Effect of phase I periodontal therapy on pro-coagulant state in chronic periodontitis patients – a clinical and haematological study

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Précis

This study validates the effect of periodontal therapy in reducing systemic inflammation, thus indirectly affecting the risk of cardiovascular disease.

Abstract

Statement of the problem: The increase in white blood cell count (WBC) and platelet count due to systemic inflammation and infection is considered a risk factor for cardiovascular diseases. These parameters increase in periodontal disease. A decrease in WBC and platelet counts by periodontal therapy may decrease the risk for cardiovascular disease.
Purpose of the study: The present study is a treatment intervention model to investigate the effect of non-surgical periodontal therapy on total leucocyte count (TLC), differential leucocyte count (DLC) and platelet count in patients with chronic periodontitis.
Materials and methods: Thirty systemically healthy patients were included in the study. Probing pocket depth (PPD), clinical attachment loss (CAL), bleeding on probing (BOP), TLC, DLC, platelet count, bleeding time (BT) and clotting time (CT) were evaluated at baseline and at two weeks after phase I therapy.
Results: A statistically highly significant decrease in the percentage of sites exhibiting BOP was observed, i.e., from 78.1% at baseline to 18.1% two weeks postoperatively (p=0.000). There was also a statistically significant decrease in TLC from 7595/mm^3 at baseline to 6690/mm^3 two weeks following phase I therapy (p=0.02). There was also a statistically highly significant decrease in platelet count from 2.1 lac/mm^3 preoperatively to 1.9 lac/mm^3 at two weeks postoperatively (p=0.003).
Conclusion: The present study depicts the importance of periodontal therapy to reduce the TLC and platelet count, thereby possibly decreasing the risk for the development of cardiovascular disease by lowering the established risk factors for periodontal atherosclerosis.

Key words: WBC count, platelet count, oral bacteria, periodontal therapy, atherosclerosis.

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Introduction

For decades, blood has been used as a diagnostic body fluid for assessing various infections and systemic diseases. For the past two decades, periodontitis has been linked to systemic disorders and is known to change the cellular and molecular components of blood.[1] Various observational studies have established an association between periodontal disease and cardiovascular disease (CVD).[1] Periodontitis may affect cardiovascular tissues directly or indirectly by 'metastatic infection', 'metastatic inflammation' and 'metastatic injury' due to dissemination of microbes and their products into the systemic circulation.[2]

White blood cells (WBCs) are an integral part of the innate immune system. These cells are recruited in higher numbers during episodes of bacteraemia or lipopolysaccharide (LPS) leakage into the systemic circulation.[1] Leucocyte count has been demonstrated in several epidemiological studies to be an independent predictor of prospective coronary heart disease.[3]

Inflammatory and infectious processes can result in an increase in the number of active thrombocytes.[1] This phenomenon is known as 'reactive thrombocytosis'. So, it is reasonable to assume that periodontal disease can also lead to an increased number of circulating platelets.[1] A large body of evidence supports the role of platelets in linking bacteraemia to atherothrombosis.[4] The aim of the present study was to investigate the effect of phase I (non-surgical) periodontal therapy on total leucocyte count (TLC), differential leucocyte count (DLC) and total platelet count in patients with generalised chronic periodontitis.

TABLE 1: Characteristics of the participants in the study.

Number of completed cases	30
Average age of subjects	40.37 years
Gender	9 male, 21 female

Materials and methods

Thirty systemically healthy patients with chronic periodontitis aged between 25 and 45 years were selected randomly among patients reporting to the Department of Periodontics, Modern Dental College and Research Centre, Indore. Patients having probing depths ≥5mm in conjunction with attachment loss in more than 30% of the sites were selected. Patients with score of 2 or 3 of the Loe and Silness

TABLE 2: Effect of phase I therapy on periodontal parameters.

