Effect on Procoagulant State after Phase-1 Periodontal Therapy in Chronic Periodontitis Patients: A Clinical and Haematological Study

Dr. Nitin Sangwan[1],Dr.C.S Baiju[2], Dr. Sumidha Bansal[3] , Dr. Gunjan Gupta[4], Dr.Karuna Joshi[5]

Department of Periodontics, Sudha Rustagi College of Dental Science &Research , Faridabad , Haryana, India
Corresponding Author:Dr. Nitin Sangwan

Abstract: *Periodontitis is a chronic inflammatory disease characterized by the destruction of alveolar bone, and the loss of the connective tissue supporting the teeth. Increase in the number of white blood corpuscles (WBCs) in response to LPS and bacteria are considered a strong independent predictor of future coronary heart diseases. It has been hypothesized that platelets and leukocytes being more sensitive to stimulation by periodontal pathogens, might contribute to procoagulant state of blood.*

AIM*: The aim of this study was to assess the effect of non-surgical periodontal therapy on procoagulant state in chronic periodontitis patients.*

Material and method*: Total number of 55 systemically healthy male patients with chronic periodontitis were selected. On first visit blood samples had been taken for total leucocyte count (TLC), differential leucocyte count (DLC) and platelet count, bleeding time and clotting time. Scaling and root planing was completed within 24 hours. Probing pocket depth (PPD), clinical attachment loss (CAL) and bleeding on probing (BOP) gingival index and plaque index was recorded by using the UNC-15 periodontal probe at baseline. Patients were recalled after 2 weeks post-therapy to resample venous blood and to record clinical measures.*

Results: *Significant reduction in Total leucocyte count (TLC) and platelet, whereas differential leucocyte count (DLC), bleeding time and clotting time didn't show any statistical significance.*

Conclusion : *Decreasing periodontal inflammation may be a successful key to decrease procoagulant state which may further decrease the risk of CVD.*

Key-words : *procoagulant ,periodontitis , total leucocyte count , coronary heart disease*

I. Introduction

Bacteria with varying pathogenicity have been identified and correlated with various forms of periodontitis.[1] It has been shown that periodontal bacteria or their products can directly invade the periodontal tissues, through the ulcerated pocket epithelium around the teeth and gain access to the systemic circulation.[2] It has been proved in various studies that periodontitis has been associated with increase in procoagulant state of blood.

Term procoagulant is defined as a precursor of a natural substance necessary for coagulation of the blood (Dorland's Medical Dictionary for Health Consumers).

Procoagulant state of blood changes to hypercoagulable state due to increase in many factors for example WBC, platelets, fibrinogen etc[1]. White blood cells (WBCs) are an integral part of the innate immune system. A large body of evidence supports the role of platelets in linking bacteraemia to atherothrombosis.Activated platelets are believed to be important contributors of the phosphatidylserine rich (PS) surface for the procoagulant membrane-dependent reactions in thrombosis and hemostasis. Procoagulant platelets formed upon strong platelet stimulation, usually with thrombin plus collagen, are large "balloons" with a small (1 μm radius) "cap"-like convex region that is enriched with adhesive proteins. The function of the procoagulant platelets is likely to be acceleration of coagulation reactions and not adhesion/aggregation. Spatial distribution of blood coagulation factors on the surface of procoagulant platelets was investigated using confocal microscopy in which all of them including factors IXa, Xa/X, Va, VIII, prothrombin and PS-sensitive marker annexin V were distributed non-homogeneously.[3] Diseased periodontal tissues represent sites of continuous localized activation of the blood coagulation cascade. The majority of biochemical reactions of blood coagulation do not occur in solution, but are rather surface-dependent, and take place on the membranes provided by cells and particles of blood and vasculature.[3]

Periodontitis has also been shown to be associated with an increase in plasma fibrinogen which might contribute to a pro-coagulant state and there is an increased risk for atherosclerosis and cardiovascular disease.[4]Besides its role in tissue homeostasis, excessive fibrinogen production can increase pro-inflammatory cytokines and attract more leukocytes at the sites of inflammation.[4] Data from several epidemiologic studies have shown an association between periodontal diseases and coronary heart disease (CHD) suggesting that periodontal diseases (PDs) are a risk factor for CHD. [6] This study was undertaken because W.B.C and platelets has been associated with atherosclerosis in a number of epidemiological studies.[5] The purpose of this study was to assess the effect of non-surgical periodontal therapy on pro-coagulant state in chronic periodontitis patients.

