European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 9, Issue 7, Summer 2022

"Impact Of "Non- Surgical Periodontal Therapyon Plasma Homocysteine Levels In Patients With Chronic Periodontitis"

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Abstract

Understanding the etiology and pathogenesis of periodontal diseases and their chronic, inflammatory and infectious nature necessitates recognizing the possibility that these infections may have effects somewhere in the body. The concept that oral diseases and systemic diseases influence each other goes back to the theoryof "*focal infection*". In 1891, Miller published his theory regarding focal infection in which he indicated that microorganisms and their products are able to access parts of the body that are adjacent to or distant from the mouth. The proponents of this concept assume that microorganisms present in dental plaque and their metabolic products may enter the bloodstream, thereby causing many systemic diseases and sometimes resulting in degenerative conditions. Associations have been reported between periodontal disease and cardiovascular disease (CVD), stroke, diabetes, preterm low birth-weight babies, respiratory infections and rheumatoid arthritis (RA).[2]

Inflammation from chronic infections has found to have a prominent role in several systemic diseases/conditions like cardiovascular disease, autoimmune conditions like rheumatoid arthritis etc.[3-8]The release of proinflammatory cytokines like IL-1β, TNF α, IL-6 etc and the activation of the host immune response is found to be linked to the underlying destructive mechanisms in these diseases. Recently among the several novel risk factors proposed for cardiovascular diseases chronic infections and infection related biomarkers such as C-reactive protein (CRP), and elevated homocysteine levels etc have been suggested.[9,10] Infections are one of the potential newer risk factors implicated in pathogenesis of atherosclerosis/CAD.[3-7] Periodontitis is a chronic inflammatory disease which serves as a reservoir of gram negative, anaerobic organisms, lipopolysaccharides and inflammatory mediators. According to the National Oral Health Survey and Fluoride Mapping conducted in 2002-2003 the total prevalence of periodontitis in the state of Maharashtra was found to be as high as 78%.[11,12]

Keywords: etiology, pathogenesis, periodontal, chronic, inflammatory, microorganisms, cardiovascular disease, rheumatoid arthritis, cytokines, periodontitis, lipopolysaccharides.

Introduction

Apart from the local destruction of the periodontal tissues, a large surface area of the ulcerated pocket epithelium allows exchange between bacterial and host products. It has been noted that periodontal pathogens like Porphyromonasgingivalis are able to invade gingival tissues[13] and from there, are able to gain access to the systemic circulation.[14] These host-bacterial interactions produce a resultant impact on various systems in the human body.[15,16,17]Several mechanisms may participate in this interaction including those induced by oralorganisms, and those associated with host response factors (such as interleukin IL- 1, IL-6 and tumor necrosis factor [TNF]-α).[15] This periodontal-systemic disease relationship is believed to be mediated through systemic inflammatory reactants such as acute-phase proteins, and immune effectors.[16,17]

Plasma homocystiene (Hcy) has drawn much attention as an emerging risk biomarker in literature. Elevated levels of plasma homocysteine/ hyperhomocysteinaemia (HHcy) has been linked to the oxidative damage of the vascular endothelium, proliferation of vascular smooth muscles, and lipid peroxidation which could result in atherothrombosis, peripheral arterialdisease etc.[18-25] Increased plasma Hcy level has also been observed in patients with chronic renal disease increasing their morbidity and mortality to atherosclerotic cardiovascular disease.[26,27]

Plasma Hcy has a definite role in the systemic inflammatory pathway. IL-6 may interfere with vitamin B6 metabolism, thereby increasing plasma Hcy levels.[28,29] IL-6 stimulated reactive oxygen species (ROS)[29] and bacterial lipopolysaccharides induced RANTES (regulated upon activation, normal T cells expressed and secreted) in monocytes could also result in HHcy.[30]

The role of inflammation in systemic diseases utilizing plasma Hcy as a biologic marker has been assessed in multiple studies on rheumatoid arthritis (RA) subjects.[31-34] A positive relation exists between the concentration of Hcy and some bio-humoral parameters of inflammation, such as the circulating levels of soluble receptors for cytokines,[31-33] adhesion molecules (sICAM-1),[32] and C-reactive protein(CRP).[33,34]

Periodontitis also shares a common immuno-inflammatory profile with rheumatoid arthritis (RA).[35,36] Chronic periodontitis, an inflammatory disease is associated with increased circulating levels of CRP and IL-6.[37] Therefore, a similar association could exist between chronic periodontitis and plasma Hcy.

Hence we undertook the following study to evaluate the effect of non-surgical periodontal therapy on plasma Hcy values.

Aims and Objectives

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.

Objectives:

1. To determine the levels of plasma homocysteine in chronic periodontitis patients.
2. To correlate the levels of plasma homocysteine to the severity ofperiodontal disease.
3. To assess the influence of nonsurgical periodontal therapy on plasma homocysteine levels in chronic periodontitis patients

Review of Literature

Hcy is formed during the course of formation of active methionine: S-Adenosyl Methionine (SAM). First methionine is activated with ATP to form SAM, here all the three phosphate bonds of ATP are hydrolyzed and the adenosyl group is transferred to the sulphur atom. The enzyme catalyzing the reaction is methionine adenosyltransferase. In methionine, the thio–ether linkage (C-S-C) is very stable. But in SAM, due to the presence of high energy bond, the methyl group is labile, and may be transferred easily to other acceptors. This methyl group is received by a methyl receptor and then S-Adenosyl Homocysteine (SAH) is formed.[39] Later the adenosyl group is removed from SAH by the enzyme adenosine homocysteinaseand homocysteine is formed.[40]

The Hcy thus formed is metabolized by two other pathways:
(1) Remethylation: in this cycle Hcy is salvaged by getting methyl group in a reaction catalyzed by methionine synthase. N^5, N^{10} methyl- tetra hydro folate (MTF) is the methyl donor in this reaction.[41]

(2) Transulphuration: in this cycle Hcy condenses with serine to form cystathione in a reaction catalyzed by vitamin B6 dependant enzyme, cystathione beta synthase and its deficiency leads to elevated plasma levels of homocysteine especially in high methionine states. Cystathione is further hydrolyzed to form cysteine and homoserine.[39] Cysteine is incorporated into glutathione or further metabolized to sulfate and is excreted in urine. Homoserine is deaminated and then decarboxylated to propionyl CoA. It finally enters into TCA cycle as succinyl CoA.[40]

Fig 1: Formation & Metabolism of Homocysteine

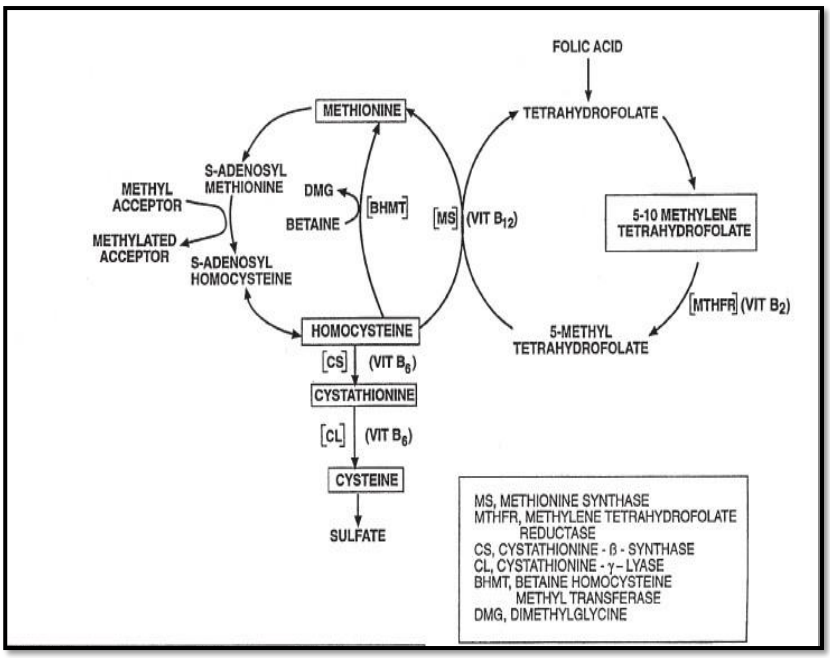


Fig 2: Structure Of Homocysteine

NH2
|
H-C-COOH
| CH2
| CH2
| SH

Hyperhomocysteinaemia - Mechanisms

Total plasma (or total serum) homocysteine (tHcy) reflects the combined pool of free,bound, reduced, and oxidized forms of homocysteine in the blood . Normal tHcy levels range between 5 and 15 µmol/L with elevations of 16 to 30 µmol/l, 31 to 100 µmol/l, and >100 µmol/l classified as mild, moderate, and severe hyperhomocysteinaemia respectively.[42] Blood levels of tHcy are optimally measured during fasting. However, measurement after methionine load may be more sensitive in identifying mild disturbances in homocysteine metabolism. Several dietary and lifestyle factors, genetic defects, nutritional deficiencies, and other etiologies can cause elevations in homocysteine.[42]

