive protein and elevated Homocysteine (Hcy) levels. Welch and Loscalzo showed that elevated homocysteine levels are indeed a risk factor for vascular disease.⁵

Also, obesity is one of the most important risk factors for cardiovascular disease^{6, 7} and generally the Hcy levels increase in obesity. It has been shown in literature that obesity and elevated Hcy both are associated with increased cardiovascular disease risk. However, the interrelation of these two risk factors is not clear yet.

Homocysteine (Hcy) is actually a sulfur containing amino acid derived from methionine during its metabolism. The B-complex vitamins are required for transformation, excretion, or both steps in the Hcy metabolism pathway. Proinflammatory cytokines, such as IL-6, may be released from inflamed periodontal pockets and may give rise to acute-phase reactants in the systemic circulation. McCarty has reported that IL-6 may interact with vitamin B6 metabolism and compromise cystathionine b-synthase activity, thereby elevating plasma Hcy concentrations.⁸ This suggests that hyperhomocysteinemia (HHcy) could be expected by the release of IL-6 from the inflamed pocket walls of periodontium.

Therefore, the aim of this study was to evaluate the levels of serum homocysteine in overweight patients with and Without Chronic Periodontitis.

Materials And Method

This was randomized clinical study carried out in department of Periodontology and Oral Implantology.

And the patients were selected from the Out-patient department of Periodontology and Oral Implantology. The study was approved by the Institute's Research Ethics Committee.

Patients were selected based on the following inclusion and exclusion criteria.

Inclusion Criteria

- 1) Systemically healthy patients
- 2) Overweight individuals with comparable body adiposity index
- 3) Patients with Chronic Periodontitis

Exclusion Criteria

- 1) Overweight individuals with any other chronic inflammatory disease
- 2) Patient with known history of vitamin B- complex deficiency
- 3) Pregnant or lactating women

Following patient selection, patients were explained about the study and a written informed consent was obtained from all the patients willing to be a part of the study. After screening overweight individuals for their body adiposity index using formula - hip circumference divided by height^{1.5} minus 18, a total of 20 patients with comparable body adiposity index were selected and were allotted to the two groups. This allotment was based on presence or absence of chronic periodontitis. In the present study Gingival index and Russell's periodontal index were used to determine the presence of chronic periodontitis. And hence the groups were as follows:

Group A (Cases): 10 overweight patients with chronic periodontitis.

Group B (Controls): 10 overweight patients without chronic periodontitis.

All individuals in both the groups were then subjected to estimation of serum Homocysteine (Hcy) levels using enzyme immunoassay. And after acquisition of laboratory

reports, level of serum Hcy in both groups were compared.

Statistical Analysis

Data obtained was compiled on a MS Office Excel Sheet (v 2010) and was subject to statistical analysis using Statistical package for social sciences (SPSS v 21.0, IBM). Descriptive variables like mean age and percentage & frequency of males and females overall and between the groups has been depicted. Comparison of Homocysteine serum levels, Body adiposity index no, Gingival index & Russell's Periodontal index between cases & controls has been done using t test for small samples. Comparison of mean Homocysteine serum levels as per BAI class (overall) has been done using one way ANOVA since the outcome is continuous. Bivariate correlation between independent and dependent variables has been performed. Comparison of frequencies of subjects of each class of independent variable with homocystiene class has been done using chi sqaure test. Curve estimation using a linear regression model has been done for variables showing highest & significant correlation. Finally a linear regression has been performed including all independent variables & co-variates. For all the statistical tests, $p < 0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%.

Results

A total of 20 individuals with comparable body adiposity index were included in study, out of which 10 had chronic periodontitis and 10 were healthy controls.

Basis statistics for age is presented in Table: 1

The age varied from a minimum of 30 years to a maximum of 63 years, mean age of the patients being 46.50 years with a standard deviation of 11.204years.

All cases and controls were matched for their age, which is represented in Table: 2 as, there was a statistically non-

significant difference in the age between cases & controls ($p > 0.05$), hence age as a confounder is ruled out.

