

## A COMPARATIVE EVALUATION OF C-REACTIVE PROTEIN GCF LEVEL IN PERIODONTITIS PATIENTS AFTER SCALING AND ROOT PLANING WITH AND WITHOUT DIODE LASER

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### Abstract

**Background and Objectives:** C-reactive protein (CRP) is a type I acute phase reactant. A number of studies have reported elevated gingival crevicular fluid (GCF) CRP levels in periodontitis subjects, which decrease following periodontal therapy. Effect of diode laser as an adjunct to Scaling & Root planing is also well established. The aim of the present study was to evaluate the effect of periodontal treatment SRP with diode laser, on CRP levels in GCF in patients with chronic periodontitis.

**Materials and Methods:** A total of 40 subjects with moderate periodontitis based on community periodontal index scores, were included in the study.

Periodontal therapy was performed dividing each side of jaw as a group (Split mouth); one side SRP alone & another Diode laser with SRP. GCF was collected from each subject at

Baseline (prior to treatment) and 1 month after periodontal therapy. The collected sample was subjected to biochemical analysis to detect CRP levels by using a commercially available highly sensitive kit.

**Results:** The present study demonstrated that the mean CRP values at baseline were found to be 0.11043mg/l in side-I (side treated with SRP alone); 0.11042mg/l in side-II (side treated with SRP & laser) of the patient, which reduced to 0.4148 mg/L in side I and 0.3985mg/L in side II after treatment, which are highly significant according to statistical analysis but the changes between two sides were non-significant statistically.

**Interpretation and Conclusion:** Within the limitations of this study, it can be concluded that periodontal therapy is able to reduce the GCF C reactive protein level significantly, but there was no statistically significant result in CRP level, between sides treated with SRP & SRP with laser.

**Key words:** Chronic periodontitis, C-reactive protein, gingival crevicular fluid, Scaling and root planing, Diode laser

### Introduction

Periodontitis is defined as “an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with increased probing depth formation, recession, or both.”<sup>1</sup> The pathogenic role of subgingival microbiota in initiation & progression of periodontitis is widely accepted. Periodontal pathogens affect both local and systemic immune and systemic responses. Locally body response to pathogenic bacteria or bacterial products is characterized by infiltration of inflammatory cells including polymorphonuclear neutrophils, macrophages, lymphocytes, and plasma cells in the periodontal tissues. Activated macrophages release cytokines. Sometimes individual respond to microbial challenge also cause high delivery of such inflammatory mediators as Prostanoid (PGE<sub>2</sub>), Interlukin-1(IL-1), Tumor

necrosis factor (TNF). These cytokines cause destruction of both the periodontal connective tissue and alveolar bone.

C-reactive protein (CRP) is an type-1 acute phase reactant plasma protein produced in response to diverse inflammatory stimuli. The acute-phase response is a nonspecific process, initiated and coordinated by a large number of diverse inflammatory mediators that may occur in the initial host response to injuries, infections, ischemic necrosis or malignancy. It is produced in response to periodontitis and regulated by cytokines such as interleukin-6 (IL-6), IL-1 $\beta$ , and tumor necrosis factor  $\alpha$ . C-reactive protein (CRP), fibrinogen, and acute-phase proteins are sensitive markers to evaluate the inflammatory status. Amongst all the acute phase reactants, CRP in particular has been the focus of attention as a key marker of atherosclerosis. Since the levels of CRP rise earlier than those of other reactants, CRP has been used as an early marker of tissue damage also.

CRP is predominantly synthesized by liver hepatocytes. Its half-life is approximately 6-4 hours. CRP in plasma is normally present <0.3 mg/dl but may increase dramatically to hundreds of micrograms per milliliter within 72 h of tissue injury. The amount decreases with the subsidence of the disease process and the recovery of the patient.

C-reactive protein was discovered in Tillett and Francis<sup>2</sup> laboratory in 1930 during the course of study of patients with *Streptococcus pneumoniae* infection. Forty years later, Volanakis and Kaplan identified the specific ligand for CRP in the pneumococcal 'C' polysaccharide as phosphocholine, part of the teichoic acid of the pneumococcal cell wall. Although phosphocholine was the first defined ligand for CRP, a number of other ligands have been identified.