DISORDERS OF TRANSULPHURATION:

i Cystathione Beta Synthase deficiency

Also known as **classical homocystinuria**. This is the most common inborn error of methionine metabolism.[43] Deficiency of the enzyme, Cystathione Beta Synthase(CBS) causes increased concentrations of methionine and homocysteine in body fluids and decreased

concentrations of cystine and cysteine.[44] Heterogenous mutation of CBS gene is present in different families. The G 307 S mutation is associated with lack of pyridoxine, whereas 1278 T mutation correlates with pyridoxine responsiveness and a milder clinical phenotype. Homocysteine interferes with the normal cross linking of collagen — an effect that likely plays an important role in ocular, skeletal and vascular complications.

ii **DISORDERS OF REMETHYLATION:**

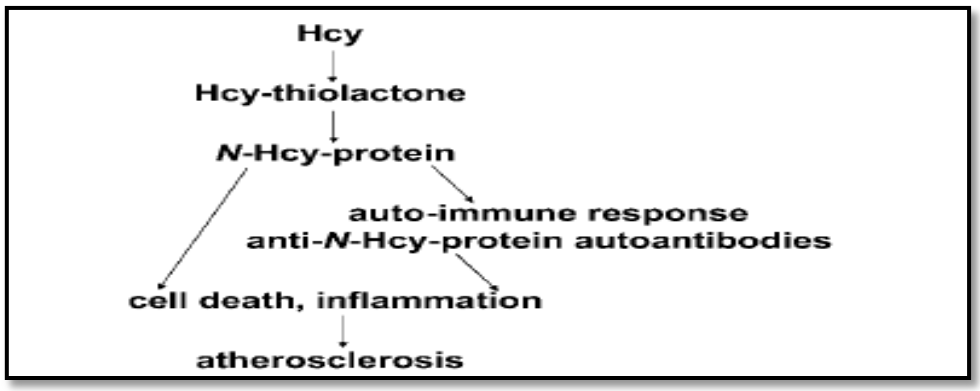
a) **N^5, N^{10} methyl- tetra hydro folate reductase (MTFR) deficiency** N^5, N^{10} methyl-tetra hydro folatereductase (MTFR) is involved in the synthesis of 5-methyl THFA, a cofactor in the formation of methionine from homocysteine. The severity of the enzyme defect and the clinical manifestation varies considerably in different families. The gene for this enzyme is located on short arm of chromosome. The condition is transmitted as an autosomal recessive trait.[43] Complete absence of this enzyme leads to neonatal apnoea, myoclonic seizures and ultimately leads to coma and death. Partial deficiency of this enzyme leads to more chronic clinical picture manifested by mental retardation, convulsions, microcephaly and spasticity. Premature vascular disease or peripheral neuropathy has been reported as the only manifestation of this enzyme deficiency in some patients.

b) **Deficiency of cobalamide (vitamin B12) co enzyme synthesis** Methylcobalamin is the cofactor for the enzyme methionine synthase, which catalysesremethylation of homocysteine to methionine. There are at least 5 distinct defects in the intracellular metabolism of cobalamin that may interfere with the formation of methylcobalamin. These 5 defects are designated as cb/C, cb/D, cb/E (methionine synthase reductase),cb/F, cb/G(methionine synthase). Patients with cb/C, cb/D and cb/F defects have methylmalonicacidemia in addition to homocysteinemia because of formation of both adenosylcobalamin and methyl cobalamin is impaired. Patients with cb/E and cb/G defects are unable to produce methylcobalamin and develop homocysteinemia without methylmalonicacidemia; only a few patients with these two defects are known.[43]

PATHOGENESIS OF HYPERHOMOCYSTEINEMIA

Homocysteine-thiolactone is cytotoxic. Early evidence suggesting that Hcy- thiolactone is cytotoxic to the cardiovascular system was obtained in the 1970s, well before metabolism of Hcy-thiolactone in humans was deciphered and its physiological significance established (Fig 2). Infusions with Hcy-thiolactone have been used as an early model of clinical homocystinuria. For instance, Harker et al 1974[23] found that baboons chronically infused with Hcy-thiolactone developed patchy desquamation of vascular endothelium and atherosclerosis. Similar vascular changes occurred in baboons in response to infusions of Hcy which is metabolically converted to Hcy-thiolactone. Hcy-thiolactonase activity of the intracellular enzyme bleomycin hydrolase is also likely to vary between species and contribute to their sensitivity to Hcy-thiolactone.[45]

Fig 3: <u>The Hcy –Thiolactone Hypothesis</u>:
Possible Mechanism Underlying the Involvement of Hcy in Atherosclerosis



N-linked protein Hcy constitutes a significant pool of Hcy in the human body. Human plasma levels of N-linked protein Hcy are much higher than the plasma levels of Hcy-thiolactone. The concentrations of plasma N-linked protein Hcy vary from 0.1 mmol/L to 13 mmol/L and comprise up to 25% of plasma total Hcy.[46]

The mechanism underlying N-Hcy-LDL toxicity may involve a decrease in endothelial Na1,K1-ATPase activity, leading to an overload with sodium and, subsequently, with calcium.[47] This in turn causes reduced production of nitric oxide and generation of peroxynitrate a highly reactive nitrogen metabolite. Taken together, these observations suggest that protein N-homocysteinylation may contribute to endothelial dysfunction, a key event initiating the development of atherosclerotic plaque.[49]

<u>PATHOHYSIOLOGIC MECHANISMS RELATING HOMOCYSTEINE TO ATHEROTHROMBOSIS</u>

[Adapted from Kaul S et al 2006 J Am CollCardiol 2006; 48:914 –23][50]

a) Atherogenesis

(1) Induces vascular inflammation via expression of TNF – α and i NOS
(2) Increases oxidative stress
(3) Induces DNA hypomethylation and gene expression for cell growth and differentiation
(4) Promotes oxidation of low density lipoprotein
(5) Enhances uptake of modified lipoprotein by macrophages
(6) Increases endothelial dysfunction through oxidative stress,increased ADMA, increased inflammation, decreased NO
(7) Promotes lipid accumulation through induction of HMG Co-A reductase
(8) Stimulates vascular smooth muscle cell DNA synthesis and proliferation
(9) Directly toxic to endothelial cells

a) Thrombogenesis

(1) Induces tissue factor activity
(2) Promoted leukocyte endothelial interaction via MCP-1 ,IL-8 expression

(3) Enhances endothelial cell associated factor V activity
(4) Impairs inactivation of factor Va by activated protein C
(5) Inhibits binding of antithrombin III to endothelium
(6) Reduces endothelia binding sites for tissue plasminogen activator
(7) Enhances binding of lipoprotein (a) to fibrin
(8) Decreases cell surface thrombomodulin and protein C activation
(9) Increases platelet aggregation

ADMA= Asymmetric Dimethylarginine, HMG Co-A= 3-hydroxy-3- methyl glutaryl Co A, i NOS= inducible nitric acid synthase

Linking HCY with various Diseases

The initial link between homocysteine and vascular disease was made by McCully,[52] over 35 years ago. He observed that an infant who died as a result of abnormal cobalamin metabolism with homocystinuria exhibited widespread, severe arteriosclerosis analogous to the lesions seen in cases of homocystinuria caused by a genetic cystathione-β-synthase deficiency. Because hyperhomocysteinemia (HHcy) was the only condition common to these 2 metabolic disorders, McCully proposed that HHcy resulted in arteriosclerotic disease.

