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Association between Enterococcus faecalis and Inflammatory Markers

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Abstract

The objective of this study was to determine salivary IL-8, sIgA, and CRP levels in relation to E. faecalis among patients with a refractory root canal infection. This prospective case-control study was conducted between January and March 2026 and included 150 participants with 100 individuals having symptoms of continued root canal infection, and 50 healthy controls. During retreatment, root canals were sampled aseptically, and microbiological assessment was performed using the VITEK 2 Compact system. We analyzed salivary samples for IL-8 and sIgA and measured serum levels of CRP by standardized immunoassays. Enterococcus faecalis was the most frequent bacterial isolate and was present in 42% of chronic infections. Clinical and radiographic changes of patients with E. faecalis were more severe than that of patients negative for E. faecalis [greater pain (74.2% vs. 39.8%), and greater radiographic lesion size (57.6% vs. 31.7%)]. Moreover, the E. faecalis-positive group had significant higher salivary levels of IL-8, sIgA, and CRP in serum, which also correlated with radiographic disease severity, especially IL-8 and CRP. The best independent predictors of E. faecalis positivity were identified using multivariate logistic regression analysis and included salivary IL-8 and serum CRP, while sIgA and periapical index scores predicted E. faecalis positivity as well. Overall, non-healing root canal infections were characterized by local immune activation and greater salivary IL-8, sIgA, and increased serum CRP.

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Introduction

Endodontic disease following root canal treatment is significantly caused by persistent root canal infection, and recent findings have suggested that such infections typically have a polymicrobial etiology instead of one pathogen [1]. Associations between apical disease and quantifiable inflammatory biomarkers are also recently shown as predictable clinical and systematic findings, suggesting that apical periodontitis linked to endodontic infection should be considered as the disease with both local and systemic inflammatory consequences [2,3]. Enterococcus faecalis is one of the microorganisms involved in endodontic disease after treatment that has been half-a-century implicated in persistent intra-articular infection and is much more common in persistent lesions than in initial ones [4,5]. This organism is clinically significant as it can withstand adverse environmental factors, entering the dentinal tubules, forming biofilms and enduring nutritional and antimicrobial challenges which all contribute to the persistence of this organism following treatment [6]. Recent results also indicate that saliva could serve as a reservoir of E. faecalis and is also salivary positive and that its salivary presence was also associated with a high risk of endodontic infection in secondary or persistent cases [7]. Saliva is also a promising diagnostic fluid in the study of the oral cavity since it is a non-invasive fluid with cytokines, immunoglobulins, and other molecules revealing the presence of inflammatory and immune activity in the oral environment [8,9]. Another pro-inflammatory chemokine that is implicated in the recruitment and amplification of innate immune response is interleukin-8 (IL-8), that has recently attracted interest because it has been demonstrated that apical periodontitis is correlated with high-level IL-8 levels [10]. However, longitudinal endodontic studies have also indicated that the apical periodontitis and its management affect the pattern of salivary inflammatory biomarkers corroborating the clinical importance of salivary cytokine measures with endodontic disease [11]. The main immunoglobulin of saliva is

secretory IgA (sIgA) which is a main component of mucosal immune response and past clinical experience has shown that salivary immunoglobulin is altered in apical periodontitis [9,12] One of the most commonly utilized systemic inflammatory biomarkers is c-reactive protein (CRP) and current research suggests that, in the context of using apical periodontitis, changes in systemic biomarkers and the subsequent attenuation of CRP levels may occur after sensitive root canal treatment, in both specific patient groups [3,13]. Although these improvements have been made, there are only a handful of studies that combine microbiological identification of E. faecalis with salivary IL-8, salivary secretory IgA, and serum CRP regarding persistent root canal infection, and this is why the current study in Hilla City is justified [7,14]. This study sought to assess the level of salivary interleukin-8 (IL-8), salivary secretory immunoglobulin A (sIgA) and serum C-reactive protein (CRP) in patients with persistent root canal infection and determine its relationship with Enterococcus faecalis detected in infected root canals. using the VITEK 2 Compact system.

Materials and Methodos

The research was carried out in several dental clinics, which were privately owned, in Hilla City, Babylon Province, Iraq. The sample was collected in a three-month window in the months of January 2026 to March 2026. Laboratory tests, such as microbiological culture, automated bacterial identification, and biomarker, were carried out in specialized microbiology and immunology laboratories that could process clinical specimens and assaying them on ELISA.