CRP can also activate the classical complement pathway, stimulate phagocytosis, and bind to immunoglobulin receptors (FcγR). In humans, plasma levels of CRP may rise rapidly and markedly, as much as 1000-fold or more, after an acute inflammatory stimulus, largely reflecting increased synthesis by hepatocytes.

The goal of periodontal therapy is to arrest the inflammatory processes by reducing the number of pathogenic periodontal microorganisms. Removing these pathologic substances ensures biologic compatibility between the diseased periodontal radicular surface and new connective tissue attachment.

Non-surgical periodontal therapy, which is the primary recommended approach to control periodontal infection, is based on the removal of supragingival and subgingival calculus and bacterial biofilms using hand instruments and ultrasonic scalers. However, complete removal of the bacteria biofilm and their endotoxins in deeper areas of the pockets and furcation sites is often difficult to achieve with both methods.

Laser therapy has been proposed as an alternative or adjunctive treatment to conventional periodontal therapy. Moreover, Moritz *et al.*<sup>3</sup> in 1997 showed a significant reduction in the amount of bacteria and inflammation by using a diode laser in combination with SRP. Soft tissue lasers reduce the microbial population while providing coagulation at the treatment site when used adjunctively in periodontal therapy after the hard accretions have been removed from the tooth and root surfaces. Maiman *et al.* in 1960<sup>4</sup> invented the ruby laser. Evidence suggests its use in initial periodontal therapy, surgery, and more recently, its utility in salvaging implant opens up a wide range of applications. Laser therapy using a Diode lasers is reported to have bactericidal and detoxification effects and is capable of removing biofilms.

CRP has been detected in both the serum and Gingival crevicular fluid of periodontitis patients and levels were significantly higher than those of non-periodontitis subjects. Furthermore, there is mounting evidence that effective periodontal therapy can lower CRP levels. A more accurate and non-invasive site for the evaluation of inflammatory

biomarker in subjects with periodontal disease is gingival crevicular fluid (GCF), since it reflects the ongoing events in the periodontal tissues that produce it. Hence, this study was undertaken to evaluate the effect of Scaling and root planing with and without diode laser on CRP levels in GCF in patients with chronic periodontitis.

#### Materials and method:

A total of 40 dentate and systemically healthy subjects with Moderate Periodontitis (CPI score 3,4 ) with 21-60 years of age, were recruited for split mouth study. Subjects were treated in 2 groups: Group I - side treated with SRP alone & Group II - side treated with SRP and diode laser(980 nm). Patients having any systemic diseases or with history of any periodontal surgery in past 6 months or any history of trauma and recent tooth extractions. Subjects with chronic smoking habit, coronary artery disease, hypertension, metabolic disorders such as diabetes mellitus and with severe infections and other inflammatory conditions like rheumatoid arthritis , pregnant and lactating females were excluded.

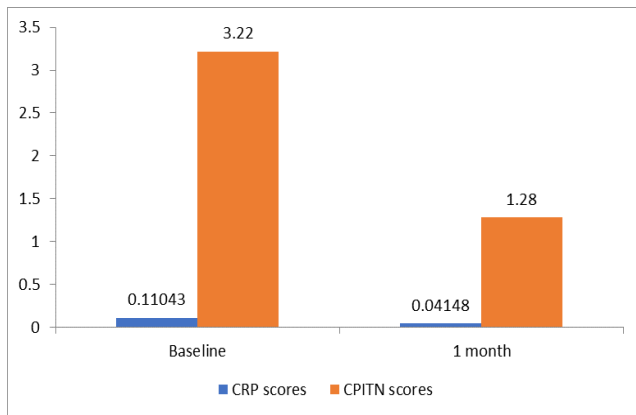
**TREATMENT PROTOCOL-** The present in study was conducted in the Department of Periodontology and Implantology, D.J. College of Dental College and Research, Modinagar (U.P.) All the patients were informed about the purpose of the study prior to participation & consent form, duly signed by each patient were obtained. Gingival Crevicular fluid was collected from each of two quadrants prior to treatment (Baseline) from all the subjects. Patients underwent scaling and root planing and randomly one quadrant was treated using a diode laser with a 980 nm fiber-optic delivery system that will be introduced parallel to the cement surface with apical- cervical scanning movements for 20 seconds per tooth.1 months after periodontal therapy GCF sample was again collected from both site and stored in freezer at -20°C till analysis. Gingival Crevicular Fluid, CRP level was quantified using a commercially available highly sensitive CRP kit, for each sample. Diagnostic reagent kit is used for the in vitro detection of CRP in human GCF by quantitative test.