I) INFLAMMATION AND HOMOCYSTEINE

The immuno-pathological processes supporting AD frequently produce a relevant inflammatory state that is representative of the disease activity. The most recent dataseem to suggest a strict and bi-univocal relationship among immune activation,inflammation, and Hcy levels.

II) Immune activation and inflammation as a cause of hyperhomocysteinemia

The mechanisms involved in the development of HHcy as a consequence of a persistent immuno-inflammatory activation, although not completely clarified, are probably multiple and intriguing.

III) INFLAMMATION LINKED TO HHCY

Tetrahydrofolate (THF) and Vitamin B12 are very prone to oxidation, with irreversible structural modifications making the recovery of the molecule impossible. In the course of Th1-immune response, activated T-cells release great amounts of IFN-γ which, in turn, strongly stimulates the formation of reactive oxygen species (ROS) by monocytes/macrophages.[29] The effects of these latter molecules, responsible for cellular damage, are normally counteracted by several specific antioxidant agents (superoxide dismutase, catalase, glutathione, ascorbic acid, α-tocopherol); however, if these detoxifying systems are overloaded as a consequence of a chronic immune activation, other oxidative-sensitive molecules, such as THF and B12, become a target for ROS.[29] Moreover, several data suggest that not only oxidative stress but also the active proliferation of immune-competent cells may contribute to the vitamin consumption observed in autoimmune diseases like rheumatoid arthritis, Bechets disease etc.[56-59] In fact, folate and B12 play an essential role in DNA synthesis and repair, and the high turnover of the cells involved in the immune

response sustaining these disorders implies an increased demand of such molecules.[33]

IV) .Hcy as a pro-inflammatory and immuno-modulating molecule

A large amount of in vitro studies on several vascular cell-types confirmed the pro-inflammatory and immuno-modulating properties of Hcy, extending also thespectrum of the molecules involved. In particular, many authors demonstrated that Hcy was able to induce chemokine (IL-8 and/or MCP-1), and chemokine receptor expression by human vascular cells and monocytes.[61,62] These results, corroborated by the data obtained also in vivo in subjects with HHcy [62], suggest the importance of Hcy in the enhancement of monocyte chemotaxis into the arterial wall representing one of the key events during atherogenesis.

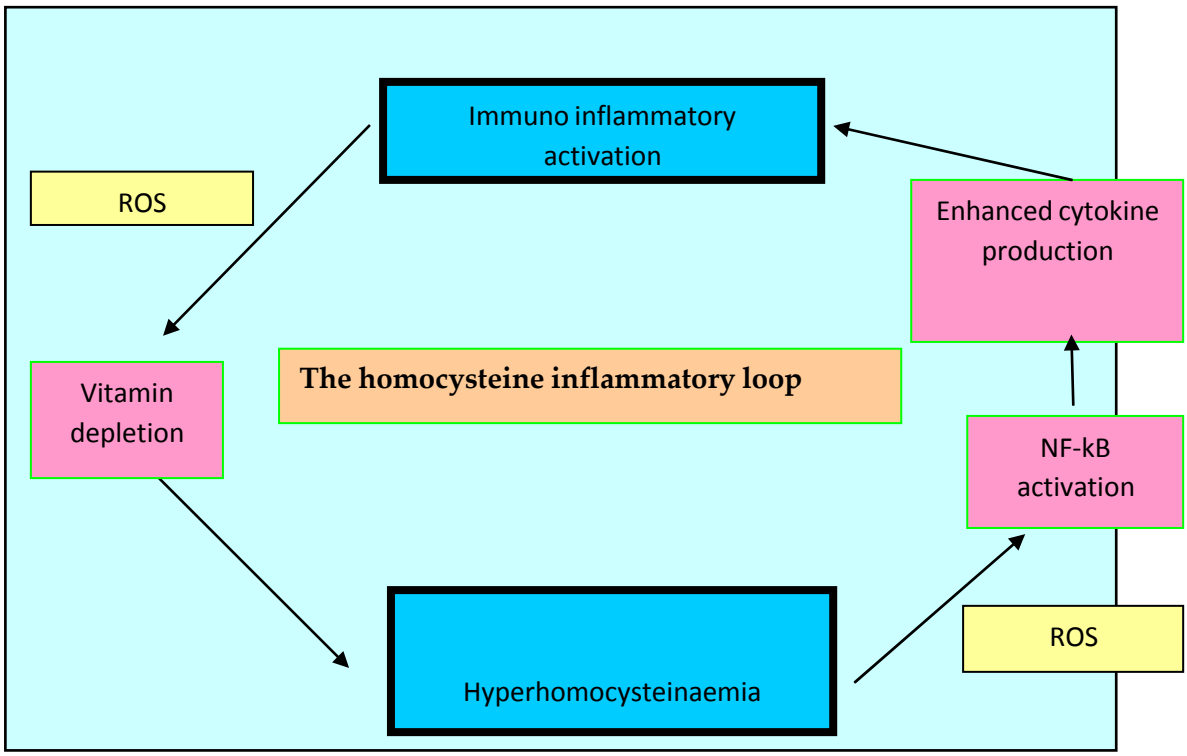


Figure 4 : The "Homocysteine –Inflammatory Loop"

[Lazzerini et al. Autoimmunity Reviews 6 (2007) 503–509]

In fact, a bi-directional link seems to connect Hcy and the immuno-inflammatory activation characterizing autoimmune diseases, in which immuno-inflammatory activation may contribute to Hcy increase, and Hcy, in its turn, may act as a pro- inflammatory and immuno-stimulating molecule putatively cooperating at the injury ofthe disease-specific target organs (fig 3).[18] Moreover, Hcy may be also a trigger of autoimmune reactions through its capability to bind and structurally modify specific proteins, then resulting in neoantigens formation, potentially relevant either in the onset of specific diseases and in the progression of the associated cardiovascular damage.

PERIODONTITIS AS A RESERVOIR OF IMMUNO INFLAMMATORY CYTOKINESAND ENDOTOXINS

Periodontitis has very similar cytokine profile as rheumatoid arthritis consisting of persistent

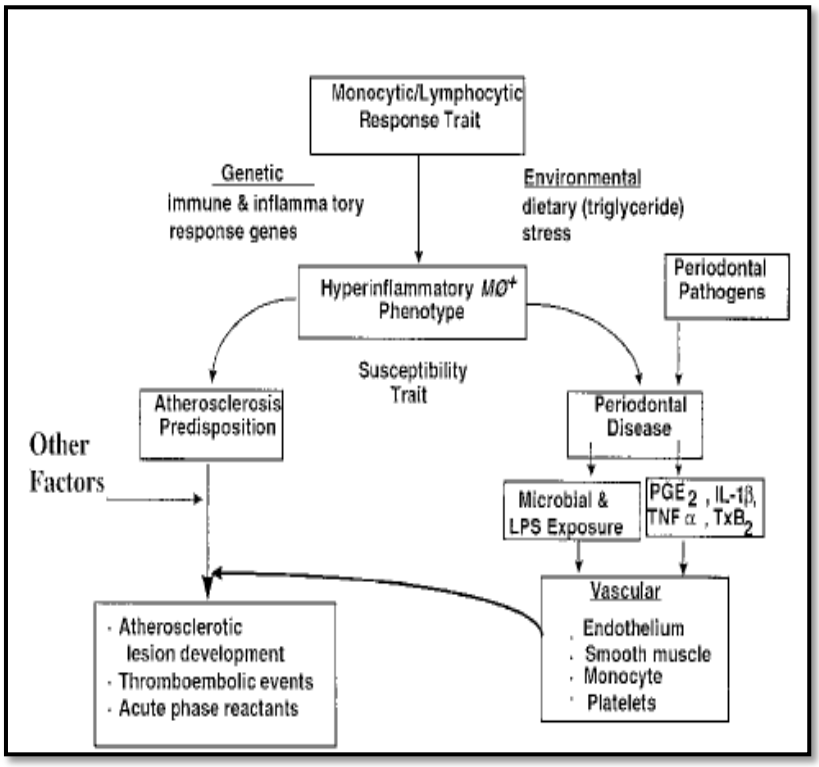
high levels of proinflammatory cytokines including IL-1β and TNF α. These cytokines together with low levels of tissue inhibitor of matrix metalloproteinases (TIMPs) and high levels of matrix metalloproteinases (MMPs) and PGE2 are associated with the active stages of Periodontitis.

