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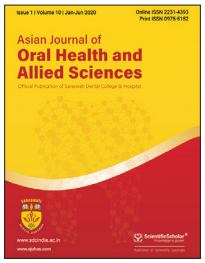

Original Article

Pre- and post-treatment levels of serum high-sensitivity C-reactive protein in patients with lesions of endodontic origin: A clinical pilot study

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ABSTRACT

Objective: The objective of the study was to evaluate the impact of lesions of endodontic origin (LEO) and their treatment on the patients' serum high-sensitivity C-reactive protein (hs-CRP) levels.

Materials and Methods: A total of 20 healthy patients with radiographic evidence of LEO in at least one tooth were recruited for the study, of which 11 were finally evaluated. Before initiating the endodontic treatment, 10 ml of patient's venous was taken from the antecubital vein to assess the pre-operative serum hs-CRP levels (T_0). Canals were prepared with K-files till a suitable size and irrigated with 2.5% sodium hypochlorite. At the subsequent appointment after 1 week, teeth were obturated. The patients were then recalled 30 days after the completion of endodontic therapy. Again blood samples will be taken at time-frame T_1 to assess the serum hs-CRP levels. The change in pre- and post-treatment values for T_0 and T_1 was statistically evaluated to assess the effect of treatment on serum hs-CRP levels.

Results: The mean CRP (mg/L) at T_0 ± SD (Range) at baseline was 6.18 ± 3.72 (0.96–11.02) and the mean CRP at T_1 ± SD (Range) was 3.92 ± 3.59 (1.108–11.04) and mean change in CRP ± SD (Range) after 30 days follow-up was −2.26 ± 3.04 (−8.26–1.16). Significance of change in CRP levels (Paired *t*-test) was $t = 2.458$; $P = 0.034$.

Conclusion: The results of the present study indicate that root canal treatment reduced the levels of hs-CRP in the serum of the patients having LEO. Timely diagnosis and treatment of these lesions may have some contribution in reducing systemic inflammatory burden.

Keywords: Lesions of endodontic origin, Pro-inflammatory biomarkers, High-sensitivity C-reactive protein

INTRODUCTION

The discussion on the suggestive role of inflammation in the development of inflammation in the pathogenesis of atherogenous plaque and subsequent cardiac heart diseases (CHD) has gained momentum in the recent past. CHD or coronary artery disease results from the deposition of plaque in the blood vessels, which transport blood to the heart. If unchecked, it may lead to constriction of the vessels, angina, myocardial infarction, or heart failure.[1,2] Age, male gender, familial history, diabetes, smoking, high blood pressure, obesity, hypercholesterolemia, sedentary lifestyle, and abdominal fat are major predisposing factors responsible for the development of CHD.[3] Although the major risk factors have been recognized, still the increase in the number

of CHD cases is alarming. Thus, other possible risk factors such as conditions associated with a state of inflammation have drawn the attention of the clinicians.[4] In recent years, a relationship between a chronic low-grade infection and atherogenous plaque formation is being extensively studied.[5] Considering that inflammatory processes have a major effect on plaque stability, it can be stated that biomarkers of inflammation might be used to predict the magnitude of risk and stratify patients accordingly.

Chronic, low-grade infections of the oral cavity such as periodontitis contribute to the inflammatory burden of the body and a question that "can oral infections cause cardiovascular damage," has been often asked in the recent past.[6,7] Chronic periodontitis, an inflammatory disease of tooth-supporting structures, is already being recognized for its association with an amplified systemic inflammatory burden as proven by a rise in the levels of various biomarkers compared with healthy control populations.[8] Lesions of endodontic origin (LEO) or chronic apical periodontitis, on the other hand, are the result of microbial invasion of the periapical area from an infected root canal resulting in inflammation at the root tip and subsequent resorption of periapical bone. Although dissimilar in etiology and pathogenesis, periodontal disease and endodontic disease show resemblance in the manner they incite an inflammatory response. Hence, like the periodontal disease, a link between endodontic disease and systemic inflammation is also possible.[9-11] In fact, many cross-sectional studies have already shown an increase in inflammatory biomarkers such as interleukin (IL)-1, IL-6, matrix metalloproteinases, and tumor necrosis factor-α in patients with endodontic lesions compared to the control group.[12-14]

