Evaluation of inflammatory markers in endotoxins induced root canal infection

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ABSTRACT

Aim: The aim of this study is to evaluate the inflammatory markers in endotoxins induced root canal infection.

Introduction: Root canal infection is mainly caused by an opportunistic infection of the pulp space with commensal oral microorganisms. Depending on the state of inflammation, different treatment regimes are currently advocated. The role of several players of the host response in root canal infection is as follows: Cytokines, proteases, inflammatory mediators, growth factors, antimicrobial peptides, and others contribute to pulpal defense mechanisms; these factors may serve as biomarkers that indicate the status of the pulp. Endotoxins are potent inflammatory agents, which activate classical and alternative pathways of complement system. Materials and Methods: The sterility of the internal surface of the access cavity was checked and all procedures were performed aseptically to the study participants. A new sterile and apyrogenic bur were used accomplished by irrigation with sterile apyrogenic water to access the canal. The endotoxin sample was taken introducing sterile pyrogen-free paper points into the full length of the canal and retained in position during 60 s. Immediately, the paper point was placed in a pyrogen-free glass and at 80°C for future cell culture stimulation. Results: C-reactive protein (CRP) shows agglutination for endotoxin group when compared to that of apyrogenic group. The mean value of fibrinogen in serum of endotoxin-induced group was 400 mg/dl which was found to be higher than apyrogenic group. The level of inflammatory markers such as CRP, fibrinogen, and uric acid was significantly high ($P < 0.001$) in serum sample. Our results suggest that inflammatory marker significantly increases in patients and correlates with infection severity. Conclusion: The pathogenesis of root canal infection is accepted widely to involve inflammation. Hence, dental practitioners should have concerns that root canal treatment may provide a source of inflammation and increase the risk of treatment failure.

KEY WORDS: C-reactive protein, Fibrinogen, Inflammatory markers, Root canal infection, Uric acid

INTRODUCTION

Root canal infection is mainly caused by an opportunistic infection of the pulp space with commensal oral microorganisms. Depending on the state of inflammation, different treatment regimes are currently advocated. The role of several players of the host response in root canal infection is as follows: Cytokines, proteases, inflammatory mediators, growth factors, antimicrobial peptides, and others contribute to pulpal defense mechanisms; these factors may serve as biomarkers that indicate the status of the pulp. Endotoxins are potent inflammatory agents, which activate classical and alternative pathways of complement system.

Endotoxin liberated by Gram-negative bacteria has been detected in primary root canal infections. [1–5] High levels of endotoxin have been associated with the development of spontaneous pain [1,3,6,11] and clinical symptoms such as tenderness to percussion [5] and pain on palpation. [10] Due to the high toxicity of endotoxin in vivo, [1,3,6,9] developing periapical inflammation and alveolar bone resorption, and in vitro, [10] stimulating cells to release proinflammatory cytokines that lead to tissue destruction, its removal/neutralization from infected root canals during endodontic treatment seems to be important for the healing process of periapical tissues. Clinical studies have detected endotoxins in infected root canals, and most of them have suggested a correlation between endotoxin levels and development of clinical symptoms, whereas others have not demonstrated this association. [1,3,6,11–27] Endotoxins are potent inflammatory agents, which activate classical and alternative pathways of
Complement activation releases biologically active peptides, which mediate a number of aspects of the inflammatory process. LPS may evoke pain through activation of the Hageman factor or through neurotoxic properties when acting on presynaptic nerve terminals. Direct sensitization of nociceptors, and sensitzation and upregulation of the transient receptor potential cation channel, subfamily V, member 1 (TRPV1).

Clinical studies have detected endotoxins in infected root canals, and most of them have suggested a correlation between endotoxin levels and development of clinical symptoms, whereas others have not demonstrated this association. Endotoxins are potent inflammatory agents, which activate classical and alternative pathways of complement system. The levels of high-sensitivity C-reactive protein (hs-CRP), fibrinogen, erythrocyte sedimentation rate, and uric acid (UA) were measured in endotoxins induced root canal infection groups.

A significant difference was found between groups in hs-CRP, fibrinogen, and UA. Our results suggest that inflammatory markers significantly increase in patients and correlate with infection severity.

**MATERIALS AND METHODS**

A total of six participants were used in this study. The sterility of the internal surface of the access cavity was checked as previously described, and all procedures were performed aseptically. A new sterile and apyrogenic bur were used accomplished by irrigation with sterile apyrogenic water to access the canal. The endotoxin sample was taken introducing sterile pyrogen-free paper points into the full length of the canal and retained in position during 60 s. Immediately, the paper point was placed in a pyrogen-free glass and at 80°C for future cell culture stimulation.

**Blood Collection and Analysis**

Venous blood was collected in all subjects (endotoxins induced root canal infection subjects compared with apyrogenic control) for biomarker measurements following an overnight fast, shortly after the conclusion of the overnight PSG. All venous samples were centrifuged, and serum was separated into multiple aliquots and stored at −80°C until assay. CRP levels and uric acid concentration was analyzed. Fibrinogen levels in plasma were measured by coagulation method.

**CRP test by Agglutination Method Principle**

The CRP test is based on the principle of the latex agglutination. When latex particles complexed human anti-CRP is mixed with a patient’s serum containing CRP, a visible agglutination reaction will take place within 2 min.

Positive: Agglutination of latex particles indicating the presence of CRP at a significant and detectable level.

