Original Article

Comparision of interleukin-1β concentrations in posttreatment endodontic disease and other pulpal and periapical conditions – A clinical study

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Abstract

Aim: To evaluate interleukin (IL)-1β concentrations in periapical tissue fluid (PTF) in persistent apical periodontitis requiring endodontic retreatment and to compare the levels of IL-1β with chronic apical periodontitis, symptomatic irreversible pulpitis (SIP), normal pulpal, and periapical tissues.

Materials and Methods: The patients were selected based on inclusion and exclusion criteria and divided into 4 groups based on the pulpal and periapical status: Posttreatment endodontic diseases (PTED): Teeth with PTED due to failed primary root canal treatment having periapical radiolucency. PNAP: Teeth requiring root canal treatment due to pulpal necrosis having periapical radiolucency. SIP: Teeth with symptoms of SIP with healthy periapical tissues). Intentional root canal treatment (IRCT): Teeth requiring IRCT (healthy pulp and periapical tissues). The access cavity was redefined and the preexisting filling was removed using H-files. The root canals were minimally enlarged followed by collection of PTF using paper points, in the case of group PTED. For groups PNAP, SIP, and IRCT, conventional access cavity preparation was done followed by enlargement of canals till 20, 0.02. PTF was collected using 15, 0.02 size absorbent points 2 mm beyond the apex. Levels of IL-1β was assessed by enzyme-linked immunosorbent assay.

Results: A statistically significant difference was seen in levels of IL-1β in all the groups. The highest concentration was seen in group PTED (85.07 ± 11.57 pg/mL) followed by group PNAP (37.60 ± 10.94 pg/mL), group SIP (8.40 ± 1.99 pg/mL), and the least was seen in group IRCT (3.47 ± 1.36 pg/mL).

Conclusion: The levels of IL-1β were highest in PETD cases followed by PNAP, SIP, and IRCT. This indicates the severity of inflammation in PETD cases as compared to other endodontic diseases.

Keywords: Inflammatory mediators; interleukin 1β; posttreatment endodontic disease

INTRODUCTION

Root canal treatment has a success rate of 86%–98%.[1] However, studies have reported that the prevalence of posttreatment endodontic diseases (PTED) such as persistent, recurrent, or emerging apical periodontitis can exceed up to 15%.[2] PTED is defined as an "endodontic failure" that warrants a clinical decision and action.[3] PTED is an inflammatory condition that develops as a result of interaction between microbial challenge and the host immune system.[4-6] There are numerous potential contributors and systemic consequences for the complex and varied process of PTED.[7] Endodontic failure can be due to root canal complexity, insufficient cleaning, shaping and obturation, and breach in asepsis protocol.[8] Endodontic retreatment is one of the treatment options in these cases.[9] During this process, numerous mediators

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of inflammation like interleukins (IL), tumor necrosis factor (TNF), and matrix metalloproteinases (MMPs) are released and play a role in periapical bone destruction.[10,11]

IL-1β may be increased in PTED.[1,2] It is a proinflammatory cytokine having numerous effects in inflammatory cascade like increasing the expression of collagenolytic enzymes, and MMPs, which contribute to extracellular matrix degradation and in turn lead to bone resorption and tissue destruction.[12-14] Furthermore, it upregulates RANK-receptor and thus stimulates osteoclast genesis.[15] All these mechanisms lead to bone resorption and thus, formation of periapical radiolucency.

Numerous investigations have found these inflammatory mediators in periapical tissue fluid (PTF).[16] The main goal of identifying these inflammatory markers is to understand the disease process. This can act as a potential tool for aiding in the diagnosis of the severity of a disease condition and also evaluating the treatment prognosis.

