CONVENTIONAL MECHANICAL PERIODONTAL THERAPY - A PIONEERING AND NONTOXIC EXPERIMENTAL MODEL FOR THE STUDY OF ACUTE HUMAN INFLAMMATORY RESPONSE*

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In the past decade, the association of periodontal diseases with the development of systemic diseases has received increasing attention. A variety of recent hypothesis driven investigations have compiled various results showing that periodontal infection and subsequent direct oral-haematogenous spread of bacteria are implicated in the development of inflammation and various systemic diseases. Chronic periodontitis causes a low-grade systemic inflammatory response; its standard treatment, however, induces an acute inflammatory response. The aim of this study was to describe the systemic inflammatory reactions to an intensive periodontal treatment regimen.

Keywords: Inflammation, Periodontitis, Periodontal therapy

Acute systemic inflammation encompasses a series of complex defensive mechanisms and events (plasma proteins level changes. behavioural, biochemical and nutritional changes) but all potentially harmful to the host.[1]

Over the past few years several investigators have attempted to study human inflammation developing and testing different experimental models. These include - The endotoxin model (use of parenteral injection of lipopolysaccharide in healthy subjects) which is probably the most accepted and adopted model, the physical training model involving strenuous exercise & the vaccination model all of which have proved to be equally useful.[2-11]

All the above mentioned models included a study of changes observed in the serum markers like C-reactive protein (CRP), Interleukin- 1(IL-1), Interleukin -6(IL-6) for the study of systemic inflammatory response.[12]

Periodontal disease is one of the most common infectious diseases seen in humans. It is caused primarily by anaerobic gram-negative bacteria protectively enclosed in a biofilm over the root surface in subgingival region.[13] It is characterized by gingival inflammation, as well as loss of connective tissue and bone from around the roots of the teeth, which leads to eventual tooth exfoliation.

The standard treatment protocol for periodontitis include mechanical removal of subgingival calculus and covering biofilm which leads to decrease in the microbial load and local inflammation[14]. Mechanical instrumentation during periodontal therapy in the subgingival regions result in soft tissue damage of the periodontium and transient bacteraemia[15,16]. The spread of these gram negative organisms via oral-haematogenous route results in the introduction of the systemic inflammatory response in the same manner as the endotoxin injection.

The concept that periodontitis and its treatment during mechanical therapy could represent a novel model to study acute human inflammation stems from the fact that conventional periodontal mechenotherapy contributes to the systemic inflammatory burden which has been documented by study reports confirming the raise in serum levels of inflammatory markers (CRP, IL-6)[17-21]. Studies by D'Aiuto et al have proved that standard periodontal treatment leads initially to an increase in the level of systemic inflammatory markers, thus giving strength

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to the concept that standard, intensive mechanical treatment of periodontal disease transiently contributes to systemic inflammatory burden of an individual[21,22].

AIM & OBJECTIVE

The aim of this pilot investigation were to describe the transient changes in acute systemic inflammatory parameters following an intensive periodontal therapy by studying the changes associated with acute phase inflammatory marker –CRP and complete blood counts in initial days following treatment associated bacteraemia.

MATERIAL & METHOD

Study population and design

A sample size of 16 patients with chronic generalized periodontitis having probing depths of 5mm or more and generalised marginal alveolar bone loss greater than 30% were selected from the Outpatient Department of Periodontics and Oral Implantology, Sri Govind Tricentenary Dental College, Hospital & Research Institute, Gurgaon, as per the following criteria:

INCLUSION CRITERIA

1. Chronic generalised periodontitis patients with pocket probing depth of 5 mm or more and marginal alveolar bone loss greater than 30%.
2. Patients willing to follow recommended plaque control and follow up regimen.

Exclusion Criteria

1. Patients with known systemic diseases.
2. History and/or presence of other infections.
3. Systemic antibiotic treatment in the preceding 3 months.
4. Treatment with any medication known to affect levels of serum inflammatory markers.
5. Pregnant or lactating females.

Each subject underwent a full mouth periodontal probing and charting, along with Orthopantomograph (OPG) & Radiographic bone loss was recorded. Blood samples were taken before treatment, subjects

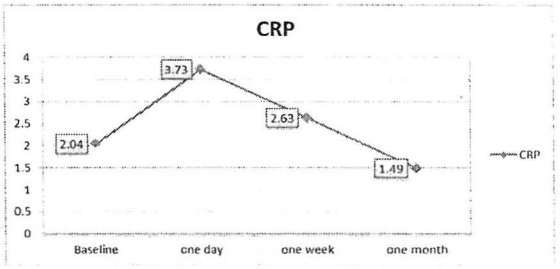


Fig. 1: C-Reactive Protein (CRP) Values (mg/l)