II. Materials and methods

The study was conducted in outpatient Department of Periodontics and Oral Implantology, Sudha Rustagi College of Dental Sciences and Research, Faridabad .Informed consent was obtained prior to the procedure of each subject. The study group comprised of 55 systemically healthy male patients with chronic periodontitis aged between 25-45.

Inclusion criteria:

Systemically healthy male patients with age group 25-45 years with generalized (moderate to severe) chronic periodontitis with probing depth $\geq$ 5mm in conjugation with CAL $\geq$ 3mm on more than 30% of sites were selected. Selected patients had not undergone any periodontal therapy for the last 6 months.

Exclusion criteria:

Patients with a history of any acute infection and /or antibiotic therapy in the last six months, current smokers / tobacco chewers, undergone recent extraction and tooth trauma, recent history of immunization and female were excluded from the study.

III. Methodology

Patients were selected on the basis of inclusion and exclusion criteria. On the first visit, clinical parameters were recorded which included Probing pocket depth (PPD), clinical attachment loss (CAL) and bleeding on probing (BOP) using UNC-15 periodontal probe .

Venous blood sample were withdrawn for the analysis of TLC, DLC, platelet count, bleeding time (BT) and clotting time (CT).After recoding base line clinical and haematological parameters, scaling and root planing was performed within 24 hours. Patients were advised to use chlorhexidine mouth rinse twice daily as an adjunctive home care measure. Patients were then recalled after a period of 2 weeks for re-evaluation of clinical and haematological parameters.

Blood sampling:

3 ml of venous blood sample was drawn from anticubital vein by venipuncture using a standard 5-ml syringe from each subject and was stored in vials containing EDTA and sent for haematological analysis (TLC,DLC, platelet count, BT,CT) .The blood samples were taken at baseline and then again 14 days after treatment. The blood samples were analyzed using semi-automatic analyzer.

IV. Statistical analysis

Data was entered into Microsoft Excel spreadsheet and was checked for any discrepancies. Summarized data was presented using tables and graphs. The data was analyzed by SPSS (21.0 version). Data was normally distributed as tested using the Shaperio-Wilk W test (p-value was more than 0.05).Therefore, analyses were performed using the parametric tests i.e. paired 't' test (for comparing more two groups). This was used for variables- Total leucocyte count (TLC), differential leucocyte count (DLC) and platelet counts, bleeding time and clotting time and for the clinical parameters as well (Gingival index , Plaque Index , Bleeding on probing, Probing depth, Loss of clinical attachment). Level of statistical significance was set at p-value less than 0.05.

V. Results

A total of 55 systemically healthy patients with chronic periodontitis aged between 25-45 years were analyzed (mean age – 35.81 years), The total sample comprised only male patients .

Table 1: Overall summary: The effect of phase I therapy on blood parameters before and two weeks after treatment.

| Parameters	Pre-treatment (baseline)	Post-treatment (After 2 weeks)	P value	Significant/ Non-significant
TLC (per mm^3)	8425.45	6258.18	0.00*	Significant
DLC				
Neutrophil %	61.50	60.47	1.520	Non-significant
Lymphocyte %	33.2	34.5	-1.819	Non-Significant
Eosinophils %	3.47	3.23	1.459	Non-Significant
Monocytes %	1.90	1.90	0.00	Non -Significant
Basophils %	Not applicable			
BT (min)	3.47	3.40	0.108	Non- Significant
CT(min)	5.07	5.15	0.174	Non- Significant
Platelet-count (lacs/mm^3)	2.51	1.86	0.00*	Significant
Plaque index	1.96	0.65	0.00*	Significant
Gingival Index	1.83	0.85	0.00*	Significant
CAL(mm)	3.83	3.60	0.10	Non- Significant
PD(mm)	3.70	3.53	0.19	Non -Significant
BOP(%age)	1.91	0.90	0.00*	Significant