#### Result:

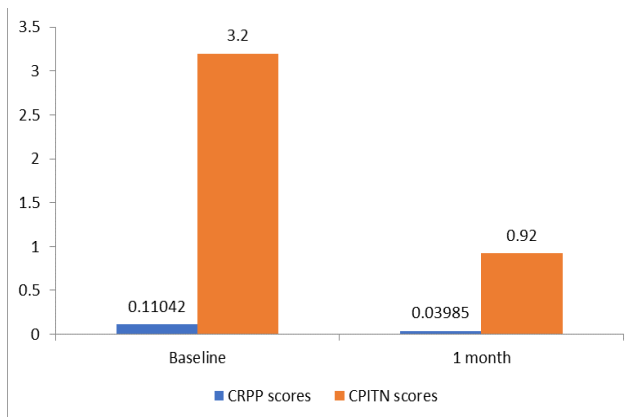
The study was carried out on a total of 40 systemically healthy subjects with moderate periodontitis. Gingival crevicular fluid(GCF) CRP was assessed in all the subjects before therapy and after periodontal therapy. The subjects included in the study had a mean age of  $36.9 \pm 10.5$  years. For all the subjects one quadrant was treated with SRP alone (Side -I) and another quadrant was treated with SRP with diode laser (Side-II).The mean CPITN score was found to be 3.22 in side-I, 3.20 in side-II before periodontal therapy. The mean CRP values at baseline were found to be 0.11043mg/l in side-I; 0.11042mg/l in side-II of the patient. The CPITN score and CRP value after treatment reduced to 1.28 & 0.4148 mg/L in side I and 0.92 & 0.3985mg/L in side II accordingly which are highly significant according to statistical analysis using Student 't' test.

**Table 1:** Descriptive statistics of CRP and CPITN scores of SRP side

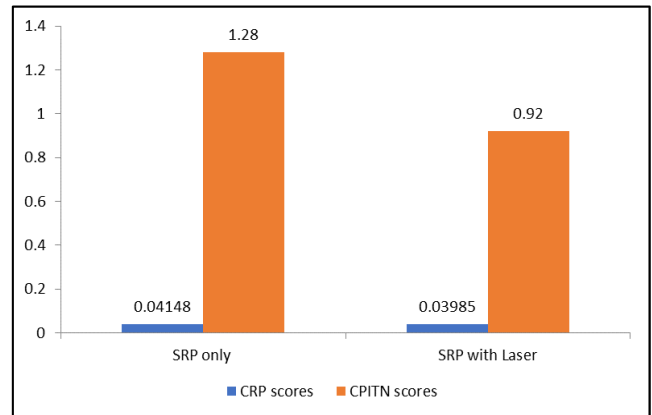
Time interval	Mean	P VALUE
<b>SRP alone side</b>		
CRP at Baseline	.11043	
CRP at 1 month	.04148	
CRP Baseline - 1 month	.068950	0.000*
<b>SRP with laser side</b>		
CRP at Baseline	.11042	
CRP at 1 month	.03985	
CRP Baseline - 1 month	.070575	0.000*
Mean difference of CRP score before and after treatment		
SRP only	.04148	
SRP with Laser	.03985	
Intergroup comparison of CRP score		
CRP scores	.001625	0.3**



**Graph 1:** CRP and CPITN mean scores of SRP side



**Graph 2:** CRP and CPITN mean scores of SRP with Laser group



**Graph 3:** CRP and CPITN mean scores after treatment in both of the studied sides



**Figure 1:**



**Figure 2:**



**Figure 3:**



**Figure 4:**

So here we can see a significant difference in the CPITN score of SRP and SRP with laser side but a very less (No significant) difference in CRP level of both the sides after treatment.