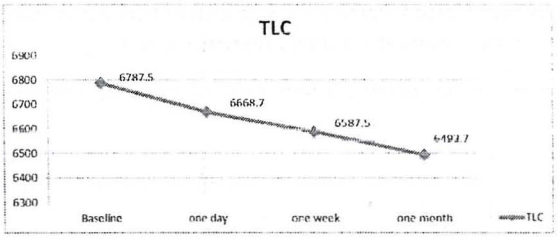


Fig. 2: Total Leukocyte Count (TLC) (109/l)

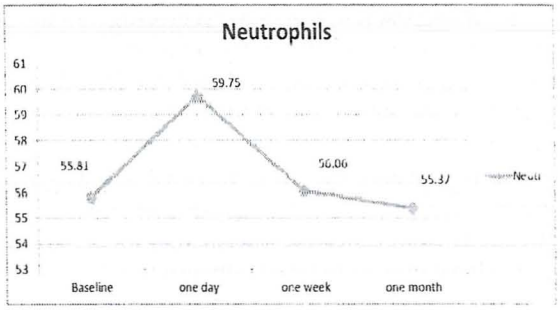


Fig. 3: Neutrophil Counts (109/l)

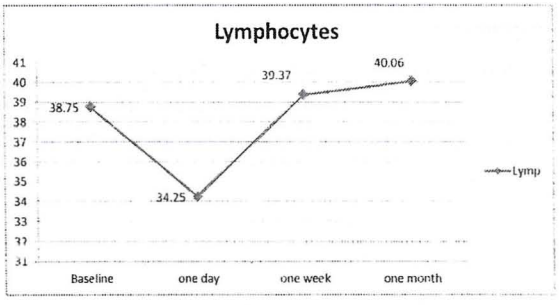


Fig 4: Lymphocyte count(109/l)

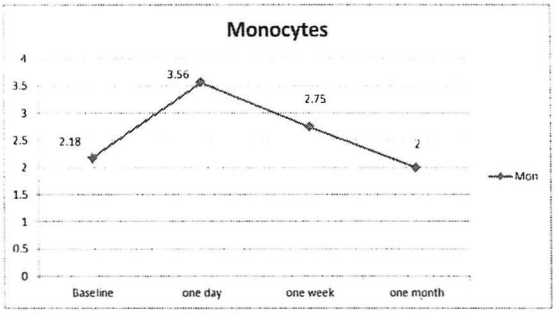


Fig. 5: Monocyte count(109/l)

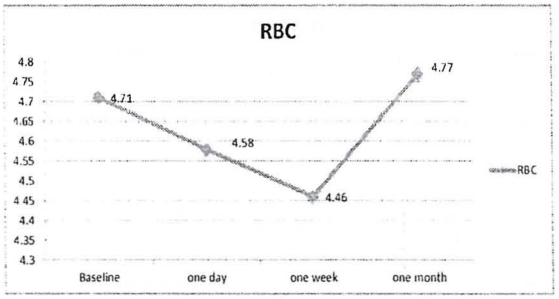


Fig. 6: Red Blood Cell (RBC) Count (1012/l)

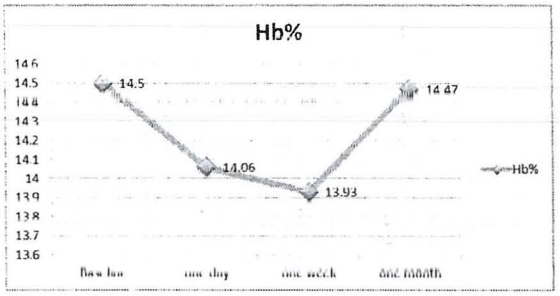


Fig. 7: Haemoglobin percentage (Hb %) (g/dl)

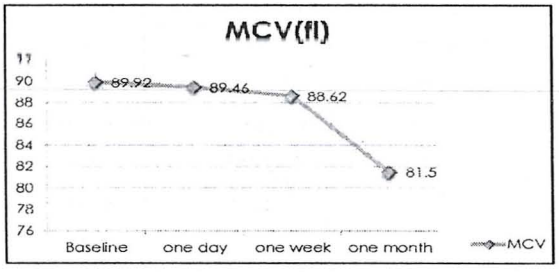


Fig. 8: Mean Corpuscular Volume (MCV) (fl)

thereafter received one single intensive session of subgingival mechanical instrumentation under infiltration anaesthesia using a piezoelectric instrument (EMS, Nyon, Switzerland) equipped with fine tips for access in the subgingival environment. Teeth with hopeless prognosis were extracted during the same session following standard clinical protocol. Blood samples were again taken one day, one week, & one month after treatment for haematological and serum analysis to assess inflammatory markers and complete blood counts for each draw.