Mounting evidence points to an association of periodontal disease to clinical and sub clinical cardiovascular vascular disease. Many authors over the last 2 decades have conducted studies to determine the association between periodontal disease and cardiovascular diseases like atherosclerosis, myocardial infarction and stroke.[67-70]

The Hyperinflammatory Monocyte Theory

The concept of monocytichypersecretory trait has been described for rheumatoid arthritis patients (**Shore et al 1986**[71], **Ollier et al 1992**[72]) and also for periodontal disease susceptibility patients. **Beck et al**[73] in 1996 hypothesized that the association between periodontal diseases and atherosclerosis may be due to an underlying inflammatory response trait through the HyperInflammatory monocytetrait, which places an individual at high risk for developing both diseases (Fig 5).

Fig 5: Model proposed by Beck et al 1996 –Hyperinflammatory monocytetheory



Periodontitis is a chronic inflammatory disease characterized by the destruction of tooth supporting structures caused by bacteria and modified by host immune response. It forms one of the most common chronic infectious diseases of humans.[74] The current era of evidence based medicine provides an increasing body of scientific evidence suggesting that periodontitis may affect an individual systemically, and may contribute to cardiovascular

disease, rheumatoid arthritis, diabetes mellitus, pre-term low birth weight etc. through similar pathways. These findings together have resulted in the rapid emergence of a new branch of periodontology focusing on the wealth of new data establishing a strong relationship between periodontal health or disease and systemic health or disease termed "**Periodontal Medicine**", as first suggestedby Offenbacher.[75] These points to the fact that periodontitis is a reservoir of immuno- inflammatory cytokines and endotoxins and inflammation from the periodontium havedistinct impact on systemic diseases/conditions like CAD/atherosclerosis etc which could be assessed with the help of emerging biomarkers of inflammation like CRP, plasma homocysteine etc.

Studies Evaluating The Influence Of Periodontal Intervention On The Risk Of Cardiovascular Disease

Various systematic reviews and meta-analyses have described the relationship between periodontal infection and cardiovascular disease and have suggested that periodontitis may contribute to cardiovascular disease and stroke in susceptible patients.[81-83]

Influence Of Periodontal Intervention On Serum Lipid Profiles

Recent studies have shown an association between periodontitis and elevated atherogenic lipid fraction levels and/or decreased anti-atherogenic lipid fraction levels.[86-88] Most of these were cross-sectional studies, and it is still unclear whether there is a causal relationship between periodontitis and hyperlipidemia. Improvement of serum lipid profiles after periodontal treatment may indicate a causal relationship between periodontitis and hyperlipidemia, and may suggest the possibility of reducing the risk of coronary heart disease by effective periodontal intervention.

A number of interventional studies have evaluated alterations in serum lipid levels after periodontal treatment. As expected, periodontal parameters improved significantly after various therapies in all studies. Interestingly, serum lipid profiles also improved after periodontal treatment in many of the studies, although the markers that were altered and the degree of improvement varied greatly. One reason for this variability is that the subjects included in these studies differed in terms of their general health status with regard to hyperlipidemia, hypertension or other cardiovascular diseases.

INFLUENCE OF PERIODONTAL INTERVENTION ON INFLAMMATORY MARKERS

A) Acute-phase reactants

(1) C-reactive protein

Recently, evidence has accumulated demonstrating the association between periodontitis and C-reactive protein. In ameta-analysis of case–control studies, it was found that subjects with periodontitis had 1.65 mg/l higher serum C-reactive protein concentrations compared to individuals without periodontitis.[93] A number of studies have assessed the serum C-reactive protein levels in patients with cardiovascular disease or cardiovascular risk factors. The results show that periodontitis patients with cardiovascular disease or hypertension have significantly higher serum high- sensitivity C-reactive protein concentrations than patients

without periodontitis.

(2) Fibrinogen

Fibrinogen is the main coagulation protein in plasma, a co-factor for platelet aggregation and an acute phase reactant. It has been reported that there is an association between elevated plasma fibrinogen levels and coronary heart disease.[94] The effects of periodontal treatment on fibrinogen levels are not consistent among the available intervention trials. In some studies, no change in fibrinogen levels were found following periodontal treatment , however, in a periodontal intervention study performed in patients with generalized aggressive periodontitis that comprised initial periodontal therapy and antibiotic therapy 8 weeks later, the plasma fibrinogen level had decreased at 6 months after the antibiotic therapy.[95]

(3) Cytokines

Many cytokines play a role in the pathogenesis of both coronary heart disease and periodontitis. These include interleukin-1, interleukin-6, interleukin-8,tumor necrosis factor-a, intercellular adhesion molecule- 1 (ICAM-1), P-selectin and E-selectin. The following interventional studies have indicated that periodontal therapy can reduce the levels of these pro-inflammory cytokines, and thus periodontal treatment may lower the cardiovascular disease risk.

(4) Interleukin-6

Interleukin-6 is involved in promoting coagulation, which may result in the development of atherosclerosis. In a prospective study of 14916 apparently healthy men, the interleukin-6 levels in 202 men who subsequently had a myocardial infarction were higher than in 202 matched control without myocardial infarction during a 6-year follow up (1.8 vs. 1.5 pg／ml, P = 0.002)[97]. This indicates that interleukin-6 levels may be a predictor of risk of future myocardial infarction in apparently healthy men.

(5) Tumor necrosis factor-α

Tumor necrosis factor-α is a cytokine with a wide range of humoral and cellular immune effects relating to inflammation, and is involved in the initiationand development of coronary artery disease. Tumor necrosis factor-a levels are increased in patients with periodontitis. The influence of periodontal treatment on circulating tumor necrosis factor-α levels is not clear, with some studies reporting no effect following periodontal intervention, and others reporting significant decreases in tumor necrosis factor-a levels after periodontal therapy, even though all of the studies considered confounding factors and the subjects in the various groups were well matched.[98,99]

B) Adhesion moleculesE-selectin

E-selectin is a glycoprotein that is expressed in activated vascular endothelium and plays a role in initiation of the inflammatory process. The circulatinglevel of E-selectin is used as a surrogate marker of endothelial function. High levels of E-selectin may predict the development of cardiovascular disease.[100]

The results of periodontal intervention treatment on the plasma levels of soluble E-selectin in various studies have been consistent.

Influence Of Periodontal Intervention On EndothelialDysfunction

Endothelial dysfunction is a fundamental step in the development of atherosclerosis, and can be measured by several methods, including flow-mediated dilatation of the brachial artery. Endothelial dysfunction as determined by measurement of brachial flow-mediated dilatation is considered to be a good predictor of cardiovascular outcomes.[101]

Periodontal disease is associated with endothelial dysfunction as measured by brachial flow-mediated dilatation. Endothelial function has been reported to be significantly lower in patients with periodontitis than in control subjects.[102] In addition, endothelial dysfunction in hypertensive patients with periodontitis is more severe compared to hypertensive patients without periodontitis.[103] Recently, endothelial function was evaluated in healthy and periodontitis patients with coronary artery disease.[104]

Influence Of Periodontal Therapy On Intima–Media Thickness OfThe Arterial Wall

The intima–media thickness of the arterial wall is a parameter of atherosclerosis. The carotid intima– media thickness is highly correlated with coronary artery disease and cerebral disease. The studies related to effects of periodontal intervention on intima– media thickness of the arterial wall are briefed in the review of literature below.