Periodontal parameters	T1 (pre-treatment) (% sites)	T2 (post-treatment) (% sites)	
Bleeding on probing (BOP)	78.1 (33-100)	18.1 (0-50)	Highly significant (p=0.000)
PPD <3mm	43.1 (0-100)	59.0 (0-100)	Non-significant (p>0.05)
PPD 3.1-5mm	33.2 (0-16)	26.9 (0-58.3)	Non-significant (p>0.05)
PPD >5.1mm	25.5 (0-100)	14.3 (0-100)	Non-significant (p>0.05)
CAL <3mm	61.4 (0-100)	68.1 (0-100)	Non-significant (p>0.05)
CAL 3.1-5mm	24.2 (0-58.3)	21 (0-50)	Non-significant (p>0.05)
CAL >5.1mm	16.8 (0-100)	11.0 (0-100)	Non-significant (p>0.05)

PPD – probing pocket depth; CAL – clinical attachment loss.

TABLE 3: Effect of phase I therapy on blood parameters.

Blood parameters	T1 (pre-treatment)	T2 (post-treatment)	
Total leucocyte count (per mm^3)	7595 (5100-11250)	6690 (4900-9200)	Significant (p=0.02)
Neutrophil count (%)	63.03 (52-72)	61.9 (50-70)	Non-significant
Lymphocyte count (%)	31.37 (21-42)	30.4 (23-36)	Non-significant
Eosinophil count (%)	4.9 (1-5)	4 (1-4)	Non-significant
Monocyte count (%)	2.17 (1-4)	2.03 (1-9)	Non-significant
Basophil count (%)	0	0	Non-significant
Platelet count (lacs/mm^3)	2.1 (1.6-2.8)	1.9 (1.5-2.4)	Highly significant (p=0.003)
Bleeding count (min)	1.2 (1-2.5)	1.1 (1-1.5)	Non-significant
Clotting time (min)	3.8 (2.6-6.2)	3.8 (3-4.4)	Non-significant

Gingival Index were included. Patients with any systemic disorders, pregnant or lactating women, patients with a history of any acute infection and/or antibiotic therapy in the last six months, patients with a recent history of immunisation, and present and past tobacco users (smokers as well as tobacco chewers) were excluded from the study.

The study protocol consisted of full-mouth scaling and root planing completed by a single operator in two visits within 24 hours, along with chlorhexidine rinsing twice a day for seven days as an adjunctive home care measure. Probing pocket depth (PPD), clinical attachment loss (CAL) and bleeding on probing (BOP) were recorded by another calibrated operator using the Williams periodontal probe at baseline and at two weeks postoperatively. Preoperative (baseline) and two weeks postoperative venous blood samples were obtained at the same time of the day, and were immediately transported and processed. The laboratory analysis of TLC, DLC, platelet count, bleeding time (BT) and clotting time (CT) were performed by a blinded pathologist. Results obtained were subjected to statistical analysis. The study was approved by the Ethics Review Committee of the Modern Dental College and Hospital. Written informed consent was obtained from all the study participants.

Results

Results are depicted in **Tables 1**, **2** and **3**.

Table 1 shows the mean age of participants, i.e., 40.37 years. Out of 30 subjects, nine were male and 21 were female. **Table 2** shows the effect of scaling and root planing on periodontal parameters at baseline and at two weeks postoperatively. A statistically highly significant decrease in the percentage of sites exhibiting BOP was observed, i.e., 78.1% of sites showed BOP before treatment, which was reduced to 18.1% postoperatively (p=0.000). In all other periodontal parameters, there was no statistically significant difference. **Table 3** shows the effect of phase I therapy on blood parameters before and two weeks after treatment. There was a statistically significant decrease in TLC two weeks after scaling and root planing (at baseline TLC was 7595/mm^3, and at two weeks' follow-up TLC was 6690/mm^3, p=0.02). There was a statistically highly significant decrease in platelet count from 2.1 lac/mm^3 preoperatively to 1.9 lac/mm^3 at two weeks postoperatively (p=0.003). There was no statistically significant difference in other blood parameters after phase I therapy.

Discussion

The present study investigated the effect of non-surgical therapy on TLC, DLCs (neutrophils, lymphocytes, eosinophils, basophils and monocytes) and total platelet count in 30 patients with chronic periodontitis. Alterations in these factors at cellular and molecular levels are known systemic risk predictors for CVD; this study was an attempt to assess the role of non-surgical periodontal therapy in reducing the risk of CVD.

Loe *et al.* (1965)[5] stated that reinstitution of oral hygiene techniques led to the disappearance of gingival inflammation within approximately one week of plaque removal. Lang *et al.* (1990)[6] stated that absence of BOP is an indicator of periodontal stability. In this 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).

