STUDY OF CLINICAL PARAMETERS IN CHRONIC PERIODONTITIS

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ABSTRACT:

Back ground: Periodontitis is a chronic inflammatory disease, causes changes in peripheral blood markers with slight abnormal lipid profile including the production of different enzymes that are released by stromal, epithelial or inflammatory cells. These changes reflect metabolic changes in the gingival and periodontium in inflammation.

Design of study: This important cohort study includes 54 subjects as chronic periodontitis patients along with 26 healthy age matched controls of both sexes, In this study, different peripheral blood markers (Neutrophils,WBC,RBC,Thrombocytes and Hb%), major inflammation markers (plasma Homocysteine, CRP),Total lipid profile (Cholesterol, TGL,HDL, LDL) and salivary enzymes (CK, LDH,AST, ALT, ALP, ACP and GGT) are studied to evaluate diagnosis, prognosis and therapeutic effects in this disease.

Results: Due to stasis of blood stream in periodontitis causes margination of central blood stream cells and finally there will be significant correlation in Neutrophils (r=0.342), WBC(r=0.431),thrombocytes(r=0.216),RBC(r=-0.183)Hblevel(r=-0.162).Inflammation markers and total lipid profile also show significant positive correlation: plasma homocystein (r=0.763),C-reactive protein(r=0.842),Total cholesterol,TGL,LDL (r=0.134,0.529,0.293) except HDL(r= -0.734). Salivary enzymes (CK-0.923, LDH-0.314, AST-0.841, ALT-0.832,ALP-0.782, ACP-0.826 and GGT-0.794) with gingival index and pocket depth.

Conclusion: By studying this simple, economical clinical parameters we can assess the damage of periodontal tissue and useful in prediction of future risk of atherosclerosis in chronic periodontal patients.

Key words: Inflammation, Peripheral Blood Markers, Salivary Enzymes, C-Reactive Protein, Atherosclerosis, Gingival Index

INTRODUCTION

Periodontitis, a bacterially induced, localized, chronic inflammatory disease, destroys connective tissue and bone that support the teeth. predominantly anaerobic Gram negative bacteria present on the tooth surface as microbial

Biofilms and other microbial substances gain access to the gingival tissue and initiate and perpetuate an inflammatory reaction, which leads to the destruction of the periodontal ligament and alveolar

bone and, finally, to tooth loss (1).

Periodontitis has even higher prevalence in developing countries and considerable global variation, although the prevalence of the severe generalized disease appears to be similar in most populations (2).

Traditional periodontal diagnosis involves measures of probing depth, gingival recession, probing attachment level using graduated periodontal probe. These are indirect measures of bone loss. Unfortunately, the sensitivity of radiographs in detecting an early osseous lesion is poor (3).Biochemical markers can detect inflammatory changes in short period of time where as longer period is required to detect measurable changes in bone density using radiographs.

A response of an organism to the periodontal infection includes production of several enzyme families and inflammation markers, which are released from stromal, epithelial, inflammatory or bacterial cells. The analysis of these enzymes in salivary secretion and inflammation markers in serum of peridontitis patients can contribute to clarification of the pathogenesis and to improvement of making a prompt diagnosis of the periodontal disease (4).
Recently, a causal relation has been demonstrated between high serum lipid levels and periodontal disease. Recent studies illustrate the existence of a relation between periodontal disorders and hyperlipidaemia, which power the probable effect of periodontal disease as an underlying factor for hyperlipidaemia. This theory is presented in Losche et al study, which demonstrated higher level of TGL and lower HDL among the patients suffering periodontitis than control group significantly (5),which was approved by some other studies (6-8);

The aim of the present study is 1) To evaluate the clinical significance of total patho and biochemical parameters in chronic periodontitis, which was not studied up to yet. 2) To prove the relation between chronic periodontitis and atherosclerosis by measuring the lipid profile and inflammation markers. 3) Statistically establish the correlation between all clinical parameters with gingival and periodontal index.

Materials and methods

The study protocol was in keeping with the ethical guidelines of the 1975 declaration of Helsinki and all the patients gave written informed consent to the study. Patients were selected from those who had visited department of dentistry, Govt. Medical College, Jagdalpur.