Influence Of Periodontal Treatment On Immunophenotypic Expression And Gene Expression Of Monocytes

Monocytes/macrophages and circulating CD4 T cells infiltrate the arterial wall, engulf the proatherogenic-modified forms of low density lipoprotein, and become foam cells. During these events, the phenotypes of the monocytes/macrophages change. In addition, many cytokines are involved and circulating monocytes and lymphocytes become important sources of these cytokines. The mechanisms involved in the association of periodontitis and cardiovascular disease are still under investigation. Recent studies have focused on the changes in immunophenotypic expression and gene expression in circulating monocytes and lymphocytes in periodontal intervention trials, because these cells are likely to be determinants of atherosclerosis.

Wilcken *et al.* (1976)[105] showed that the concentration of homocysteine-cysteine mixed disulfide after a methionine load was slightly higher in coronary heart disease (CHD) patients than in respective age- and sex matched controls. This pioneering work has led to many studies that have been the subject of a number of important review articles.

Lumeng *et al.* (1980)[106] suggested that plasma pyridoxal 5'-phosphate concentration reflects vitamin B6 status in the liver in healthy humans. A reduced plasma pyridoxal 5'-phosphate level implies that vitamin B6 status in the liver could be altered. These results suggest that the lower circulating pyridoxal 5'-phosphate levels observed in rheumatoid

arthritis could reflect a decrease in hepatic pyridoxal 5'-phosphate pools, and plasma pyridoxal 5'-phosphate is a good indicator of liver B6status during inflammation. Therefore, the abnormal vitamin B6 status in rheumatoid arthritis results from the inflammatory process, and it is unlikely that it resulted from insufficient intake or excessive excretion of vitamin B6.

Ueland *et al.* (1992)[107] summarized 17 studies that presented fasting Hcy concentrations for 1500 patients with various forms of vascular disease and a similar number of normal controls. Fasting Hcy concentrations were consistently elevated among patients with all types of vascular disease and averaged 31% greater than concentrations among controls.

Boushey *et al.* (1995)[108] in a meta-analysis involved 27 studies including prospective and population-based case-control studies. These studies concluded that elevations of plasma total homocysteine (tHcy) were considered an independent graded risk factor for arteriosclerotic vascular disease with odds ratios for 5mmol/L increase in plasma tHcy that ranged from 1.5 to 1.8 for men and women with CHD, cerebrovascular or peripheral vascular diseases.

Beck J *et al.* (1996)[109] put forward the central hypothesis that periodontal diseases, which are chronic Gram-negative infections, represent a previously unrecognized risk factor for atherosclerosis and thromboembolic events. They hypothesized that this association may be due to an underlying inflammatory response trait, which places an individual at high risk for developing both periodontal disease and atherosclerosis. The authors further suggest that periodontal disease, once established, provides a biological burden of endotoxin (lipopolysaccharide) and inflammatory cytokines (especially TxA2, IL-1β, PGE2, and TNF-α) which serve to initiate and exacerbate atherogenesis and thromboembolic events.

Wang *et al.* (1999)[110] demonstrated that in the blood, homocysteine molecules, which possess the capacity of forming chelate complexes with metallic cations including calcium ions, interact with the calcium ions of the calcium-dependent cell adhesions/junctions of the vascular endothelium. Such an action results in the release of calcium ions from some cell adhesions/junctions, causing the latter structures to dissociate and the vascular endothelium to be injured. While such factors as high levels of plasma calcium ion tend to restore the disrupted cell adhesions/junctions, other factors including blood pressure, bloodstream shearing force and high cholesterol/ phospholipid ratio in the membranes are unfavorable to the repair.

McCarty *et al.* (2000)[111] stated that smokers, patients with chronic inflammatory disorders, and the elderly are characterized by increased production of interleukin-6 as well as increased plasma Hcy levels. Analysis of cirrhotic livers suggests that interleukin-6 may stimulate the activity of pyridoxal phosphatase in hepatocytes, thereby diminishing pyridoxal phosphate levels, compromising cystathione-β- synthase activity and raising plasma Hcy.

De Nardin *et al.* (2001)[112] in a review published described mechanisms that may

help explain the association between periodontal infections and CHD. Periodontal diseases are bacterial infections associated with bacteremia, inflammation, and a strong immune response, all of which may represent significant risk factors for the development of atherogenesis, CHD, and myocardial infarction (MI). Several mechanisms may participate in this association, including those induced by oral organisms, and those associated with host response factors. This review focused on host factors. Oral pathogens and inflammatory mediators (such as interleukin [IL]-1 and tumor necrosis factor [TNF]-α) from periodontal lesions intermittently reach the bloodstream inducing systemic inflammatory reactants such as acute-phase proteins, and immune effectors including systemic antibodies to periodontal bacteria. This review describes the potential role of various inflammatory as well as immunologic factors that may play a role in periodontitis as a possiblerisk factor for CHD.

Libby P *et al.* (2002)[113] reviewed the evidence linking inflammation and atherosclerosis. Atherosclerosis, formerly considered a bland lipid storage disease, actually involves an ongoing inflammatory response. Recent advances in basic science have established a fundamental role forinflammation in mediating all stages of this disease. Substantial biological data implicate inflammatory pathways in early atherogenesis, in the progression of lesions, and finally in the thrombotic complications of this disease. These new findings provide important links between risk factors and the mechanisms of atherogenesis. Clinical studies have shown that this emerging biology of inflammation in atherosclerosis applies directly to human patients.

Chiang *et al.* (2003)[117] in 37 RA patients, demonstrated that the increase in Hcy levels after methionine load correlated with erythrocyte sedimentation rate (ESR) and CRP levels. The effect of high-dose pulsed glucocorticoid treatment on plasma Hcy concentration in patients with active RA was checked. In fact, if the inflammatory state is really implicated in the genesis of HHcy in these patients, a decrease in plasma level of Hcy following intensive steroid therapy would be expected together with the overall reduction in inflammation. Accordingly, there was a significant 26% Hcy reduction, and this effect was both rapid and long-lasting over a 6-month follow- up period. These findings, together with the concomitant decrease in CRP observed in early studies, provided a further indirect evidence of the link between inflammation and HHcy in RA patients.

Mercanoglu *et al.* (2004)[120] in a case control study evaluated the outcome of periodontal treatment on endothelial function as measurded by endothelium- dependent flow-mediated dilatation of the brachial artery(EDD) and endothelium- independent flow-mediated dilatation (EID). The maximum evidence for the improvement in endothelial function after periodontal therapy was obtained in a randomized controlled trial.

Seinost *et al.* (2005)[123] conducted an interventional study to assess the effects of periodontal therapy on endothelial dysfunction. 30 patients with severe periodontitis without systemic disease were treated with oral hygiene instruction, SRP in two sessions and 0.1% chlorhexidine mouthwash for 14 days along with systemic antimicrobial therapy for 7 days.

Results showed significant improvement in endothelium-dependent flow-mediated dilatation of the brachial artery after 12 weeks.

Elter *et al.* (2006)[126] followed a cohort of 22 systemically healthy patients suffering from periodontitis and treated them with oral hygiene instruction, SRP, periodontal flap surgery where indicated and extraction of hopeless teeth. Reduction in IL-6 levels were found 1 month after treatment.

Tuter *et al.* (2007)[130] compared SRP with SRP plus adjunctive sub-antimicrobial dose doxycycline on the level of C-reactive protein in patients with coronary heart disease. Results showed statistically significant decrease in both groups after 6 months.

Recently, in randomized-controlled trial conducted by Higashi et al, 48 patients with coronary heart disease who had periodontitis were randomly assigned to a periodontal treatment group or a control group (24 patients in each group). At 6 months after therapy, the serum concentration of high-sensitivity C-reactive protein was significantly reduced from 2.7 ± 1.9 to 1.8 ± 0.9 mg/l in the periodontaltreatment group, but no significant reduction was noted in the control group (from 2.6 ± 2.2 to 2.5 ± 2.1 mg/l).