Higher leucocyte counts have been found to be correlated with higher Gingival Index (GI) and Community Periodontal Index Treatment Needs (CPITN) scores.[7] This can be attributed to the host's immune response to microbially induced periodontal inflammation, which can be resolved by non-surgical periodontal therapy.[7] In our study, a statistically significant decrease in TLC was observed two weeks after scaling and root planing (from 7595/mm^3 at baseline to 6690/mm^3 two weeks post phase I therapy). Similar findings were also reported by Christan *et al.* (2002),[8] who reported a decrease in leucocyte counts in the course of periodontal therapy. Taylor *et al.* (2006)[9] reported 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 and monocytes, was also observed, but this decrease was statistically non-significant. No difference was found with respect to basophil count in the present study. Taylor *et al.* (2006)[9] have also reported a statistically significant decrease in neutrophil and lymphocyte counts after full-mouth tooth extraction. This difference may be attributed 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.

In several epidemiological studies, leucocyte count has been demonstrated to be an independent predictor of prospective coronary heart disease.[10] A direct dose–response relationship has been observed between increasing levels of leucocyte count and graded increase in CVD risk.[10] So, the positive effect of non-surgical periodontal therapy in reducing such factors should be welcomed in the prevention of CVD.

Higher leucocyte count also alters the blood rheology. More cells make the blood more viscous and more cells may adhere to endothelial cells lining the blood vessels, thereby decreasing the blood flow.[11] Reduced blood flow can alter cardiovascular system dynamics, especially in narrow or partly blocked arteries, due to atherosclerotic plaque formation.[11] Microbes (periodontal pathogens) and their products invade tissues to enter the bloodstream. These bacteria attach to or invade vascular endothelial cells and are deeply involved in the formation of arteriosclerotic lesions.[12] Periodontal therapy aims to reduce the number of periodontal pathogens and hence periodontal inflammation, thereby indirectly decreasing the risk of CVD.[8]

Platelets have their main function in haemostasis, but they also play a role in inflammatory and immune processes. Their number increases in chronic inflammation.[13] Griesshammer *et al.* (1999),[14] in a study of 732 patients with elevated platelet counts (>500×10^3) reported that infection was the underlying cause of thrombocytosis in 21% of the subjects studied. Wakai *et al.* (1999)[7] have also reported increased platelet counts in patients with periodontitis. An increase in the number of circulating platelets as a result of inflammatory and infectious processes is known as 'reactive thrombocytosis'.[1]

TABLE 4: Microorganisms and their mode of action on platelets.[4,16-28]

S No.	Microorganisms	Action on platelets
1.	*Streptococcus sanguinis*	Erickson and Herzberg (1993) identified a protein on the surface of platelet-activating strains of *Streptococcus sanguinis*, which was termed as platelet aggregation-associated protein (PAAP). PAAP is similar to a collagen octapeptide region required for platelet aggregation.[16] *S. sanguinis* can increase platelet aggregation, leading to increased thrombus formation. A role for IgG in *S. sanguinis*-induced platelet activation has also been suggested. The depletion of plasma IgG or the antagonism of FcγRIIA, the platelet IgG receptor, both attenuated platelet activation in response to some strains of *S. sanguinis*. These strains engage intra-cellular signalling pathways similar to those underlying traditional IgG-induced, Fc RIIA-mediated platelet activation.[17] A role of a complement system in *S. sanguinis*-induced platelet activation has also been postulated. C1q contains a sequence with high homology to the repeating regions of collagen and PAAP.[18] Platelet aggregation by *S. sanguinis* is an active process rather than a passive cross linking. It is dependent on fibrinogen binding to αIIbβ3.[19]
2.	*Streptococcus gordonii*	The activation of platelets by cell wall proteins (e.g., Hsa protein) of some strains of *S. gordonii* has also been demonstrated.[20]
3.	*Streptococcus mitis*	In some strains of *Streptococcus mitis,* surface protein Pb1A has been proposed as a platelet adhesion protein.[21]
4.	*Streptococcus epidermidis*	*Staphylococcus epidermidis* expresses a fibrinogen-binding protein, serine aspartate repeat protein G (SdrG), which causes adhesion and stimulation of platelets.[22]
5.	*Porphyromonas gingivalis*	*P. gingivalis* has also been shown to be a platelet activator, utilising several mechanisms in a strain-, donor- and thromboxane-dependent manner. It produces proteinases that have been associated with the invasive properties of the organisms.[23] Some strains of *P. gingivalis* produce trypsin-like proteinases, protease I, which can activate platelets.[24] Direct activation of platelets by *P. gingivalis* has also been reported. Arg-specific gingipains (Rgp) secreted by this microbe stimulate platelet aggregation.[25] IgG and FcγRIIA are also critical for platelet aggregation in response to *P. gingivalis*.[26] Toll-like receptors (TLRs) 1, 2, 4, 6, 8 and 9 are present on the surface of platelets. Lipopolysaccharides (LPS) can bind to TLR-4 and lead to the secretion of cytokines like TNF-α (tumor necrosis factor-α) and interleukin-1 (IL-1), which suggest a role of platelets in the innate response to bacteraemia.[27] Taken together, TLRs provide another potential mechanism by which *P. gingivalis*, either directly or via the liberation of LPS, stimulates platelet activation.[4] Animal studies have shown that *P. gingivalis* is as effective as a high cholesterol diet in inducing atherosclerosis.[28]