Table 1shows that the mean Total leukocyte count (per cubic millimeter of blood) at baseline and after 2 weeks was 8425.45 /mm^{3}and 8577.95/mm^3 respectively. A comparison for Total leukocyte count was done using Paired't' test between the two (for example) at baseline and after 2 weeks. This difference reached the level of significance. After 2 weeks mean Total leukocyte count was significantly less as compared to baseline readings. The mean platelet count at baseline and after 2 weeks was found to be 2.51 lac/mm^3 and 1.86 lac/mm^3 respectively. This difference reached the level of significance. After 2 weeks mean platelet count was significantly less as compared to baseline readings. Whereas differential leukocyte count (DLC), bleeding time and clotting time didn't show any statistical significance. Similarly comparative evaluation between baseline and 2 weeks readings of clinical parameters such as - Gingival index, Plaque Index and Bleeding on probing were found to be significant but the Probing depth and Loss of clinical attachment failed to reach the level of significance.
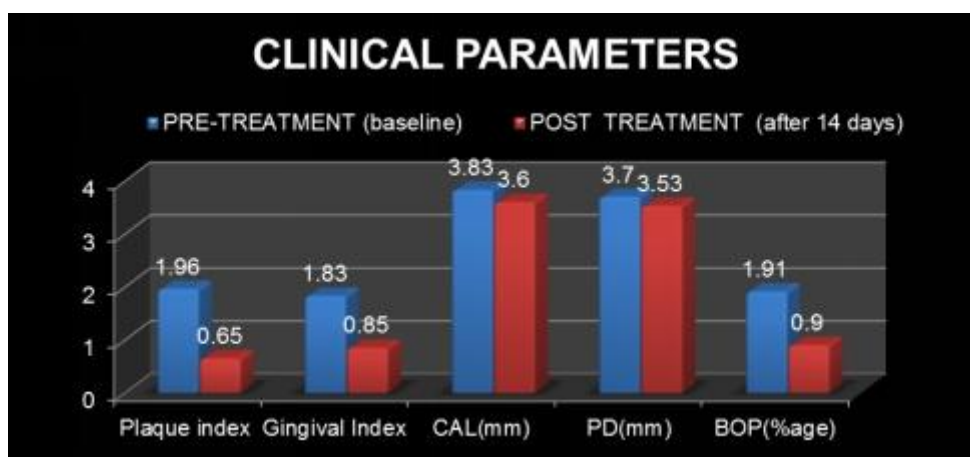
Graph 1: Combined graph of clinical parameters

Table 2: Neutrophil -lymphocyte ratio (NLR)

| Neutrophil -lymphocyte ratio (NLR)	
Baseline	1.85
14 Days	1.75
p value	0.700
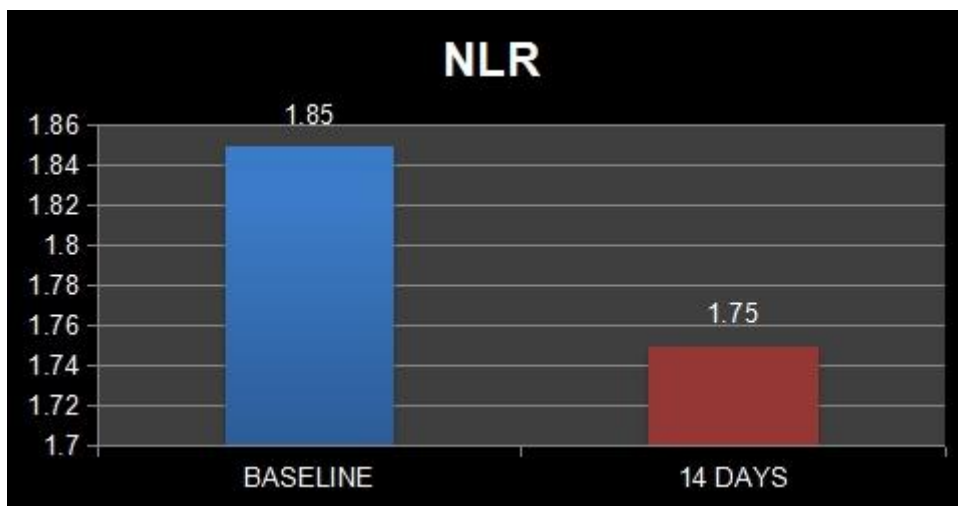
Graph 2: Neutrophil -lymphocyte ratio (NLR)

Table 2 and Graph 2

      In the present study, for neutrophil -lymphocyte ratio (NLR) ratio was 1.85 at baseline and 1.75 after 14 days. Hence a decrease in neutrophil-lymphocyte ratio (NLR) was seen but the comparison was not found to be statistically significant. (p, 0.700)

Table 3: Platelet-lymphocyte ratio (PLR)