Also, it may pave the road to targeted therapy. These inflammatory mediators can be identified by using various molecular diagnostic tools like enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR).[17]

Studies comparing concentrations of IL-1β in PTED and comparing it with other conditions like symptomatic irreversible pulpitis (SIP), pulpal necrosis with apical periodontitis, and normal pulp cases are lacking. Therefore, this study aims to compare the levels of IL-1β in PTF from root canals in PTED and other endodontic disease conditions like pulpal necrosis, SIP and healthy periapical tissue (requiring intentional root canal treatment [IRCT]) using ELISA.

MATERIALS AND METHODS

Study design

This observational study was carried out in the outpatient unit of the Department of Conservative Dentistry and Endodontics, Meenakshi Ammal Dental College, Chennai. Ethical clearance was obtained from the Institutional Ethical Committee (MADC/IEC-I/26/2022) and consent was obtained from patients in the vernacular language. Patients of age 18–45 years, having no systemic diseases and having periapical radiolucency with periapical index (PAI) score more than 2 in case of pulpal necrosis with periapical radiolucency and PTED with periapical radiolucency. The exclusion criteria for this study were anti-inflammatory and antibiotic therapy up to 1 week before starting endodontic therapy, teeth having complex anatomy, or nonrestorable.

Based on clinical signs and symptoms and radiographic assessment, the patients were categorized into the following groups:

- Group PTED: Permanent molar teeth (maxillary and mandibular) with failed primary endodontic treatment having periapical radiolucency (PAI score >2) i.e., symptomatic persistent apical periodontitis, acute/chronic periapical abscess
- Group Pulpal necrosis with apical periodontitis (PNAP): Permanent molar teeth requiring root canal treatment due to pulpal necrosis with apical periodontitis (PAI score >2) i.e., symptomatic and asymptomatic apical periodontitis
- Group SIP: Permanent molar teeth with symptoms of SIP without apical periodontitis
- Group IRCT: Permanent molar teeth requiring IRCT (healthy pulp and periapical tissues).

Sample size calculation

A pilot study was conducted with a sample size of five in each group and using the results obtained, the final sample size was calculated with the power of 80% and alpha error of 0.05, resulting in a sample size of 15 per group i.e. $n = 60$.[18]

Methodology

All patients who fulfilled the eligibility criteria and signed an informed consent form before the treatment were included in the study. Local anesthesia was administered with 2% lignocaine with 1:80,000 epinephrine (Lignox 2%) and Rubber dam (Coltene Rubber Dam Kit Hygenic, Coltene Whaledent Pvt. Ltd, Maharashtra) isolation was done. For each group following protocol was followed.

Group posttreatment endodontic diseases: Teeth with failed primary endodontic treatment (having periapical radiolucency)

The cavity was redefined with a high-speed airotor handpiece (NSK Nakanishi Inc., Japan) and water coolant. The preexisting root canal filling was removed with the help of H-files (Mani Inc., Tochigi, Japan) and ProTaper retreatment files (Dentsply Sirona, Ballaigues, Switzerland) without using chemical solvent,[18] followed by, physiological saline irrigation. An electronic apex locator (J Morita, Europe GVBH, Frankfurt, Germany) and intraoral radiograph (Soredex™ Digora™ optime Intraoral X-ray, KaVo Dental, Biberach and der Riss, Germany) were used to re-establish working length. Apical patency was established using a 10-size K-file (Mani Inc., Tochigi, Japan). Minimal enlargement of canals was done till the 25 K size file (Mani Inc., Tochigi, Japan). The canals were flushed with normal saline to remove the debris which was then dried with the absorbent points. A PTF sample was collected. After cleaning and shaping till 25, 0.06, calcium hydroxide as intra-canal medicament was placed for 1 week. Subsequently, obturation was done using a cold lateral compaction technique with the help of AH plus sealer and gutta-percha.