As mentioned earlier, all the individuals included in the study had comparable body adiposity index. Therefore, there was statistically non-significant difference between the means of Body adiposity index between the cases and controls ($p > 0.05$)

The values of Gingival index and Russell's periodontal index between cases and controls varied because controls had healthy periodontium and cases had chronic periodontitis. Therefore, there was a statistically highly significant difference between the means of Homocysteine serum levels, Gingival index and Russell's Periodontal index between the cases and controls ($p < 0.01$) with values higher in the cases than controls. It is presented in Table:3 As shown in TABLE: 4, as the value of Gingival index and Russell's Periodontal index increase, there is an increase in Homocysteine serum levels. Hence, there was a positive, high correlation between Homocysteine serum levels and Gingival index & Russell's Periodontal index ($r > 0.7$, $p < 0.01$, highly significant)

Discussion

Homocysteine is a sulfhydryl-containing amino acid which is an intermediate product in the normal biosynthesis of the amino acids methionine and cysteine.⁹ Its chemical properties showed a similarity to cysteine, and therefore the name homocysteine. The amino acid methionine is the only known source of Hcy in the human body. Under normal circumstances, most but not all of the Hcy formed in transmethylation reactions is remethylated back to methionine or converted into cysteine in transsulfuration reactions.

Hyperhomocysteinemia has been defined as a medical condition which is characterized by an abnormally high level (above 15 $\mu\text{mol/L}$) of homocysteine in the blood.¹⁰

It is classified as moderate (16-30 $\mu\text{mol/L}$), intermediate (31-100 $\mu\text{mol/L}$) and severe (above 100 $\mu\text{mol/L}$).¹¹

Elevated levels of plasma Hcy/hyperhomocysteinemia (HHcy) have been linked to the oxidative damage of the vascular endothelium, proliferation of vascular smooth muscles, and lipid peroxidation, which could result in atherothrombosis and peripheral arterial disease. And hence it can be considered as a risk factor for atherothrombotic and thromboembolic disease.^{12, 13, 14}

Also, it is well established that obesity is considered as an independent risk factor for cardiovascular disease.^{6, 7} And generally the Hcy levels are increased in obesity. Marchesini et al. reported that Hcy levels were moderately increased in obese individuals when compared with the normal population and higher in males than in females, but not different in relation to the severity of obesity.¹⁵ Similarly in a study by Tungtrongchitr et al, statistically significantly higher levels of serum Hcy concentrations were found in the overweight subjects, and serum Hcy concentrations in overweight and obese males were significantly higher than females.¹⁶ However it is unclear if these two risk factors are interrelated. Therefore, the present study was therefore undertaken to estimate the levels of serum homocysteine in overweight patients in healthy individuals with and without chronic periodontitis. In the present study overweight patients with any other chronic inflammatory disease and patients with known history of vitamin B- complex deficiency were excluded to remove bias, as these conditions may influence the levels of serum homocysteine. A total of 20 patients with mean age of 46.5 were included who were age and sex matched.

In the present study, the results showed a statistically significant difference between the means of Homocysteine serum levels, Gingival index and Russell's Periodontal index between the cases and controls ($p < 0.01$) with values

higher in the cases than controls. Similar results were obtained from the study conducted by Rosamma Joseph et al (2011) where the authors showed elevated plasma Hcy in patients with chronic periodontitis.¹⁷

Also, it was found that overweight patients with chronic periodontitis had elevated serum Hcy levels. This demonstrates an association between periodontal disease and serum Hcy. The possible mechanisms linking between chronic periodontitis and serum Hcy could be as follows: (1) Proinflammatory cytokines, such as IL- 6 are released from inflamed periodontal pockets which may interact with vitamin B6 metabolism and compromise cystathionine b-synthase activity, thereby elevating plasma Hcy concentrations.⁸ (2) Also, IL-6 through activation of the Thelper-1 immune response stimulates the formation of reactive oxygen species. These are normally counteracted by several specific antioxidant agents. But if these detoxifying systems are overloaded as a consequence of a chronic immune activation, other oxidative-sensitive molecules, such as vitamin B12 and tetrahydrofolate (which are essential for the metabolism of Hcy), become a target for reactive oxygen species. And hence leading to significant accumulation of Hcy.¹⁸ (3) The periodontal tissue damage may also accelerate specific remethylation reactions of DNA, RNA, and various proteins during tissue repair, which leads to the generation of S-adenosylhomocysteine and release of plasma Hcy.¹³ Hence it can be hypothesized that the inflammatory processes of chronic periodontitis may act as pathway for elevation of Hcy concentrations in serum. There are some limitations to this study. First, the sample size was small and only the patients reporting to institute were included so it might not give true representation of the entire population. Second, overweight patients were not classified nor was the level of hyperhomocysteinemia and so the exact level of correlation Was Not Achieved.

Conclusion

In this study, there is a positive relationship between serum homocysteine (Hcy) levels and chronic periodontitis in patients with comparable body adiposity index. As Hcy is considered as a risk factor for CVD, this association between periodontal disease and Hcy could act as a link between periodontal disease and CVD. However, large prospective multicenter clinical trials are needed to confirm this association. Also, this association can further be proved by studying the effect of periodontal therapy on serum Hcy levels.

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