#### **Discussion:**

Chronic periodontitis (CP) is a chronic inflammatory response to the accumulation of microbial plaque and calculus on the root surface of the tooth; this condition leads to breakdown of the surrounding periodontal tissues.<sup>5</sup> While most studies of periodontitis have emphasized the local nature of periodontitis, it appears that systemic manifestations of this disease are also detected through the production of CRP and other acute-phase proteins and pro-coagulant mediators.<sup>6,7</sup> As a response to the presence of bacteria and bacterial products, such as lipopolysaccharides, cell-mediated inflammation is triggered and a number of proinflammatory cytokines (tumor necrosis factor, interleukin IL-1 and IL-8) are synthesized. Systemic inflammation primed by periodontal infection and the release of lipopolysaccharides into the periphery activates both inflammatory cells and endothelial cells and cytokines are carried to the liver where they induce the production of acute-phase proteins such as CRP.

Gingival crevicular fluid (GCF) is proved to be a more specific sample for analysis of periodontal disease activity. GCF can be easily and non-invasively collected and contains products of the host, plaque and their interactions. Analysis of GCF in our study showed elevated C- Reactive protein (CRP) levels in periodontitis patients at baseline which decreased dramatically after non-surgical treatment. (Both the SRP side and SRP with diode laser side).

C-reactive protein is a phylogenetically highly conserved plasma protein, with homologs in vertebrates and many invertebrates that participate in the systemic response to inflammation. It is a pattern recognition molecule, binding to specific molecular configurations that are typically exposed during cell death or found on the surfaces of pathogens. Its rapid increase in synthesis within hours after tissue injury or infection suggests that it contributes to host defense and that it is part of the innate immune response. It is produced in response to many forms of injury other than periodontitis, such as other infections, trauma and hypoxia,

and it is regulated by cytokines such as interleukin-6, IL-1 $\beta$ , and tumor necrosis factor  $\alpha$ . CRP levels have an association with smoking, obesity, triglycerides, diabetes, and periodontal disease. It is proposed that changes in cellular and molecular components of peripheral blood can be found in patients with periodontitis because of inflammatory changes of the periodontal tissues. C Reactive Protein was identified because of its ability to precipitate with the Capsular- polysaccharide extract of streptococcus pneumonia.

CRP is a very strong acute phase protein. In healthy, young subjects and resting situations the serum concentration is <1.5 mg/l. In acute phase situations, the concentration can increase up to thousand folds. CRP is mainly synthesized by hepatocytes, but mRNA and CRP have been shown to be present in monocyte derived macrophages in atherosclerotic plaques, lymphocytes and alveolar macrophages.<sup>8,9</sup> This synthesis is regulated by interleukin (IL-1, IL-6) and TNF- $\alpha$ . Peak value of CRP is usually disappear within a few days of inflammatory stimulus. A study evaluated CRP value in morning and afternoon and found no difference, suggesting that there is no diurnal variations.<sup>10</sup> The CRP concentration is associated with cardiovascular diseases along with other inflammatory disease, such as rheumatoid arthritis. Also several components of insulin resistance syndrome, such as obesity and increased blood pressure, are associated with altered CRP values and which has been confirmed by several other groups.<sup>11</sup>

Recent studies have investigated the potential role of periodontal therapy on systemic inflammation, but the results are somewhat conflicting.<sup>12</sup> Patients treated by non-surgical mechanical periodontal therapy showed a significant increase in plasma CRP, TNF- $\alpha$  and IL-6 levels immediately after the intervention, indicating a systemic acute-phase response, apparently due to a massive bacterial inoculation in conjunction with instrumentation<sup>13</sup> followed by a steady decrease. The meta-analysis conducted by Ioannidou *et al.*<sup>14</sup> did not show any statistically significant reduction in serum CRP following periodontal therapy whereas systematic review by Paraskevas *et al.*<sup>15</sup> showed modest evidence of lowered serum CRP. Study by Pradeep *et al.*<sup>16</sup> have shown increased GCF levels of CRP in obese and non-obese patients with chronic periodontitis. Another study by the same group of authors showed a decrease in both serum and GCF CRP levels following short term non-surgical periodontal therapy in type 2 diabetes mellitus patients. Megson *et al.*<sup>17</sup> have deduced that the CRP detected in the GCF is of systemic origin and cannot be produced locally due to the absence of CRP messenger ribonucleic acid (mRNA) in periodontal tissues. They also stated that genetic susceptibility to inflammation may also modify CRP levels in response to periodontal destruction or systemic disease thus explaining the dissimilar correlation of CRP levels found in healthy and diseased groups. The authors stated that the elevated CRP levels were not due to local production but due to systemic or periodontal inflammation.