Collection of periapical tissue fluid sample

The PTF in the root canal was then obtained by using a

sterilized absorbent point of size #15, 0.02 (DiaDent Group International, South Korea) which was inserted into the root canal 1 mm beyond the established working length. The absorbent point was held for 60s with the help of a tweezer and a digital stopwatch was used to record the time. This procedure was repeated three times. In case of bleeding, the absorbent point containing the streak of blood was discarded, and after 1 min, the PTF was collected.[19]

For groups PNAP, SIP, and IRCT, access opening was performed using a high-speed airotor handpiece (NSK Nakanishi Inc., Japan) with water coolant. An electronic apex locator (J Morita, Europe GVBH, Frankfurt, Germany) and intraoral periapical radiograph (Soredex™ Digora™ optime Intraoral X-ray, KaVo Dental, Biberach an der Riss, Germany) were used to determine the working length. Apical patency was determined using a 10 size K-file (Mani Inc., Tochigi, Japan). After completing minimal canal enlargement till 20 K file (Mani Inc., Tochigi, Japan), the canals were irrigated with physiological saline and canals were dried with absorbent points. PTF was collected as in group PTED. The canals were obturated using a cold lateral compaction technique using AH plus sealer and gutta percha after cleaning and shaping till 25, 0.06.

Sample collection and storage

After sample collection it was stored in 1x phosphate buffer solution and samples were stored at −20°C. The ELISA test was conducted for the samples using EliKine™ Human IL-1β ELISA.

Statistical analysis

Statistical analysis was done using SPSS (Statistical Package for Social Sciences, version 23, IBM Corp., Armonk, New York, USA). Student's *t*-test was used to test the significant differences among the groups. All data was expressed in mean standard deviation and percentage. The correlation between the observed parameters was detected using the Pearson Correlation ($P < 0.05$) in group PTED and PNAP. In group SIP and IRCT correlation was not applicable.

RESULTS

Sample were collected from a total of 60 patients (38 females, 22 males; mean age 35 years) that is 15 patients in each group having multi-rooted teeth (molars) were included.

In PTED, retreatment was done in 15 patients due to under-obturation in 8 patients, missed canals in 5 patients, and untreated canals in 2 patients.

The concentration of IL-1β was statistically significant in different endodontic disease conditions as shown in Table 1. The highest concentrations were in cases that presented with PTED followed by patients with pulpal necrosis with periapical radiolucency (PNAP) then SIP and the least was seen in cases requiring IRCT as shown in Figure 1.

Linear correlation was determined between the measured values of IL-1β concentration and size of the radiolucency and symptoms of patients, by applying the Pearson correlation test. The examined relationship between these values demonstrated a high positive correlation in the size of radiolucency and symptoms of patients with IL-1β concentration in PTED ($r = 0.807$, $P < 0.05$ for PAI score, and $r = 0.775$, $P < 0.05$ for symptoms) and low positive value for PNAP ($r = 0.61$ for PAI score and $r = 0.314$, $P < 0.05$ for symptoms) as shown in Table 2. For group SIP and IRCT correlation was not applicable.

DISCUSSION

Among the various important messengers involved in the progression of apical periodontitis, 2 important messengers are IL-1 and TNF-α.[20] IL-1 is a key mediator of pulpal and periapical inflammation and is released in higher levels (12 folds).[21] Moreover, IL-1β is a potent osteoclastic mediator and is significantly higher in PTF of teeth with larger radiolucent areas.[16] To our knowledge, this is the first study conducted to compare the levels of IL-1 β in PTED and primary endodontic infections like symptomatic/asymptomatic periodontitis with pulpal necrosis and SIP. Permanent molars having PAI scores of 2-3 were selected in PTED[2,22] and in patients with no systemic diseases.[23] Cleaning and shaping were done till 20, 0.02, and following this samples were immediately collected to decrease the influence of cleaning and shaping on IL-1β levels.[24,25]

Most periapical inflammatory mediators have been investigated either at the transcriptome (i.e., mRNA) using PCR or proteomic levels (i.e., actual released protein or metabolite like inflammatory markers, antigen, antibody, etc.) with ELISA[26] due to posttranscriptional or translational changes, a weak correlation between concentrations of protein and its respective mRNA has been seen.[27] Since ELISA assesses the protein itself, it is a better method for evaluating IL-1β than PCR. Hence in this study, ELISA (sandwich technique) was used due to its higher sensitivity.[28]