Platelet-lymphocyte ratio (PLR)	
Baseline	89.731
14 Days	86.14
p value	0.700
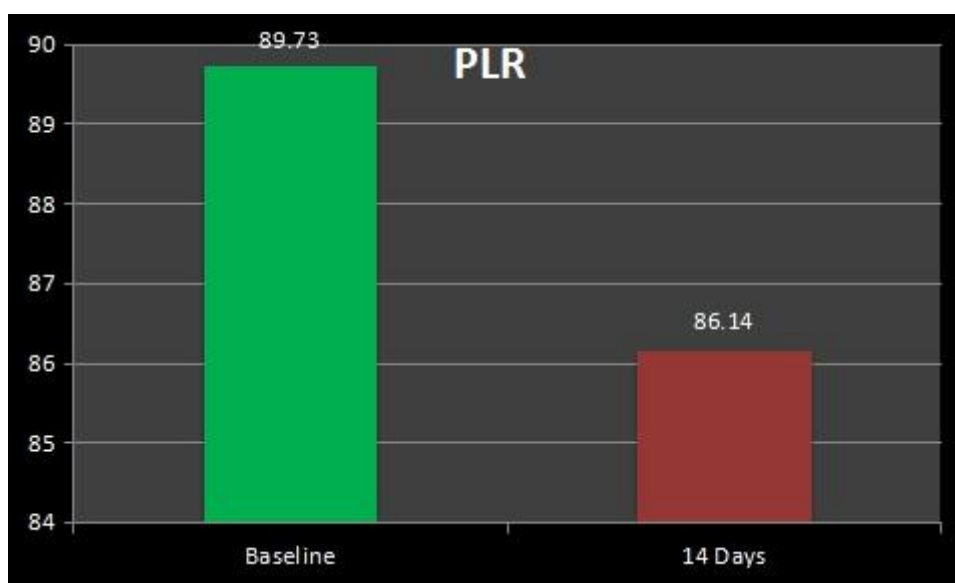
Graph 3: Platelet -lymphocyte ratio (PLR)

Table 3 and Graph 3:

      In the present study, Platelet -lymphocyte ratio (PLR) ratio was 89.73 at baseline and 86.14 after 14 days. Hence a decrease in Platelet -lymphocyte ratio (PLR) was seen but the comparison was found to be statistically non- significant. (p, 0.026*)

VI. Discussion

The purpose of this study was to assess the effect of non-surgical periodontal therapy on pro-coagulant state in chronic periodontitis patients. In our study, a statistically significant decrease in TLC was observed two weeks after scaling and root planing (from 8425.45/mm^3 at baseline to 6258.18/mm^3 two weeks post phase I therapy).

Similar results were found by Christan et al. (2002)[6]they reported a decrease in leucocyte counts in the course of periodontal therapy. Taylor et al.(2006) observed a statistically significant decrease in WBC counts after full mouth tooth extraction. In the present study, a reduction in counts of individual WBCs, i.e neutrophils, lymphocytes, eosinophils andmonocytes, was also observed, but this decrease was statistically non-significant.[8]In present study no statistically significant difference was found with respect to basophil count . Similar findings were observed by Taylor et al. in(2006)[8] .They reported decrease in neutrophil and lymphocyte counts after full-mouth tooth extraction. This difference could be due to the differences in follow-up period, which was 12 weeks in the study conducted by Taylor et al ,as compared to two weeks in our study. Similar to our study R. Banthia et al (2013) also observed no significant difference in the differential leukocyte counts after nonsurgical periodontal therapy conducted for 2 weeks.[9]Leucocyte count has been shown to be an independent predictor of prospective coronary heart disease in many epidemiological studies.[10] A direct relationship has been reported between increasing levels of leucocyte count and increase in CVD risk. So, the positive outcome of non-surgical periodontal therapy in reducing such factors should be welcomed in the prevention of CVD. Higher leucocyte count also changes the blood rheology. Increase in cell count make the blood more viscous and many cells may adhere to endothelial cells lining the blood vessels, which could decrease the blood flow.[11]

Main function of platelets is to maintain haemostasis, but they also play an important role in inflammatory and immune processes. Their number increases in chronic inflammation. [12] Griesshammer et al.(1999)[13] in a study of 732 patients with elevated platelet counts (>500x10^3) reported that infection was the underlying cause of thrombocytosis in 2 1% of the subjects studied. Wakaiet al.(1999) have also reported increased platelet counts in patients with periodontitis.[14] As a result of inflammatory and infectious processes the number of circulating platelets increases and the phenomenon is known as 'reactive thrombocytosis' .[2] Platelets have been reported to be activated in response to a variety of orally derived microorganisms, and the underlying mechanisms are highly species dependent. Several orally derived bacteria like Streptococcus sanguinis. Streptococcus mutons.Streptococcus agalactiae. Streptococcus pyogenes, Streptococcus gordonii, Pseudomonas aeruginosa and Porphyromonas gingivalishave been known to interact with platelets and alter the pro-coagulant state of the body.[15]As many of these species are platelet activators, it is possible that they act synergistically to stimulate platelet adhesion at a site of endothelial activation or damage, providing the surface for migration of immune cells and a focus for thrombus formation. [16]