Periodontitis is the most prevalent bacterially induced inflammatory condition in the world.[15] So, it is reasonable to assume that platelet count increases in periodontal disease patients.

Platelets have been shown to activate 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 mutans, Streptococcus agalactiae, Streptococcus pyogenes, Streptococcus gordonii, Streptococcus*

pneumonia, Streptococcus mitis, Staphylococcus epidermidis, Staphylococcus capitis, Pseudomonas aeruginosa and *Porphyromonas gingivalis* have been known to interact with platelets and alter the pro-coagulant state of the body.[4] Some of the mechanisms are discussed in **Table 4**.[4,16-28]

The interaction of one or multiple organisms with platelets upregulates adhesive receptors on the platelet surface, thereby facilitating their binding to damaged or activated endothelial cells early in the atherogenic process. The enhanced release of platelet contents and the presence of bacteria facilitate the accumulation of both platelets and monocytes at the site of injury. All of these provide a surface for the adhesion and locomotion of monocytes prior to their translocation through the endothelial barrier.[29]

A variety of microorganisms like *Streptococcus mutans, Aggregatibacter actinomycetemcomitans, Streptococcus sanguinis, Porphyromonas gingivalis* and *Treponema denticola* have been reported in specimens of heart valves and aneursym walls, including aneurysmal thrombi. DNA from a number of different bacterial species have been found in atherosclerotic plaques. It has been suggested that the presence of these bacteria and bacterial DNA in atherosclerotic plaque is the result of bacteraemia.[30] As many of these species are platelet activators, it is possible that they act synergistically to stimulate platelet adhesion at a site of endotheial activation or damage, providing the surface for migration of immune cells and a focus for thrombus formation.[4]

Thaulow *et al.* (1991)[31] found that platelet counts were positively related to the risk of cardiovascular death. So, an increase in platelets might be another underlying mechanism for the possible link between periodontal inflammation and cardiovascular disease.

In the present interventional study, there was a statistically highly significant decrease in platelet counts two weeks after non-surgical periodontal therapy, i.e., 2.1 lacs/mm^3 to 1.9 lacs/mm^3. Similar results were reported by Christan *et al.* (2002),[8] who showed a decrease in platelet counts after periodontal therapy from 2.54×10^3 to 2.25×10^3/µl. Similar results were observed by Taylor *et al.* (2006),[9] who also reported a statistically significant decrease in platelet count after full-mouth tooth extraction.

Conclusion

Patients with chronic periodontitis exhibit signs of a subclinical systemic inflammatory condition.[32] The results of the present study support this notion. In the current study, statistically significant reductions in TLCs and statistically highly significant reductions in platelet counts were observed following periodontal treatment. Periodontitis 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. Therefore, it can be concluded that decreasing periodontal inflammation may be a successful key to decrease the risk of coronary heart disease. These systemic markers may prove to be useful tools for the assessment of cardiovascular risk in patients with periodontitis.

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