Among the various inflammatory biomarkers, C-reactive protein (CRP) is most linked as a causal factor and as a risk-predictor of CHD. It is an acute phase marker of inflammation and infection is highly sensitive and closely reflects the systemic inflammatory burden with remarkable accuracy, CRP levels are constant over an extended time interval, do not show a diurnal change, and can be quantified at low cost with easily available high-sensitivity assays.[15]

Although cross-sectional studies have been conducted to correlate the endodontic lesions and inflammatory biomarkers present in gingival crevicular fluid (GCF), none have been conducted post-treatment to longitudinally follow the effect of endodontic treatment on the levels of the inflammatory biomarkers. Thus, the aim of this study was to evaluate the impact of LEO and their treatment on the patients' serum high-sensitivity CRP (hs-CRP) levels.

Study population and study design

The study was a prospective, longitudinal, and intervention trial. Each patient served as his own control. The study protocol was approved by the institutional ethical committee (Ref No. 77th ECM B-IMR-faculty/P2). All the patients were explained the procedure and purpose of the study and gave written informed consent before entering the study.

The patient pool consisted of healthy patients (ASA-1) with radiographic signs of LEO of one or more than one teeth not affecting any anatomical structure (within age range of 16–27 years). Patients on any antibiotic or anti-inflammatory medication in the past 3 months, systemic inflammatory conditions such as diabetes mellitus, smoking, or obesity, <25 teeth or poor oral hygiene were excluded from the study. A total of 20 patients who met the inclusion criterion were recruited and were made to sign an informed consent before starting the procedures.

The patients fulfilling the selection criteria were screened for clinical (pulp sensibility tests, percussion, and palpation response) and radiographic periapical status according to periapical index (PAI) index defined by Ostravik *et al*.[16] baseline parameters. The decayed, missing, and filled teeth index were recorded in accordance to the WHO criteria.[17] Before initiating the endodontic treatment, 10 ml of patient's venous was taken from the antecubital vein to assess the pre-operative serum hs-CRP levels (T_0).

MATERIALS AND METHODS

All clinical procedures were performed by a single operator to remove inter-operator bias. Endodontic treatment (root canal treatment) was started under rubber dam isolation. Canals were prepared with K-files (Dentsply, Maillefer, Switzerland) till a suitable size and irrigated with normal saline and 2.5% sodium hypochlorite. The canals were filled with a non-setting, calcium hydroxide based intra-canal medicament (Metapex, Meta Dental Manufacturing Inc., Korea). At the subsequent appointment after 1 week, teeth were obturated with gutta-percha cones and AH-plus sealer (Dentsply, DeTry). The access cavities were filled with a composite resin. Clinical and radiographic parameters at the time of completion of treatment were recorded. The patients were instructed not to take any medication without consulting the treating endodontist. The patients were recalled 30 days after the completion of endodontic therapy. Again blood samples will be taken at time frame T_1 to assess serum hs-CRP levels. The pre-treatment and post-treatment values for T_0 and T_1 were statistically evaluated to assess the effect of treatment serum hs-CRP levels. Furthermore, the change in the levels of hs-CRP was correlated to the clinical healing and PAI score and the data were statistically analyses. The patients who developed acute pain or swelling, fever or any other inflammatory condition during or after completion of the treatment were excluded from the study.

Serum hs-CRP estimation

The method adopted for hs-CRP estimation was in accordance to the instructions on the enzyme immunoassay test kit (Chemux BioScience, Inc., San Francisco), and the same technique used by few earlier studies also.[8] The serum sample was assayed for quantitative determination of hs-CRP levels by the based on the principle of a solid phase enzyme-linked immunosorbent assay. It utilizes a distinctive monoclonal antibody directed against a distinct determinant on the hs-CRP molecule. The antibody (horseradish peroxidase) enzyme conjugate solution contained goat anti-hs-CRP antibody. Simultaneously, the sample to be tested was allowed to react with the two antibodies, resulting in sandwiching of hs-CRP molecules between enzyme-linked and solid phase antibodies. After 45-min incubation at room temperature, the wells were washed with water to eliminate any loose labeled antibodies. Then, after adding tetramethylbenzidine (TMB) reagent, the sample was incubated for 20 min, which resulted in the development of a blue color. The development of color at 450 nm was measured spectrophotometrically.