54 subjects ranging age from 30 – 65 years with chronic periodontitis and 26 healthy age matched controls of both sexes were taken, all subjects with good general health with no history of systemic disease. As the initial examination, each subject completed a detailed medical questionnaire and received a complete periodontal examination, which included: gingival index (GI), bleeding on probing (BOP), probing depth (PD).

the selection criteria taken for this study is at least 18 teeth had to be present, excluding third molar; of which at least 12 had to be posterior teeth. Definite diagnosis of chronic periodontitis. was performed based on the existence of calculus an plaques, at least one pocket above 6mm depth in every quadrant, and bone destruction.

Diabetic, cardiac heart disease, hypertension, anti hyperlipidemic consumption, tobacco smoking and women who were pregnant or receiving hormone or vitamin treatment were excluded from this study

In all these cases, the peripheral blood were taken drawn for routine investigation, that is total white blood cell count, red blood cells count, thrombocytes count and Hb level. Plasma was obtained after centrifugation at 3000rpm at 20 mins and stored at -4ºc are used for plasma Homocysteine (Homocysteine Enzyme Immunoassay Kit, Bio-Rad Lab, Oslo, Norway).and C-reactive protein estimation(C-reactive protein, Enzyme Immunoassay Kit, Bio-Rad Lab, Oslo, Norway)(9)Totalcholesterol(cholesteroloxidase-peroxidasemethod)andSerumTGL(glycerophosphateoxidase method), HDL(phosphotungstate), LDL (friedwald's equation) (10,11,12) are analyzed by using Hitachi 704 fully automated analyzer with reagents supplied by Boehringer Mannheim diagnostics.

Samples of a un stimulated, mixed saliva were taken, 3minutes after mouth cleansing and before breakfast, directly from the mouth of the patient by an automatic pipette (Salivette, Sarsstadt, Germany) (13) and were collected in a sterile test tubes. After that the saliva samples were centrifuged at 10000rpm for 10 minutes. The activity of enzymes in saliva was determined spectrometrically by the IFCC method on the Hitachi 704 Automatic analyzer.

RESULTS

Table -1. Demographic profile: Age, Height, Weight and Body Mass Index (BMI) of normal healthy and Chronic Periodontal disease

Values are mean±SD of number of observations (n). * indicates statistical significance when compared with normal healthy controls.

Parameter	Control group (n=26)	Case group (n=54)	P-value
Age(yrs)	45±3.5	52±2.2	p<0.01*
Height (cm)	163.13±0.8	161.32±0.8	p<0.05*
Weight (kg)	58.32±0.15	63.37±0.65	p<0.1
BMI (kg/m2)	23.21±0.15	21.04±0.18	p<0.05*
Clinical loss of Attachment (in mm)	1.4± 0.1	8.7±1.2	p<0.05*

Table -2: Total number of peripheral blood parameters with Plasma Homocysteine and CRP levels in Chronic Periodontitis and Healthy controls.

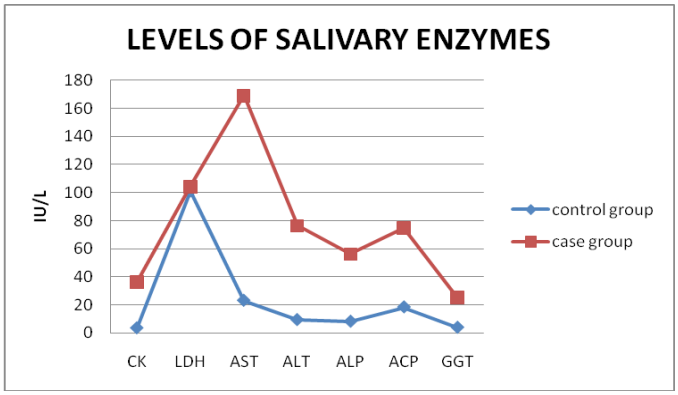
Parameter	Control group (n=26)	Case group (n=54)	Correlation significance
Total cholesterol	194±34.3	210.3±32.4	r= 0.134, P<0.1
TGL	132.4±38.6	171.2±33.5	r= 0.529, P<0.02
HDL	48.8±11.4	42.6±9.3	r= -0.734, P<0.01
LDL	121.4±21.3	129.4±36.4	r= 0.293, P<0.1
Plasma Homocystiene	8.4±1.53	18.44±5.23	r= 0.763, P<0.01
Plasma CRP	1.69±0.32	4.2±1.32	r= 0.842, P<0.001
Neutrophil count(*10^9/L)	4.1±1.7	6.3±1.5	r= 0.342, P<0.05
Total WBC count(*10^9/L)	5.8±1.1	8.1±1.4	r= 0.431, P<0.05
Total thrombocytes(*10^9/L)	1.9±1.2	2.4±1.2	r= 0.216, P<0.05
Total RBC count(*10^{12}/L)	4.8±1.6	4.2±1.4	r= - 0.183,P<0.01
Hb level(g/dl)	10.2±1.5	9.3±1.3	r= - 0.162,p<0.05