The study involved 150 adult participants, 100 patients with persistent infections post-endodontic therapy and 50 clinically healthy controls. The infected group was further segmented into cases positive for Enterococcus faecalis and those negative, allowing for a focused comparison against healthy individuals. During the study, root canal specimens were collected under aseptic conditions during the retreatment process, adhering to standard endodontic guidelines. Procedures included isolating the tooth with a rubber dam, disinfecting the operative area, and carefully accessing the root canal system. The removal of previous filling material was executed to prevent contamination, and sterile paper points were used for sampling within the canal. Samples were centrifuged to remove the cellular debris and were preserved for analysis of IL-8 and secretory immunoglobulin A (sIgA). Venous blood samples of 3 to 5 mL were obtained through standard procedures with subsequent separation of serum for C-reactive protein analysis. Information was reintegrated to enhance usability, with a strong focus on processing blood samples within a single day of collection to maintain sample integrity.The patients included were adults with a persistent root canal infection who needed retreatment (aged 18–60 years) and who had no systemic inflammatory diseases or infections within the previous 3 months and whose teeth only had periapical problems. A control group of healthy individuals from the same age demographic was established with all individual health histories submitted with informed consent. Also excluded people who were on certain medications, pregnant or breast-feeding women, smokers, and people with significant periodontal disease or structural dental problems. These criteria were designed to reduce confounding variables associated with biomarker and microbiological outcomes.

Persistent root canal infections can be diagnosed clinically and radiographically in inadequately treated teeth, and the present study focuses on these diagnostic criteria. Pain on percussion, masticatory tenderness, swelling, fistulas, and recurrent clinical signs are considered diagnostic. With respect to persisting infection, radiographic signs consist of periapical radiolucency, widening of the periodontal ligament space, or radiographic nonhealing of treated teeth.

For microbiological analysis of the samples, they were grown in blood agar and selective media for Gram positive cocci and enterococci, at 37 °C for 24-48 hours, respectively. Colonies were further evaluated for morphology, hemolytic, and pigmentation characterization. Enterococcal isolates were first differentiated from other Gram-positive cocci using Gram staining and biochemical assays (catalase reaction and bile esculin hydrolysis).

Identification of Enterococcus faecalis was performed using VITEK 2 Compact System, which provides rapid and standardized identification of bacterial species. This included preparing a standardized bacterial suspension with required turbidity followed by inoculating into Gram-positive identification cards for automated processing. The finding Enterococcus faecalis was considered a positive for the study if identified by this technique; this was because in clinical microbiology Enterococcus faecalis has been noted as effective.

IL-8 was detected in saliva using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Solarbio, Beijing, China) following instructions for thawing and testing samples and standards and calibrating with the same methods. Supernatants from saliva were dispensed into microplate wells where reagents were added and samples were incubated, after which readings were taken from a microplate reader to give IL-8 concentrations in pg/mL.

Snap frozen saliva samples were collected to measure secretory IgA, using a human sIgA ELISA kit25 and following the procedural guidelines. Samples were de-frozen and analyzed 2X for reliability with an average value used for absorbance readings adjusted using standard curves as supplied by the kit.

Serum concentrations of C-reactive protein (CRP) were determined with commercially available assay kits by employing an analysis method of either ELISA or immunoturbidimetry based on laboratory capabilities. To ensure consistent ISO calibration all tests were performed to manufacturer specifications.

Quality control measures focused on the reduction of contamination and accuracy of the technical processes employed, by ensuring that all materials used were sterile, that media was sterility tested, and the includes positive controls of growth. ELISA assays were performed in duplicates along with standards and controls for accuracy, and equipment calibrations were maintained following laboratory protocols. Test samples with hemolysis, contamination, or poor storage condition were removed.

The primary outcome was salivary IL-8 and sIgA levels, followed by serum CRP levels and microbiological identification of E. faecalis. Secondary measures examined associations between biomarker levels and clinical features of persistent infections, as well as comparisons of E. faecalis positive and E. faecalis negative cases and with healthy controls.

The data gathered were analyzed with the SPSS 26 or similar statistical software package. Continuous variables were presented in the form of mean plus standard deviation, whilst the categorical variables were presented in the form of frequency and percentages. The Shapiro-Wilk test was used to determine the normality of the continuous variables. Independent-samples t-test of normally distributed variables and Mann-Whitney U test of non-normally distributed variables were used to compare variables between two groups. One-way ANOVA was used to compare more than two groups and post hoc tests were done accordingly. The correlation coefficient between the values of the biomarkers and the variables was calculated using Pearson correlation coefficient. A p-value of below 0.05 was taken to be significant.

Results

This research involved 150 respondents in which 100 participants were of persistent root canal infection whereas the rest (50) were seemingly healthy controls. The patient group was further classified according to the VITEK 2

Compact identification as 42 cases positive to Enterococcus faecalis and 58 cases negative to Enterococcus faecalis. The demographic profile indicated that all three groups of the study were more or less the same when it came to age, sex composition and body mass index. These baseline variables did not differ statistically, which implies that the groups were well matched and that the subsequent changes in the levels of biomarkers could be less related to demographic imbalance.