Table 1: Expression of interleukins-1β in different clinical scenarios

Group	Mean±SD	SE	*P*
PTED (*n*=15)	85.07±11.57	2.9848	0.000
PNAP (*n*=15)	37.60±10.94	2.8230	0.000
SIP (*n*=15)	8.40±1.99	0.5146	0.000
IRCT (*n*=15)	3.47±1.36	0.3501	0.000

SD: Standard deviation, SE: Standard error, PTED: Posttreatment endodontic diseases, SIP: Symptomatic irreversible pulpitis, IRCT: Intentional root canal treatment, PNAP: Pulpal necrosis with apical periodontitis

Table 2: Correlation between symptomatic cases and periapical index score with interleukins-1β levels

Group	Symptoms	Pearson correlation	Radiolucency size	Pearson correlation
PTED (*n*=15)	Pus discharge (6)	0.775	PAI score (PAI score 2 - *n*=6, PAI score 3 - *n*=9)	0.807
PNAP (*n*=15)	Pain on percussion, pain (7)	0.314	PAI score (PAI score 2 - *n*=8, PAI score 3 - *n*=7)	0.061

PTED: Posttreatment endodontic diseases, PAI: Periapical index, PNAP: Pulpal necrosis with apical periodontitis

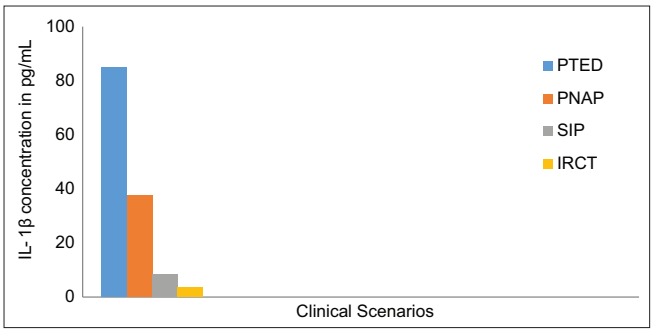

Figure 1: Graphical representation of interleukin-1β concentration in different clinical scenarios. PTED: Posttreatment endodontic diseases, SIP: Symptomatic irreversible pulpitis, IRCT: Intentional root canal treatment, PNAP: Pulpal necrosis with apical periodontitis

Bacterial infection is the primary cause of PTED.[29] Most often, an infection is found inside the root canal system (intra-radicular infection),[30] although it can spread to the peri-radicular tissues (extra-radicular infection). The persistence or secondary nature of the intra-radicular infection depends on the time at which the bacteria enter the root canal system. Microbes present during the first therapy, but not effectively eradicated or controlled likely result in persistent infection that can be attributed to either insufficient root canal debridement or an imperfect root canal seal leading to PTED.[31] In this study, the highest levels of IL-1β levels were present in PTED which is similar to the previous study.[32] In contrast to this, a study by Henriques *et al.* (2011),[33] reported lower IL-1 mRNA levels in cases of PTED. However, IL-1β levels were evaluated using PCR in that study and hence the difference in result is seen.[28]

During PTED, IL-1β is a key cytokine in apical periodontitis that causes increased local blood flow, neutrophil infiltration, and leucocyte recruitment in the inflammatory site.[12] MMPs, collagenolytic enzymes, and cytokines like PGE2 and RANKL that aid in the breakdown of extracellular matrix and ultimately cause bone resorption and tissue damage, are upregulated in expression when exposed to IL-1β.[12,34] Hence, increased production of IL-1β could be the reason for the patient presenting with symptoms of pain, tenderness on percussion, and swelling.