Analysis of inflammatory marker of CRP and blood counts

For the selected patients venous blood samples was obtained from the veins through the skin in the cubital fossa. 5 ml of blood was drawn using a disposable syringe and 23 gauge needles. The blood was distributed for CBC Count and 3ml of blood was centrifuged at 3,000 R.P.M (revolutions per minute) for 5 minutes and serum was separated. The serum thus obtained was used for testing the CRP levels using the commercial latex kit *(CRP TURBI, Reactivos GPL, SPAIN)* for the quantitative measurement of the C-reactive protein level (CRP) in human serum with the help of a semiautomatic biochemic analyzer. Differential blood counts were performed using standard clinical haemetology procedures on an automated analyzer..

STATISTICAL METHODS

Data were reported as mean+/- standard deviation (SD) for variables normally distributed or median and interquartile range (IQR). Non parametric statistical comparisons were applied. All statistical comparisons were performed using Friedman test, Wilcoxon signed ranks test (alpha value set at 0.05) using software – SPSS, version[17].

RESULTS

16 subjects (mean age 30-58 years) were included in the trial. All subjects were systemically healthy except for the presence of chronic generalised periodontitis. The group comprised of 4 females and –12males. They had an average body mass index of

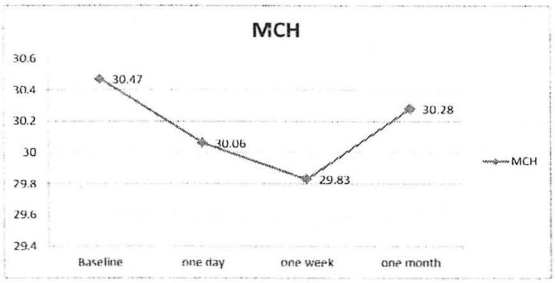


Fig. 9: Mean Corpuscular Haemoglobin (MCH) (pg)

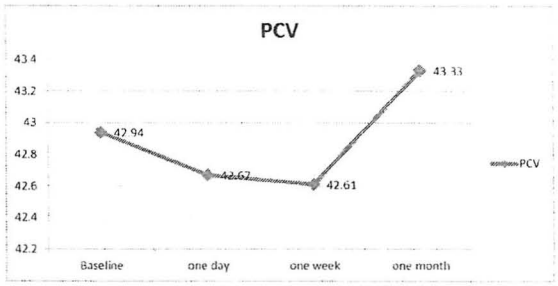


Fig. 11: Packed Cell Volume (PCV) (l/l)

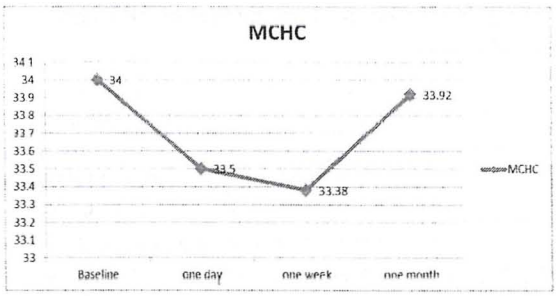


Fig. 10: Mean Corpuscular Haemoglobin Concentration (MCHC) (mg/dl)

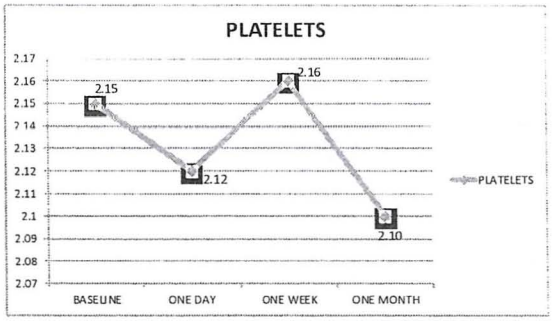


Fig 12: Platelet Count (109/l)

Table 1: RESULTS -CRP values & Blood Counts before and after Periodontal Therapy

Blood Parameter	Baseline	One Day	One Week	One Month	p-value
CRP(mg/l)	2.04	3.73***	2.63*	1.49**	<.0001
TLC (109/l)	6787.5	6668.7	6587.5	6493.7	0.455(ns)
L(109/l)	38.75	34.25**	39.37	40.06	<.001
N(109/l)	55.81	59.75**	56.6	55.37	<.001
M(109/l)	2.18	3.56**	2.75**	2	<.001
E(109/l)	2.93	2.31*	2.25*	2.12*	0.011
RBC (1012/l)	4.71	4.58**	4.46***	4.77*	<.001
Hb% (g/dl)	14.5	14.06*	13.93**	14.47	<.001
MCV(fl)	89.92	89.46***	88.62***	81.50**	<.0001
MCH(pg)	30.47	30.06**	29.83***	30.28**	<.0001
MCHC(mg/dl)	34	33.5***	33.38***	33.92	<.0001
PCV(l/l)	42.94	42.67***	42.61**	43.33**	<.0001
PLT(109/l)	2.15	2.12**	2.16**	2.10	<.001

26+/- 4 kg/m2. During the study all patients remained stable with no alterations in lifestyle, habits, exercise, diet and medications.