Reagents and materials

Antibody-coated wells (1 plate, 96 wells) consisting of mouse monoclonal anti-CRP layered microtiter wells were used. A reference standard set (1.0 mL/vial) contained 0 mg/L CRP, 0.005 mg/L CRP, 0.010 mg/L CRP, 0.025 mg/L CRP, 0.050 mg/L CRP, and 0.100 mg/L CRP within xs m.p phosphate-buffered bovine serum albumin (BSA) solution along with preservatives. CRP sample diluent (50 mL/vial) consisted of phosphate-buffered BSA solution along with preservatives. CRP-enzyme conjugate reagent (12 mL/vial) was made from goat anti-CRP conjugated to horseradish peroxidase along with preservatives. TMB reagent (11 mL/bottle) and stop solution (1 bottle, and 11 mL/bottle) contained one-step TMB solution and diluted hydrochloric acid (1N HCl), respectively.

Assay procedure

Each patient sample was diluted 100 folds before use. The required number of coated wells in the holder was secured. Ten microliters of CRP standards, diluted samples and diluted controls were dispensed into the appropriate wells. One hundred microliters of hs-CRP-enzyme conjugate reagent were then dispensed into every well. The reagents were then thoroughly mixed for 30 s. Incubation was performed at room temperature (18–25°C) for 45 min. Flicking the plate contents into a waste box eliminated the incubation mixture. The microtiter wells were rinsed 5 times and flicked using distilled or deionized water. To remove all the residual water droplets, the wells were struck sharply onto absorbent paper or paper towels. One hundred microliters of TMB solution were dispensed into each well, gently mixed for 5 s and incubated at room temperature for 20 min. One hundred microliters of stop-solution were added to every well to stop the reaction, and then mixed gently for half a minute. When the entire blue color changed to yellow, the absorbance at 450 nm was read within 15 min using a microtiter well reader.

Calculation

For each set of reference standards, controls and specimens, the mean absorbance value (OD 450) was calculated. By plotting the mean absorbance obtained for each reference standard on the vertical (y)-axis versus its concentration in milligrams per milliliter on the horizontal (x)-axis, a standard curve was constructed. The corresponding concentration of CRP (mg/L) was determined for every sample from the standard curve using the mean absorbance value. To obtain CRP results in, milligrams per liter, the values of the control sera and the patient specimens were multiplied by the dilution factor of 100. Further 10-fold dilution, after the initial 100-fold dilution (total dilution 1:1000) was performed in specimens having CRP concentrations >10 mg/L, and to obtain CRP results in mg/L, the final CRP values were multiplied by 1000.

RESULTS

Out of total 20 patients recruited for the study, only 15 fulfilled the eligibility criteria. Of this, two patients failed to turn for follow-up while two developed acute pain during treatment and were excluded from the study, thus finally 11 patients were evaluated. The mean age ± SD (range) in years was 21.09 ± 3.24[16-27] [Table 1]. Data were analyzed using Statistical Package for the Social Sciences version 21.0. Paired *t*-test was used to evaluate the significance of change in CRP levels between two intervals.

The mean CRP (mg/L) at T_0 ± SD (range) at baseline was 6.18±3.72 (0.96-11.02) and the mean CRP at T_1 ± SD (range) was 3.92 ± 3.59 (1.108–11.04)a mean change in CRP ± SD (range) after 30 days follow-up was −2.26 ± 3.04 (−8.26–1.16). Significance of change in CRP levels (Paired *t*-test) was $t = 2.458$; $P = 0.034$ [Table 2].

DISCUSSION

The literature is replete with studies indicating a positive correlation between long- standing, chronic oral inflammatory conditions, and systemic conditions. The first Surgeon General's report on "Oral Health in America" published in 2000 asserted on the significance of oral/dental health to achieve overall general health and addressed the

Table 1: Demographic characteristics of study population.

Mean Age±SD (Range) in years	21.09±3.24 (16–27)
Gender (%)	
Male	9 (81.8)
Female	2 (18.2)
Tooth No. (%)	
11	5 (45.5)
12	1 (9.1)
21	1 (9.1)
22	2 (18.2)
37	2 (18.2)
Mean DMFT±SD (Range)	3.73±1.35 (1–5)
Mean PAI±SD (Range)	3.09±0.54 (2–4)

DMFT: Decayed, missing, filled teeth, PAI: Periapical index, SD: Standard deviation

Table 2: Mean change in serum CRP levels (mg/L) from baseline (T_0) to 30 days (T_1).