Higashi *et al.* (2008)[138] in a randomized clinical trial evaluated the effects of periodontal therapy in patients with hypertension and found beneficial effects of the same. Periodontitis patients with hypertension were divided into a periodontitis treatment group (17 patients) and an untreated group (9 patients). There was no significant difference in body mass index, blood pressure, total cholesterol, triglyceride, HDL and LDL cholesterol or glucose between the groups. The endothelial function was evaluated in terms of forearm blood flow responses to acetylcholine. The untreated group showed no significant change between baseline and 3 months follow-up.

Piconi *et al*. (2009)[141] conducted a recent study to evaluate effect of periodontal treatment on intima–media thickness of the arterial wall, in this longitudinal study, 35 otherwise healthy subjects with mild to moderate periodontitis were enrolled. Non- surgical debridement was performed and completed within 4 weeks. Echo-Doppler cardiography of the carotid artery was evaluated before and 1, 6 and 12 months after the periodontal treatment. The results showed that the carotid intima–media thickness was significantly reduced at 6 and 12 months after treatment, and the decrease in the carotid intima–media thickness was detected at multiple sites along the carotid axis: at the carotid bifurcation and at 1 and 2 cm from the bifurcation.

Dhadse, *et al*.(2010)[142] designed a study to update the potential association, that forms the basis of understanding for a (causal) role for PD to cardiovascular events; as reported by various observational (case-control, cohort, cross-sectional) studies, epidemiological and interventional studies, not considering the other number of systemic health outcomes like cerebrovascular disease, pregnancy complications, chronic obstructive pulmonary disease**,** diabetes mellitus complications, osteoporosis, etc. A brief overview has been included for

atherosclerosis (ATH), its pathophysiology and the association of periodontal infections as a risk factor for causing ATH, which seems to be a rational one; as development of ATH involves a chronic low-grade inflammation and moreover, it has long been set up prior to development of ischemic heart disease and thus provides potential contributing mechanisms that ATH may contribute singly or in concert with other risk factors to develop ischemic heart disease.

Efeito *et al* (2011)[143] C-reactive protein (CRP) is a marker of inflammation that is naturally present in the plasma at levels that may rise due to inflammatory processes, associated with a greater risk of cardiovascular events such as acute myocardial infarction. Periodontal disease is responsible for a host immune- inflammatory response, contributing towards clarifying its association with cardiovascular disease. The aim of this study was to investigate the effect of periodontal therapy on the levels of this inflammatory marker. The sample consisted of 62 patients of both genders, between the ages of 30 to 60 years, who were referred to dental treatment at the Bahia Foundation for the Development of Sciences, and they were divided into two groups: with and without periodontitis.

Caúla *et al.* (2013)[145] determined the influence of non-surgical mechanical periodontal treatment on inflammatory markers related to risk for cardiovascular disease. A total of 64 patients with severe chronic periodontitis was randomly subjected to immediately periodontal treatment (test group, n = 32) or delayed periodontal treatment, without treatment during the study period (control group, n = 32). Clinical periodontal and laboratory examinations were performed at baseline (T0), 2 months (T2), and 6 months (T6) after the initial examinations (Control group) or completion of periodontal treatment (Test group). After 2 months of periodontal treatment there was a significant reduction of erythrocyte sedimentation rate (ESR) and triglycerides ($p = 0.002$, $p = 0.004$, respectively) in the test group. Median values of C-reactive protein, ESR, total cholesterol, and triglycerides were reduced after 6 month of periodontal treatment in the test group ($p < 0.001$, $p < 0.001$, $p < 0.001$, and $p = 0.015$, respectively). The non-surgical periodontal treatment was effective in reducing the levels of systemic inflammation markers and improved the lipid profile in subjects with severe chronic periodontitis.

Goyal *et al.* (2014)[146] C-reactive protein (CRP) is an acute-phase reactant and has been proved to be a significant predictor of future cardiovascular events. Recent studies have demonstrated a correlation between periodontitis and elevated CRP levels. However, most of the studies have focused on chronic periodontitis and very few studies are done in patients with aggressive periodontitis. The aim of this study was to determine and compare the relative levels of serum CRP in aggressive and chronic periodontitis patients.

Gupta *et al.* (2015)[147] both periodontitis and cardiovascular diseases (CVD) represent chronic inflammatory conditions, and periodontal infections have been postulated to perpetuate the progression of CVD's. However, limited evidence is available to prove the causal relationship. An effort in exploring this interrelation has been made in this study. The role of two inflammatory mediators, soluble CD40 ligand (sCD40 L) and monocyte chemoattractant protein-1 (MCP-1) has been established in progression and acute

precipitation of CVD's. Due to a close link between these two mediators, the present study was designed to correlate the levels of sCD40 L and MCP-1 in serum and gingival crevicular fluid (GCF) of patients with chronic periodontitis.

Reichert *et al.* (2016)[150] investigated whether periodontal conditions and/or oral care habits are associated with new cardiovascular events among patients with coronary vascular disease (CVD). Materials and Methods In this longitudinal cohort study, 1002 in patients with CVD were included. They were examined regarding prevalence of severe periodontitis, bleeding upon probing (BOP), number of missing teeth and oral care habits. The combined endpoint was defined as myocardial infarction, stroke/transient ischaemic attack, cardiovascular death and death caused by stroke. Survival analyses were carried out after a 3-year follow-up period. Hazard ratios (HRs) were adjusted for known cardiac risk factors using Cox regression.

DATA SOURCES:

The subjects for the study were selected from the patients attending the outpatient Department of Periodontology, School of Dental Sciences, KIMSDU, Karad after due approval of the Ethical committee (Ref. No.: KIMSDU/IEC/04/2014, dated 23/09/2014).

Subjects were explained about the study procedure in detail and were included in the study after obtaining their informed consent.

The study comprised of 50 subjects who fulfilled the inclusion criteria. The subjects included both sexes in the age group of 18-45 years. The prospective study design was explained to the subjects prior to their enrollment in the study. They were evaluated before and after treatment for clinical and biochemical parameters.

A standard proforma consisting of the following data: name, age, sex, medical and past dental history, personal history, oral hygiene habits, & Gingival Index (Loe and Sillness), Oral Hygiene Index (Loe and Sillness) and clinical attachment loss for each patient was recorded. Patients identified as having chronic periodontitis were classified based on the disease severity into mild, moderate and severe categories.

1. Slight periodontitis = 1 or 2 mm CAL
2. Moderate periodontitis = 3 or 4 mm CAL
3. Severe Periodontitis $\geq$ 5mm CAL

INCLUSION CRITERIA:

1. Number of teeth present: 20
2. Age between 18-45 years
3. >30% of site with chronic generalized moderate / severe periodontitis withpresence of disease activity as recorded by Gingival Index (Loe&Silness J).

EXCLUSION CRITERIA:

1. History of periodontal therapy within 6 months

2. History of antibiotic use within 3 weeks
3. Smokers
4. History of any systemic disease
5. Pregnant and lactating females
6. Presence of any systemic infection in the last 6 months

Armamentarium for Clinical Examination, Blood Sample Collection and Treatment

A) Intraoral Examination and treatment (Fig 6)

1. Mouth-Mirror
2. Tweezers
3. #17-23 Explorer
4. Cotton swabs
5. Mouth mask
6. Gloves
7. UNC-15 periodontal probe
8. Gracey Curettes
9. Piezoelectric Ultrasonic Scaler
10. Examination gloves

B) Blood Sample Collection (Fig 7)

1. 5 ml disposable syringe
2. Plain Vacutainer
3. Cotton Swabs
4. Spirit
5. Tourniquet.