Negative: No agglutination.

**Estimation of Fibrinogen**

In a clean tube, add 1.0 ml of oxalate or heparinized plasma obtained by centrifugation of blood. Add 9.0 ml of 13% sodium sulfite solution, mix, and stand the tube in a water bath at 37°C for 10 min. A white precipitate forms which aggregate into larger masses. Centrifuge at 3000 r.p.m for 5–10 min. The precipitate will collect in the bottom of the tube in a compact mass, leaving the supernatant fluid clear. Pour off the supernatant fluid and drain the tube for 1 min, wiping off the last drops clinging to the mouth of the tube with absorbent tissue. Breakup the precipitate by spurtng in 5 ml of the sodium sulfite solution. Place caps on the tube and shake vigorously to suspend the precipitate. Remove the cap and add 3 ml of sulfite solution, washing down the sides of the tube. Centrifuge, pour off, and drain the tube. Add 8 or 10 ml of 5% sodium triphosphate solution to the tube. Place in a boiling water bath and boil until the protein precipitate is dissolved. This usually requires 10–15 min. Cool the tube in a cold water bath. Replace the evaporated basic solution to the 8 or 10 ml mark. Add 0.2 ml of 4% copper sulfate to the tube, stopper and shake vigorously for 2 or 3 min. Remove the cap and centrifuge at 3000 r.p.m for 10 min. Decant the supernatant and read in a suitable spectrophotometer at 560 nm. A water blank is run along with the samples.

**Estimation of Uric acid**

Uric acid was estimated according to the method of Caraway (1963). 5.4 ml of diluted tungstic acid was added to 0.6 ml of sample. The contents were mixed and centrifuged into the test tubes 3.0 ml of supernatant, standard, and water (as blank) was taken. 0.6 ml of sodium carbonate and 0.6 ml of phosphotungstic acid reagent were added, mixed, and placed in a 25°C water bath for 10 min. The blue color developed was read at 700 nm. The uric acid levels were expressed as nM/mg protein.

**RESULTS**

Results are expressed as mean ± standard error of the mean (S.E.M), n = 3; *P < 0.001, statistically significant as compared with apyrogenic control.

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**DISCUSSION**

Clinical studies have detected endotoxins in infected root canals, and most of them have suggested a
correlation between endotoxin levels and development of clinical symptoms when compared with apyrogenic group. From the present study, it is evident that there is a significant difference in the inflammatory markers in endotoxins induced root canal infection.

CRP shows agglutination for endotoxin group when compared to that of apyrogenic group [Figure 1]. The mean value of fibrinogen in serum of endotoxin-induced group was 400 mg/dl which was found to be higher than apyrogenic group [Figure 2]. The mean value of uric acid in serum of endotoxin-induced group was 35 nmoles/mg which was found to be higher than apyrogenic group [Figure 3]. The level of inflammatory markers such as CRP, fibrinogen, and uric acid was significantly high ($P < 0.001$) in serum sample. A significant difference was found between groups in hs-CRP, fibrinogen, and UA. Our results suggest that inflammatory markers significantly increase in patients and correlate with infection severity. Root canal treatment or endodontic treatment is the process of removing infected, injured, or dead pulp from the tooth. The main objective of endodontic treatment is to eliminate bacteria from the root canal system and to prevent them from infecting or reinfecting the root canal or the periapical tissues. Enterococcus faecalis is a recalcitrant candidate among the many causative agents of failed endodontic treatment. Chronic failure is due to the ability of Enterococcus faecalis to bind to the collagen of the dentinal tubule and remains viable within the tubules. These microorganisms have the ability to grow even in a low-nutrient environment and can survive in the root canals as a monoinfection. Eradication of E. faecalis from the root canal with chemomechanical preparation using disinfecting irrigants and antibacterial dressings is difficult. The most commonly used methods for microbial control include instrumentation, antimicrobial irrigation, intracanal dressing, adequate filling, and coronal restoration. The use of root canal filling materials with antibacterial activity is considered beneficial in the effort to further reduce the number of remaining microorganisms and to eradicate the infection. The endodontic sealers have been shown to offer the greatest antimicrobial effects immediately after spatulation, following which there will be a gradual loss of antimicrobial effects over time. The use of sealers with antibacterial properties may be advantageous, especially in clinical situations of persistent or recurrent infection.\[33\]

The LAL assay was chosen to quantify endotoxin levels before and after chemomechanical preparation of primarily infected root canals because of its extreme sensitivity for the detection of minute quantities of endotoxin.\[34\]

According to Berkiten et al.,\[35\] Gram-negative bacteria depth penetration is in a maximum of 275 mm, whereas to 800 mm depth of LPS,\[36\] representing approximately 4 times greater than the bacterial invasion, as a result of its lower molecule weight. In theory, root canal instrumentation establishing 3 size less enlargement apically might leave behind more than 50% of endotoxin-infected dentin. Such measurement is consistent with the percentage of endotoxin left after chemomechanical preparation in the present study (>47%). An enlargement of more than 500 mm would be required to attempt an optimal removal of endotoxin. However, clinically,
this procedure might not be compatible with the tooth anatomy in most of the cases.

CONCLUSION

The removal of debris during chemomechanical preparation and the amount of root canal enlargement seem to play an important role in reducing oral bacterial LPS during endodontic treatment. More research may be required to study the mechanism of action on this field of dentistry.

REFERENCES


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