With reference to periodontal parameters all participants presented with high levels of gingival inflammation, widespread periodontitis (represented by pockets of 5mm or more and loss of clinical attachment level). All the parameters showed marked improvement after completion of treatment.

All the participants reported headaches, tiredness, chills, general malaise and rise in body temperature on the evening following treatment.

CRP levels showed clear significant changes between pre-treatment, one day, one week and one month after therapy. (p <.0001, Wilcoxon signed rank–sum test). CRP levels remained significantly higher than baseline till one week after treatment and returned to values below baseline by one month.

With respect to haemetological parameters early changes in differential leukocyte counts were observed at day 1: increase in the number of circulating neutrophils (p <.001, Wilcoxon signed rank –sum test compared to baseline). There was an increase in the number of circulating monocytes observed on day 1(p <.001, Wilcoxon signed rank – sum test compared to baseline). Lymphocyte counts fell on day 1 (p <.001, Wilcoxon signed rank –sum test compared to baseline) and increased by one week. All the counts returned within normal range by one month following therapy. The changes observed in total leukocyte counts were not found to be of significance (p <.0.455, Wilcoxon signed rank –sum test compared to baseline).

The erythrocyte number, haematocrit and haemoglobin levels at one week post therapy (p <.0001, Wilcoxon signed rank–sum test) which returned to normal levels by one month. The total number of platelets decreased on day 1 and increased by one week (p <.001, Wilcoxon signed rank–sum test) and returned to normal range by one month post therapy.

DISCUSSION

Transient changes in Serological & Haemetological parameters also confirm the human body response to bacteremia induced by mechanical periodontal therapy, thus confirming an acute inflammatory response for the treatment of this chronic disease[13].

CRP a prototype acute phase marker showed marked increase after day one, (due to action of cytokines like IL1Ra, IL-6 on liver leading to increased CRP production). CRP values decreased to slightly above baseline by one week and finally settled close to baseline value by one month post treatment. This change is following the classic acute phase marker level changes with a rise in the first 24 hours[23]. Based on the evidence of significant ingress of bacteria into the systemic circulation following periodontal instrumentation[15,16,24-26], the present data defines the systemic inflammatory changes occurring after periodontal treatment. As bacteraemia and tissue damage result, local and systemic production of proinflammatory mediators (such as tumour necrosis factor alpha and IL-1b) has been described as a component of host defences[10,12,27]. In order to modulate the process, anti-inflammatory molecules (such as IL-1Ra, IL-6) appear on the scene to control inflammation and stimulate the hepatic synthesis of acute phase proteins (CRP)[28].

Changes in leukocytes and erythrocytes numbers become transiently apparent in the first few days to a week18. There was a decrease in the total leucocyte count observed which could be attributed to peripheral vascular margination of neutrophils leading to mobilization of marrow neutrophil. There is a stimulation of bone marrow colony forming unit granulocyte (CFU-G) due to the action of cytokines leading to an increase in the production of neutrophils by day one, but the number declined by one week and settled near baseline values by one week[23].

There was a mild tendency towards anemia (normocytic, normochromic) seen due to functional iron deficiency because of a shift of iron from erythropoiesis to reticuloendothelial system observed

by a week[18,23,29]. Also action of cytokines (IL1, IL-6) directly affect iron metabolism and inhibit the maturation and synthesis of erythroid progenitor cells[30-33]. A specific IL-6 induced hepatic mediator "hepcidin" is thought to be responsible for iron sequestration in macrophages thus decreasing iron absorption in small intestines[34]. The levels returned to normal by one month.

There was a transient fall in platelet count by day one, followed by an increase in number by one week .This results due to the action of cytokines on CFU-Megakaryocytes leading to thrombocytosis which returns to normal values by one month.[2]

CONCLUSION

Mechanical periodontal therapy in the absence of administration of antimicrobials represents the standard of care delivered to most patients affected by chronic periodontitis.

Intensive periodontal treatment produced an acute systemic inflammatory response of one week duration and might represent an alternative readily available pioneer, nontoxic experimental model to study inflammation as compared to the classic endotoxin-challenge or drug-induced models to study acute inflammation in humans which have proved unethical and impractical[2].

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