TGL- Triacyl glycerol, HDL- High density lipoprotein, LDL- Low density lipoprotein, CRP – c- reactive protein, RBC – Red blood cells, Hb – Haemoglobin

Table -3: Salivary Enzyme levels in Normal Healthy control and periodontal Disease.

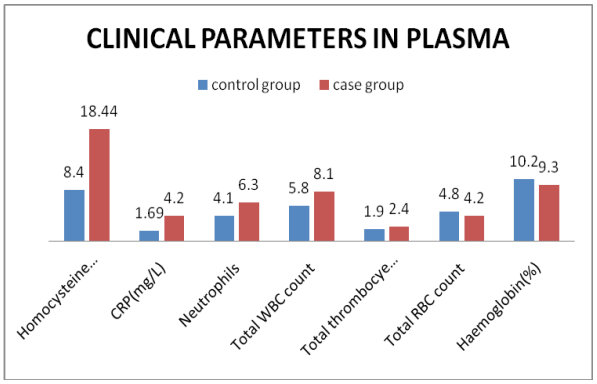
Parameter	Control group (n=26)	Case group (n=54)	Correlation significance
CK	3.50±1.82	36.34±18.3	r= 0.923, P<0.001
LDH	101.2±13.01	104.3±24.5	r= 0.314, P<0.05
AST	23.20±4.53	169.14±68.2	r= 0.841, P<0.01
ALT	9.4±2.3	76.39±34.1	r= 0.832, P<0.01
ALP	8.2±1.04	56.34±23.6	r= 0.782, P<0.01
ACP	18.45±1.02	74.93±15.89	r= 0.826, P<0.05
GGT	3.9±3.1	25.34±9.36	r= 0.794, P<0.01

CK -creatine kinase, LDH-Lactate dehydrogenase, AST- Aspartate Transaminase, ALT –Alanine Transminase, ALP- Alkaline Phosphatase, ACP- Acid Phosphatase, GGT – Gamma Glutamyl Transferase.



Graph-1: The above graph represents the levels of salivary enzymes in chronic periodontitis and in normal control group. The values are represented here as mean of specific enzyme levels.

CK -Creatine kinase, LDH-Lactate dehydrogenase, AST- Aspartate transaminase, ALT – Alanine transminase, ALP- Alkaline phosphatase, ACP- Acid phosphatase, GGT – Gamma glutamyl transferase.



Graph-2: represents the data of major clinical markers in chronic periodontitis. Neutrophils,WBC count, Thrombocytes($x10^9$/L), RBC($x10^{12}$/L) and Homocysteine(µmol/L).

Discussion

Periodontitis begins with a microbial infection, followed by a host-mediated destruction of soft tissue caused by hyper activated or primed leukocytes and the generation of cytokines eicosanoids, and matrix metalloproteinases that cause clinically significant connective tissue and bone destruction (14). Bacterial accumulations on the teeth are essential to the initiation and progression of periodontitis. In the early satge of peridontitis the rate of blood flow is increased due to vasodialation. But subsequently there is a slowing or stasis of blood stream. With stasis, changes in normal axial flow of blood in the microcirculation takes place. The normal axial flow consists of central stream of cells comprised by leucocytes and RBCs and peripheral cell free layer of plasma close to vessel wall. Due to stasis, the central stream of cells widens and peripheral zone becomes narrower because of loss of plasma by exudation. After this margination the neutrophils of the central coloumn come close to the vessel wall as a result of redistribution. All this consequences finally causes increase in neutrophils, leukocytes and thrombocytes.(15)