In the present study, diode laser therapy has been used as an adjunct to Scaling and root planning. Laser is the acronym of the words "Light Amplification by Stimulated Emission of Radiation."<sup>18</sup> Laser is one of the most captivating technologies in dental practice. The beneficial effects of non-surgical laser therapy in the treatment of periodontal diseases have been discussed in many studies.<sup>19</sup> The use of the laser as an adjunct to conventional SRP is based on the understanding that subgingival debridement and eradication of pathogenic microorganisms could provide new sites for attachment of connective tissue attachment. When used adjunctively with SRP, an 805-nm diode laser was shown to have an additive effect in reducing subgingival bacteria in periodontal pockets measuring  $\pm 4$  mm.<sup>20</sup> A study<sup>21</sup> using a 940-nm diode laser for therapy demonstrated that non-surgical laser applications modulate the behavior and induce mRNA expression of growth factors in gingival fibroblasts, but when compared with the control, laser therapy did not have a positive or negative effect on the proliferation of gingival fibroblasts. However, despite the potential benefits of the use of lasers in periodontology, limited clinical evidence has confirmed that lasers used as an adjunctive to conventional periodontal therapy provide additional benefits. A randomized controlled clinical study by Caruso *et al.*<sup>22</sup> who evaluated the efficacy of a diode laser as adjunctive therapy to SRP, showed a slight improvement in clinical parameters in the laser group. Lai *et al.*<sup>23</sup> did not observe differences in the measured clinical parameters or radiographic findings between the test (low-power helium–neon laser) and control sites after 3, 6, 9, and 12 months, although this study used laser therapy eight times during the first 3 months.

In this Split mouth study, diode laser therapy has been used as an adjunct to Scaling and root planning, in chronic periodontitis patient in one quadrant and SRP alone in another. Both the side treated showed significant decrease in clinical parameter (CPITN score) as well as CRP value after treatment but we compared two sides treated. Though the side treated with SRP and diode laser both showed better result in decrease of CPITN score and CRP value as well but the mean change was statistically non-significant.

#### Conclusion:

In the present study total of 40 dentate and systemically healthy subjects with Moderate Periodontitis were recruited for split mouth study and divided in 2 following groups for treatment: Group I - side treated with SRP alone & Group II - side treated with SRP and diode laser. Evaluation of the effect of periodontal treatment (SRP alone & SRP with diode laser), on CRP levels in GCF in patients with chronic periodontitis was performed along with comparison of levels of CRP in GCF sample in both groups after treatment. With the result of present study the following conclusion were drawn- The mean CRP value after treatment was reduced in both side which are highly significant according to statistical analysis but in intra

group comparison decrease in CRP level after treatment in between two sides are non- significant statistically.

While further studies are needed using larger sample size. Though its well documented, still there is need of further study to justify laser therapy as an adjunct to SRP in decreasing the inflammation level. Therefore the main implication is- Periodontal disease needs to be viewed more broadly in terms of systemic inflammation, either as a consequence of an underlying hyper inflammatory trait or as a factor contributing to systemic inflammation. With the limitation like short sample size and use of only one biomarker(CRP) as diagnostic tool, it is concluded that periodontal therapy could be one of the important aspect in the prevention of adverse cardiovascular events as elevated CRP level has direct relation with the same and Laser therapy as an adjunct to non-surgical periodontal therapy (SRP) lasers have limited clinical outcome.

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