Mean CRP at T_0	Mean CRP at T_1	Mean change in CRP	Significance of change
6.18±3.72 0.96–11.02	3.92±3.59 1.108–11.04	2.26±3.04 –8.26–1.16	t=2.458; P=0.034

CRP: C-reactive protein

oral diseases as an "Overlooked epidemic."[18] Considering the fact that LEO also induce an inflammatory response in the body, few studies have been conducted to evaluate the correlation of these lesions with the systemic health.[19] Most of these studies are cross-sectional, and researchers have found that LEO are associated with increased levels of biomarkers of inflammation such as IL-1, -2, and -6 compared to the healthy patients.[6,20] Still, well conducted randomized controlled trials studies, longitudinal studies or studies evaluating the pre- and post-treatment effect of these lesions on any systemic marker are missing.

In the present study, there was a significant decrease in the CRP serum levels of the patients from baseline to 1 month follow-up visit after completion of root canal treatment 2.26 ± 3.04 (−8.26–1.16), t = 2.458; (P = 0.034). This finding corroborates with the fact that chronic inflammatory diseases and low-grade inflammation lead to an increase in acute-phase biomarkers in serum and often in other tissue fluids. Serum CRP is a very sensitive systemic marker of inflammation and exhibits a rapid increase of up to 1000 times in response to various stimuli such as inflammation or injury.[21,22] LEO or apical periodontitis is a local, non-specific inflammatory responses mainly to the bacterial infection of the root canal space of teeth with necrotic pulps.[23] In a cross-sectional study by Vidal *et al.*,[24] which showed a positive correlation between serum CRP levels and presence of lesions of endodontic lesions, the authors expressed that the pin pointing a threshold level of infection from an infected root canal leading to an upregulation in the systemic inflammatory response is difficult and may vary from person to person and intensity and duration of infection. Unlike studies in periodontology, where authors have suggested that in dentate people with periodontitis involving more than 10% of sites and pockets more than 4 mm, there may be an increase in one-third mean CRP levels as compared to periodontally healthy persons,[25] there are no studies in endodontics that estimate how much threshold must be.[24]

The existence of an endodontic lesion in relation to a tooth is largely determined by the presence of a periapical radiolucency, and there is a relatively low incidence of clinical signs and symptoms associated with it.[26] More recently, peripheral body fluids such as GCF, plasma, or serum have come up as identity markers of acute and chronic periapical inflammation.[27] In a cross-sectional study, Burgener *et al.*[28] compared total protein levels and IL-1 and 6 in GCF collected from a tooth having endodontic lesion to that from a healthy contralateral tooth. In their study, protein levels in GCF collected around diseased teeth were higher than in control teeth; however, there was not a significant change in the levels of IN-1 and 6. The biomarkers of inflammation serve as a useful indicator of a person's overall metabolism. As most of the studies conducted are cross-sectional, in accordance to the suggestions by van der Waal *et al.*,[7] the present paper attempted to incorporate a more appropriate model to assess the role of inflammatory biomarkers by taking samples from patients with apical periodontitis before and sometime after treatment. Here, as each patient was his/her own control, measuring markers in a single patient also reduced the confounding factors.

Limitations

The study was a pilot project and not without its limitations. The sample size was low as it was difficult to zero-in patients that matched closely in regard to age, systemic conditions, socioeconomic, and environmental factors as all these have an effect on the CRP levels, also, there was no healthy control group. Hs-CRP is a very sensitive acute-phase inflammatory biomarker and it is indeed very difficult to say with affirmation that the increase in levels of serum CRP levels was specifically in response to LEO only and not some other systemic influence.

CONCLUSION

Whether LEO has a causative effect on the systemic inflammatory burden or not; is a question that needs a definitive answer. More carefully designed studies involving

many centers and larger sample size are required to come to a final conclusion. Nevertheless, the present study can serve as a base, for conducting further studies to decide whether LEO and their treatment can contribute to prevention of coronary heart diseases by reducing systemic inflammatory burden or not.

Declaration of patient consent

Institutional Review Board (IRB) permission obtained for the study.

Financial support and sponsorship

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Conflicts of interest

There are no conflicts of interest.

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