Fredirickson et al 1999(16) suggested low hematocrit with elevated leukocyte count in relation to periodontitis. Our study shown significant similarity with this as per the (table-1). As thrombocytes play an active role in innate immunity against micro organisms, naturally in inflammation and infectious processes there will be increased number of active thrombocytes. The number of increase in leukocytes in periodontitis has been suggested to be mainly due to an increase in polymorphonuclear leukocytes. More cell number per unit cube made blood more viscous and more cells may adhere to endothelial cells lining the blood vessels decreases the blood flow it causes atherosclerotic plaque formation especially at narrow capillaries and finally causes cardiovascular diseases.

Losche et al in 2005(17) assayed plasma TGL and total cholesterol levels and that study sates there is a significant increase in TGL and LDL along with decreased HDL values in chronic peridontitis. But in this study we got similar results except rise in LDL levels, which are statistically not significant ($p<0.1$) in chronic peridontitis. Morita et al(2005) (18) in a similar study found that the average cholesterol and LDL were not significantly higher in periodontal than healthy ones.

In our study patients lipid profile (table-2) were assessed and the serum total cholesterol ($r = 0.134, p<0.1$) ,TGL ($r =0.529, p<0.02$),LDL($r = 0.293$) and HDL ($r = -0.293, P<0.01$), which are clinically correlated with gingival index and pocket depth and matched by age and sex.

High age is a risk factor of hyper lipidemia and could be an effective factor to set a significant relation between chronic peridontitis and hyper lipidemia.

The C–reactive protein (CRP) is a part of the body's normal response to infection and inflammation; the higher plasma levels of CRP were observed in the present study in case of severe periodontitis patients in comparison with healthy control ($r =0.842, p<0.001$). it has been well documented that plasma Homocysteine ($r = 0.763, p<0.01$) and CRP levels are valuable markers in assessment of cardiovascular risk. Zhu et al (2000) reported strong association of CRP and plasma Homocysteine with chronic periodontitis. Over the past 2 decades, inflammation has emerged as an integrative CVD factor. Inflammation can operate in "all stages of this disease from initiation through progression and, ultimately, the thrombotic complications of atherosclerosis.

CK, LDH, AST, ALT. ALP, ACP and GGT are intracellular enzymes. (Table-3) these are good indicators to assess the cellular damage level. Metabolic changes in the inflamed gingival causes these enzymes to be release in high amounts in to saliva.

Periodontitis is a inflamed disease that progresses to the resorption of alveolar bone, which leads to progressive bone destruction and tooth loss. As a consequence of resorption, breakdown of products are released into periodontal tissues, migrating toward the gingival sulcus and gathering from the surrounding site in whole saliva, where several of them have been identified. Among the several host enzymes proposed as diagnostic indicators of periodontal status, ALP was one of the first to be identified. ALP is released from polymorphonuclear cells (PMNs) during inflammation and from osteoblasts and periodontal ligament fibroblasts during bone formation and periodontal regeneration respectively.

Hypertension was more prevalent in patients with severe alveolar bone loss, and significantly more hypertension occurs in patients with periodontitis compared with populations with little or no periodontal disease. Whether hypertension is a risk factor for periodontitis, however, remains uncertain. Systemic inflammation, a feature of hypertension, as evidenced by increased CRP plasma levels in patients with pre hypertension and patients with established hypertension.

Conclusion

The conventional periodontal diagnosis methods are provide limited information about patient sites and risk of future periodontal breakdown, but biochemical parameters here we studied in this study give good information on not only assessment of status of the disease. They can also predict the future risk of cardiovascular diseases in chronic periodontits patients. In this study we used saliva as a sample for estimation of all enzymes, Sampling of saliva is much easier, economical, feasible, convenient approach, bearable for the patient and it represents same diagnostic enzymes of GCF. These all things made it as a good sample parameter for the routine periodontal diagnosis. Finally all parameters are studied and we established the significance of clinical parameters in chronic periodontitis, clinically correlated with gingival index and pocket depth.

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