C) Plasma Homocysteine Analysis

1. AccurexHomocysteine kit (Fig 8)
2. Centrifuge Machine (Fig 9)
3. EM 360 auto analyzer (Fig 10)

Data Collection Methods:

i. Probing pocket depth (PPD) (Fig 11)

Measured from gingival margin to the base of gingival sulcus and was recorded at six sites around all teeth (buccal, mesiobuccal, distobuccal, lingual, mesiolingual and distolingual). The UNC (University of North Carolina) — 15 periodontal probe was inserted parallel to the vertical axis of the tooth and walked circumferentially around each surface of the tooth with constant probing force of 0.75 N

ii. Clinical attachment level (CAL) (Fig 12)

Williams graduated periodontal probe was used to assess CAL (measured from cementoenamel junction to the base of gingival sulcus/ pocket). Attachment loss was recorded at 4 sites around all the teeth. The subjects were classified as having chronic

periodontitis based on 1999 consensus classification of periodontal diseases. Chronic periodontitis patients were characterized into three categories depending on level of clinical attachment loss (CAL), slight: 1-2mm CAL, moderate: 3-4 mm CAL and severe: ≥ 5mm CAL. Only patients with moderate to severe periodontitis were included in the study.

iii. Gingival Index (G.I) (LOE AND SILNESS 1963)

Index used for assessing the severity of gingivitis. The tissues surrounding each tooth were divided into gingival scoring units i.e. distofacial papilla, mesiofacial papilla, facial margin and the entire lingual margin. A blunt instrument, such as a periodontal probe was used to assess bleeding of the gingival tissues. Each of the four gingival units was assessed according to the criteria:

0 – Normal.
1 – Mild inflammation, change in color, slight edema43, no bleeding on probing.
2 – Moderate inflammation, redness, edema and glazing, bleeding on probing.
3 — Severe inflammation, marked redness and edema, tendency to spontaneous bleeding.

The numerical scores of gingival index associated with varying degrees of clinical gingivitis are as follows:

GINGIVAL SCORES	DEGREE OF GINGIVITIS
0.1-1.0	Mild gingivitis
1.1-2.0	Moderate gingivitis
2.1-3.0	Severe gingivitis

Gingival index (GI) for a tooth = scores around each tooth

iv. Simplified Oral Hygiene Index (Ohi-S) (Greene And Vermillion1964

The Simplified Oral Hygiene Index (OHI-S) was developed in 1964 by John C.Greene and Jack R. Vermillion, the developers of the original Oral Hygiene Index (OHI). Even though the original OHI was determined to be simple, sensitive, and useful, it was time-consuming and required more decision making. Thus the more simplified version was developed with equal sensitivity. The OHI-S differs from the original OHI (The Oral Hygiene Index) in the number of tooth surfaces scored (6 rather than 12), the method of selecting the surfaces to be obtained. The criteria used for assigning scores to the tooth surfaces are same as those used for the OHI. The OHI-S has two components, Simplified Debris Index (DI-S) and the Simplified Calculus Index (CI-S). Each of these indexes, in turn is based on numerical determinations representing the amount of debris or calculus found on the preselected tooth surfaces.

Surfaces and teeth to be examined

Tooth **Surface**

16 – Upper right first molar	Buccal
11 – Upper right central incisor	Labial
26 – Upper left first molar	Buccal
36 – Lower left first molar	Lingual
31 – Lower left central incisor	Labial
46 – Lower right first molar	Lingual

Debris Index – Simplified (DI-S)

The surface area covered by debris is estimated by running the side of a explorer (Shepard's Hook) along the tooth surface being examined. The occlusal or incisal extent of the debris is noted as it is removed. Following scoroing criteria is used:

0	No debris or stain present
1	Soft debirs covering not more than one third of the tooth surface, or the presence of extrinsic stains without other debris regardless of surface area covered
2	Soft debris covering more than one third but not morethan two thirds, of the exposed tooth surface.
3	Soft debris covering more than two thirds of theexposed tooth surface

Calculus Index – Simplified (CI-S)

The no. 5 explorer (Shephard's hook) is used to estimate the surface area covered by supragingival calculus and to probe for subgingival calculus. The following scoringcodes and criteria are used for the CI-S

Score	**Criteria**
0	No calculus present
1	Supragingival calculus covering not more than 1/3 of the exposed tooth surface
	Supragingival calculus covering more than 1/3 but not more than 2/3 of the exposed tooth surface or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth or both.

3	Supragingival calculus covering more than 2/3 of the exposed tooth surface or a continuous band of subgingival calculus around the cervical portion of the tooth, or both.

Calculation of the Index:

For each individual, the debris and calculus scores are totaled and divided by the number of tooth surfaces scored. For an individual score to be calculated at least twoof the six possible tooth surfaces must have been examined. For a group of individuals, the debris and calculus scores are obtained by calculating the average ofthe individual scores. The average individual or group score is the DI-S or the CI-S. Individual scores are calculated to one decimal place, and group scores may be calculated to one decimal place, and group scores may be calculated to one or two decimal places depending on the sample size and use of the data.

Once the DI-S and CI-S are calculated separately, they are then added together for the OHI-S. The DI-S and CI-S values range from 0 to 3, which can be interpreted as

Good	0.0 to 0.6
Fair	0.7 to 1.8
Poor	1.9 to 3.0

The OHI-S value ranges from 0 to 6, which can be interpreted as:

Good	0.0 to 1.2
Fair	1.3 to 3.0
Poor	3.1 to 6.0

METHOD OF STUDY:

Each patient was examined using a mouth mirror and UNC-15 graduated periodontal probe. After recording the clinical parameters & indices, 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. After 90 days, the patient was re-evaluated for clinical parameters and the readings were recorded again. Blood was again sent for analysis of post treatment plasma homocysteine levels.

ANALYSIS OF PLASMA HOMOCYSTEINEMETHOD PRINCIPLE:

The Siemens Diagnostics enzymatic test for the quantitative Homocysteine determination (HCY) is based on a series of enzymatic reactions, causing a decrease in absorbance valuedue to NADH oxidation to NAD+. HCY concentration in the sample is directly proportional to the quantity of NADH converted to NAD+ (ΔA_{340nm}). The enzymatic reactions areas follows:

T(HCY)→(HCY)free

(HCY)free + SAM* —s-Methyltransferase→ Methionine + SAH**

SAM Hydrolase + Adenosine deaminase

SAH + NADH —Glutamatedehydrogenase→ Inosine + NAD^+

* SAM = S-Adenosyl-methionine

** SAH = S-Adenosyl-homocysteine

COMPOSITION OF DIAGNOSTIC KIT

- **REAGENT A:**

S-adenosylmethionine	0.1 mmol/l
NADH	0.2 mmol/l
TCEP	0.5 mmol/l
2-oxoglutarate	5.0 mmol/l

- **REAGENT B:**

Glutamate dehydrogenase	10 KU/l SAH hydrolase	3.0 KU/l
Adenosynedeaminase	5.0 KU/l	
HCY methyltransferase	5.0 KU/l	

CALIBRATORS: 2 levels 2x1 ml

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLES

Fresh serum or plasma is recommended sample for the HCY assay. It is important to centrifuge blood samples immediately after collection to separate the plasma from the blood cells. If immediate centrifugation is not possible, collected blood specimens should be kept on ice and centrifuged whithin an hour. Hemolysed or turbid specimens or severely lipemic specimens are not recommended for HCY assay. They should be discarded.

STABILITY:

After separation of serum/plasma from cells, HCY is stable in the sample if stored for 4 days at room temperature, 4 weeks at 2-8 °C, and for 12 months at -20 °C.

ANALYTICAL PROCEDURE

Allow the reagents to reach working temperature before using.Pipette the reagents into

disposable or well clean cuvettes for calibaration:

	Blank	CAL 1	CAL 2	Sample
Reagent A	950 µl	950 µl	950 µl	950 µl
Saline	50 µl			
Calibrator 1		50 µl		
Calibrator 2			50 µl	
Sample				50 µl

Mix and incubate for 5 minutes at 37 °C. Then add:

Reagent B	250 µl	250 µl	250 µl	250 µl

Mix and incubate at 37 °C for 2.5 minutes. Read absorbance (A1) at 340 nm andthen read again after 2.5 minutes (A2).

Note:

- Reaction volumes can be proportionally changed.
- Samples with values greater than 50 µmol/l should be diluted 1:2 and tested again. Multiply results by 2.

AUTOMATIC ANALYZERS

The present kit can be used with any type of clinical chemistry automatic analyzer.