Thaulow et al.(1991) found that platelet counts were significantly related to the risk of cardiovascular death. Increased platelets count could be another underlying mechanism for the possible link between periodontal inflammation and cardiovascular disease.[17]In the present study, there was a statistically significant decrease in platelet counts two weeks after non-surgical periodontal therapy, i.e. 2.51 lacs/mm^3 to1.86 lacs/mm^3. Similar results were reported by Christan*et al.* (2002).They observed statistically significant decrease in platelet count after full-mouth tooth extraction.[8]Loe et al. (1965) stated that reinstitution of oral hygiene techniques led to the disappearance of gingival inflammation within approximately one week of plaque removal.[15]Lang et al. (1990) reported that absence of BOP is an indicator of periodontal stability. In our study, we achieved a highly significant decrease in BOP in the maximum percentage of sites at the end of two weeks. Hence, the two-week time period may be a justifiable time frame for achieving reduction in gingival inflammation and thereby reducing systemic inflammation (reduction in TLC and platelet counts).[18]

Test for BT was done to assess platelet function and the body's ability to form a platelet plug at the site of injury. According to Duke's method the normal BT ranges from 1 to 3 min. [19]Platelets play a significant role in controlling bleeding. BT is commonly evaluated before surgery and in several hemorrhagic disorders such as dengue.

In our study a comparison for bleeding time was done using Paired't' test between the two i.e at baseline (3.47) and after 2 weeks (3.40). This difference failed to reach the level of significance. After 2 weeks mean bleeding time was less as compared to baseline readings but not statistically significant. Similar results were reported by R. Banthia et al[9] in 2013.They conducted a 2 week study on 30 patients with chronic periodontitis to investigate the effect of non-surgical periodontal therapy on blood parameters including BT and CT. The author reported non- significant decrease in bleeding time and clotting time. A significant decrease in bleeding time was reported in a study conducted by Kalsi DS (2017 which was of three week duration.) [20] However, the reason for this decrease was unclear.

In our study only male patients were included to avoid bias as in case of female there were variations in the different haematological parameters such as total leukocyte count and total platelet count during menstrual

cycle.[21]Some of the markers are gaining popularity as diagnostic tools in CVD such as platelet- lymphocyte ratio (PLR), neutrophil -lymphocyte ratio (NLR), red cell distribution width (RDW) and CRP(C-reactive proteins).It was shown that an elevated neutrophil -lymphocyte ratio (NLR) is related to early mortality in patients with pulmonary embolism.[22] RDW is strongly associated with prognosis in cardiopulmonary disorders such as coronary artery disease (CAD), acute myocardial infarction, acute and chronic heart failure, and pulmonary hypertension.[22]

CRP (C-reactive protein) has been shown to predict cardiovascular mortality in recent studies, and elevated CRP levels have been observed in middle-aged patients with periodontitis. Combination of chronic infections like periodontitis with elevated CRP is associated with higher chronic heart diseases.[23]In the present study, decrease in neutrophil-lymphocyte ratio (NLR) was noticed after 2 weeks as compared to baseline but the comparison was not found to be statistically significant. This could be due to smaller sample size group and less time duration .An elevated platelet- lymphocyte ratio (PLR) as a risk factor for arterial obstructive diseases has been evaluated in some studies. It has been shown that elevated PLR is a significant independent predictor of long term mortality after non-ST elevation myocardial infarction.[24] In present study, decrease Platelet -lymphocyte ratio (PLR) was seen after 2 weeks as compared to baseline but the comparison was found to be statistically non-significant.

The study design had some limitations which can be incorporated in further studies to improvise the study design. Some of the limitations were smaller sample size and less time duration. Large sample size and longer time duration would result in more statistically significant values. More hematological parameters could be included i.e red cell distribution width (RDW), C-reactive proteins, systemic marker along with advanced diagnostic technique to correlate the effect of non- surgical therapy on systemic conditions such as atherosclerosis .

VI. Conclusion

Chronic periodontitis exhibit signs of a subclinical systemic inflammatory condition and may influence the atherosclerotic process in human beings via increasing the WBC and platelet counts, i.e., by altering the pro-coagulant state of the body, which is found to decrease after periodontal therapy as increase in W.B.C. count can act as risk factor for many systemic diseases.

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