No direct studies are comparing IL-1β and clinical symptoms in patients requiring retreatment due to PTED. However, studies aimed at associating IL-1β levels with clinical symptoms in cases of pulpal necrosis with apical periodontitis, have shown inconsistent results. Lim *et al*. (1994),[35] demonstrated that peri-radicular lesions in humans with symptoms tended to have higher levels of IL-1β than lesions without symptoms. Kuo *et al*. (1998)[36] have documented levels of IL-1β in the exudates suppurating teeth to be 3 times greater than nondischarging canals.[16] Similar results were seen in this study, IL-1β concentration (105 pg/ml) was higher in patients having pain, tender on percussion and pus discharge. Also, it was seen that a higher PAI score (2/3) had a higher concentration of IL-1β. A high positive correlation was seen between PAI score and symptoms with levels of IL-1β concentration which was statistically significant. Although the correlation coefficient was determined but severity of the symptoms was not analyzed. Further investigation in this regard is required.

In PNAP, IL-1β values were similar to previous studies,[37] however, it was significantly lower than PTED and was significantly higher in concentration when compared with SIP and IRCT. Lower levels in PNAP than PTED can be attributed to the pathogens that can withstand adverse conditions created due to failed primary endodontic treatment than in pulpal necrosis and apical periodontitis.[38] Higher levels of IL-1β in PNAP as compared to SIP can be attributed to pulpal necrosis that leads to the progression of inflammation in periapical tissues and harbors micro-organisms that are more virulent. The highest concentration of PNAP was (56 pg/mL) in patients having PAI radiolucency 3 with symptoms of pain, and tenderness on percussion. Which is similar to PTED and previous studies.[24] Interestingly, in this group, it was seen that patients who were asymptomatic with PAI score 2 had IL-1β concentration at par with symptomatic patients. This could be the reason for the low positive correlation between the PAI score and symptoms with the IL-1β concentration. However, the correct interpretation of these data to the amount of bone loss is not possible, and a greater number of patients must be taken into consideration for the correct interpretation of results.

In SIP, the disease is limited to the pulpal tissue and not the periapical tissues, which were evaluated by clinical and radiographic examinations.[39] The presence of IL-1β in SIP is significantly higher than in IRCT, suggesting that the inflammation is progressing into the periapical tissues despite lacking any clinical or radiographic signs or symptoms of apical periodontitis. As the disease is restricted to the pulpal tissues and does not affect the periapical tissues, the concentration of IL-1β is statistically significantly less than in the PTED and PNAP groups. Since

there are no previous studies evaluating IL-1β in PTF of SIP, therefore, results of this study can be taken as a preliminary study for identifying the markers in PTF for evaluating the prognosis of treatment or as a method to diagnose apical periodontitis. However, further studies are required in this regard to device methods to diagnose the disease more accurately and efficiently, making the treatment more predictable.

In IRCT healthy pulpal and periapical tissues are present, hence the IL-1β concentration is the least in this group. This is seen in previous studies as well.[40] The presence of IL-1β in IRCT indicates that inflammatory mediators are seen in normal tissues and these inflammatory mediators increase on onset of disease. This helps in limiting the disease in the local tissues and prevents bacteremia.[41]

The strength of this study is that ELISA was used to evaluate IL-1β and concentrations of IL-1β were compared in different endodontic disease conditions. The limitations of this study are, the Peri apical (PA) radiolucency being assessed using two-dimensional imaging only, the correlation between individual symptoms and IL-1β levels was not done and multiple proinflammatory mediators could be involved in the disease progression, which requires further investigation.

CONCLUSION

Within the limitation of this study, IL-1β levels were higher in PETD cases followed by pulpal necrosis with periapical radiolucency, SIP, and cases requiring IRCT. PTED cases show a strong positive correlation between symptoms and IL-1β concentration. A weak positive correlation was seen in pulp necrosis with PA radiolucency and symptoms.

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Conflicts of interest
There are no conflicts of interest.

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