CALIBRATION

Use the calibrator 1 and 2 to establish the calibration curve. Use saline or distilledwater as calibration point 0 (0 µmol/l) where applicable or requested.

Two different values referring to two different standardizations aregiven for each calibrator:

- NIST: values standardized and traceable to NIST SRM 1955 "Homocysteine Standard Reference Material";
- CLIA: values standardized against chemiluminescent assays

Calibration is stable up to 5 days. Anyway, it is suggested to recalibrate in case of the assay Quality Control gives non acceptability

ANALYTICAL PERFORMANCES

Sensitivity

Test sensitivity, in terms of limit of detection, is 0.4 µmol/l.

Linearity

The assay is linear upto 50 µmol/l.

Precision

Precision has been evaluated by testing replicates of four samples at differentHomocysteine concentration.

The obtained results are reported in the following tables.

Within-run precision

Sample	N	Mean(umol/l)	SD	%CV
Sample 1	40	7.0	0.32	4.57
Sample 2	40	12.0	0.22	1.83
Sample 3	40	15.6	0.47	3.01
Sample 4	40	29.0	0.70	2.41

Between-run-precision

Sample	N	Mean	SD	%CV
Sample 1	40	7.0	0.42	6.00
Sample 2	40	12.0	0.59	4.92
Sample 3	40	15.6	0.80	5.12
Sample 4	40	29.0	0.75	2.59

Results and Discussion

The present study was conducted to determine the levels of plasma homocysteine in patients with chronic periodontitis and to evaluate the effectof non-surgical periodontal therapy on plasma homocysteine. The study consisted of systemically healthy patients with chronic periodontitis, which were evaluated for clinical and biochemical parameters at baseline and 90 days after treatment. Clinical parameters compared were clinical attachment levels, gingival index, and oral hygiene index whereas the biochemical parameter evaluated was plasma homocysteine. The group tested consisted of 28 males and 22 females, which consisted of 56% of males and 44% of female subjects. The mean age of the group was 37.6 ± 5.7 years. 6 patients in the group were less than 30 years of age, 25 patients were between 31 to 39 years of ageand 19 patients were between 40 to 45 years of age.

Comparison of baseline and post treatment homocysteine levels

In our study, the mean HCY value was 20.7± 3.4 µmol/L while the mean post treatment HCY values were14.7± 2.2 µmol/L. Thus our treatment resulted in a reduction of 6.0 ± 1.2µmol/L. The percentage of change was 9.19%. This means that the post treatment HCY levels are significantly lower as compared to the baseline HCY levels. In other words a significant change of 9.19% was observed from baseline to post treatment values.

Comparison of baseline and post treatment Gingival Index scores byWilcoxon matched pairs test.

In our study, the degree of severity gingival inflammation was evaluated bythe 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 was

1.1± 0.22 which indicates moderate gingivitis. There was a decrease of 0.9± 0.05 after treatment. The percentage of change was 55 %.As this data is non-parametric, the Wilcoxon matched pairs test was used. This implies that there was a significant improvement in the gingival condition following periodontal treatment. In other words there was an improvement of 55 % from baseline to post treatment values.

Comparison of baseline and post treatment OHI-S scores by Wilcoxon matched pairs test

In our study, the simplified oral hygiene index of the patients was evaluated to correlate the oral hygiene status with the other clinical parameters. Baseline simplified oral hygiene index was 2.6 ± 0.5 which indicated poor oral hygiene while after treatment it was 1.6 ± 0.9 which indicate fair oral hygiene. There was an improvement of 1.0 ± 0.5 with a percentage change of 61.5%. As this data is non-parametric, the Wilcoxon matched pairs test was used. This means that there was a significant improvement in the oral hygiene of the group following periodontal treatment. In other words there was an improvement of 61.5 % from baseline to post treatment levels.

Correlation among different clinical parameters with pre scores and post scores by Pearson's correlation:

In our study, the different clinical parameters were compared using Pearson'scorrelation. The Pearson's correlation value is represented as 'r'. The correlation gives relationship between two variables. It also gives the direction of relationship between the variables, whether positive or negative correlationexists as well as the strength of the relationship whether they are strongly associated or weakly associated.

<u>According to the Pearson's correlation:</u>

0.00- 0.19 - very weak
0.20- 0.39 - weak
0.40- 0.59 - moderate
0.60- 0.79 - strong
0.80-1.0 - very strong

- Positive values denote positive linear correlation;
- Negative values denote negative linear correlation;
- A value of 0 denotes no linear correlation;
- The closer the value is to 1 or –1, the stronger the linear correlation.

The correlation value was set as $p<0.05$. There was no correlation found between any of the HYC, OHIS and GI ($p>0.05$) for pre scores as the correlationvalues found were, HYC and OHIS: r= -0.10, HYC and GI:r= 0.12, OHIS and GI: r= -0.15. Similarly when the correlation was compared between HYC, OHIS and GI ($p>0.05$) for post scores there was no correlation as the values found were, HYC and OHIS: r= -0.02, HYC and GI: r= 0.24, OHIS and GI: r= -0.05.

Gender wise mean score of Hcy

In our study, the Hcy levels were compared based on gender. There was no statistical significant difference found between Hcy levels of males and females, both pre operatively and post operatively.

Age wise correlation scores of Hcy

A correlation analysis was carried to confirm any relationship between HYC and age. There was no correlation found between HYC and age ($p>0.05$). Chronic periodontitis has been linked to systemic diseases/conditions like coronary artery disease (CAD)/ atherosclerosis etc.[13,14] Literature now explores the immunologic-inflammatory pathway behind systemic diseases/conditions (like cardiovascular disease, autoimmune conditions like rheumatoid arthritis etc). The release of proinflammatory cytokines like IL-1β, TNF α, IL-6 etc and the activation of the host immune response has been linked to the underlying destructive mechanisms in these diseases. In addition to conventional risk factors, recently several novel risk factors have also been proposed for CAD. These novel risk factors include chronic infections and infection related biomarkers such as CRP, elevated homocysteine (Hcy) levels etc.[9,10]

The present study was undertaken to evaluate the effect of non-surgical periodontal therapy on plasma homocysteine values. A prospective study design was selected. 50 patients were selected from the Department of Periodontology. Informed consent was taken from all the patients, and clinical as well as biochemical parameters were recorded as per the study design.

Poor oral hygiene was predominant in the subjects analyzed with a mean baseline OHI-S score of 3.09 ± 0.69 and baseline gingival index of 2.44 ± 0.50 indicating severe gingival inflammation.

Our study showed with 20 cases of moderate periodontitis having mean Hcy of 16.94 ± 3.71 µmol/L and 30 patients having severe periodontitis with mean Hcy of 17.52 ± 1.92 µmol/L. However this difference was not statistically significant. The periodontal disease in these subjects result in inflammation and immune reaction and an ultimate systemic burden to the host with the resultant emergence of inflammatory reactants such as acute-phase proteins, and immune effectors like CRP, plasma homocysteine etc.

The baseline plasma Hcy values in our 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. Hyperhomocysteinemia has been traditionally classified as plasma Hcy concentrations above 15 µmol/L is considered elevated: 16 to 30 µmol/L is classified as mild, 31 to 100 µmol/L is classified as intermediate, and > 100 µmol/L is classified as severe hyperhomocysteinemia. Mild hyperhomocysteinemia has shown to be independently associated with the development of CAD, cerebral and peripheral vascular disease and deep-vein thrombosis. Thus the mean values obtained in our study group show that chronic periodontitis could be a

risk factor for CAD.

Conclusions

Thus the following conclusions may be drawn from this study:

I) An inflammatory condition like chronic periodontitis is significantly associated with elevated plasma homocysteine levels

II) However, no significant change was seen in the plasma homocysteine levels between moderate and severe chronic periodontitis.

III) There was no correlation associated with plasma homocysteinelevels and age.

IV) There was no significant difference associated with plasma homocysteine levels when compared between males and females.

V) Periodontal intervention shows statistically significant improvement in plasma homocysteine values.

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ISSN 2515-8260 Volume 9, Issue 7, Summer 2022

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