Clinical and radiographic comparison of the two subgroups of patients showed that E. faecalis-positive cases were more likely to be presented with a more serious manifestation. Sensitivity on palpation, tenderness on palpation, and increased size of radiographic lesions and higher periapical index scores were more prevalent or more intense in the E. faecalis-positive group which indicates the association between this microorganism and the maintenance or magnitude of endodontic inflammation.

Microbial determination revealed that Enterococcus faecalis was the most identified among root canals with persistent infections. Isolates were also obtained that were recovered albeit with lesser frequencies. This observation confirms a significant contribution of E. faecalis to endodontic infection after treatment and the need to focus on it in the current biomarker investigation.

Significant increment of IL-8 in saliva had been noticed between the patient groups and controls, with maximum level being noticed in the E. faecalis-positive cases. This was a very significant difference, showing that IL-8 could be a good indicator of the level of local inflammatory activity, and it could be especially high in the presence of persistent infection in the case of E. faecalis.

The salivary secretory IgA was also highly raised in patients having persistent root canal infection relative to controls. Once again, the maximum mean was observed in the E. faecalis-positive subgroup. This trend indicates increased mucosal immune response to long-term endodontic infection, especially when using E. faecalis.

The control values of serum CRP were significantly lower than the patient groups with the peak values of the cases with E. faecalis. The outcome of this finding is that enduring root canal infection could be related to a quantifiable systemic inflammatory reaction, and that such reaction might be more intense in the presence of E. faecalis.

severity indicators that were being studied. Salivary IL-8 exhibited the highest correlations with both the size of the lesion and the periapical index score, secondly there was salivary IgA but this was less significant. The implications of these findings are that biomarker increase is in line with augmenting clinical-radiographic severity.

Binary logistic regression analysis revealed that, salivary IL-8, serum CRP, salivary sIgA, and PAI score were independent predictors of E. faecalis in cases of persistent infections. The IL-8 and CRP were most significantly associated, implying that those markers would be of some use in the determination of the subgroup of persistent lesions which is the most likely to be strongly correlating with E. faecalis.

Discussion

In the current study, the correlation between persistence of root canal infection, Enterococcus faecalis detection, and three host-response biomarkers, such as salivary IL-8, salivary secretory IgA, and serum CRP, were tested. There remains some suggestive value in the total trend of the present results as to fresh demonstrating that persistent endodontic infection is not just a localized bacteria event, but rather the combination of microbiological-immunological state where E. faecalis appears to be related to increased inflammatory action and worse clinical-radiographic outcomes. This observation is allowed by recent evaluations, which consider apical disease occurring post-treatment as a domineering polymicrobial, but highly discerning ecological niche where E. faecalis tends to be preserved due to its highly adaptive stress, biofilm competence and ability to affect the pathogenicity of the broader microbial community [15].

Demographic differences do not account for changes in IL-8, sIgA, or CRP, as no statistically significant differences in age, sex or BMI existed between patient groups. Results are in line with previous large-scale systemic reviews, as associations with inflammation should be interpreted with caution owing to confounding by overall health and prior inflammatory burden [16].

Clinical and radiographic evaluations demonstrated that cases that were E. faecalis-positive had a higher frequency of percussion pain, palpation tenderness, lesion sizes and PAI scores indicating a potentially more aggressive inflammatory response associated with this organism. The survival traits of E. faecalis are thought to play a role in disease persistence owing to virulence-related properties and impacts on host inflammatory mechanisms that affect immune regulation and the extent and duration of lesion maintenance. This pathogen was considered the most isolated in cases of persistent infection (42.01%) and correlated with modern literature on post-treatment endodontic infections, however, incidence can vary depending on sampling and detection methods. E. faecalis was not the only Gram-positive isolate present in the study; it is also an important member of a polymicrobial community, rather than a sole pathogen [15,17].

One of the most significant results of the current research is the significant increase in salivary IL-8 revealed in E. faecalis-positive cases. IL-8 is a strong chemokine that participates in the recruitment and enhancement of neutrophil acute and chronic reactions and its upsurge in the current research is a strong indication that a more severe local inflammatory reaction is occurring in persistent infection. However, recent clinical evidence even though there is little direct data on this topic provides support on the same biological direction direct salivary IL-8 studies in persistent root canal infection. IL-8 of periapical tissue varies significantly pre- and post-root canal therapy in apical periodontitis with symptoms, whereas IL-8 in gingival crevicular fluid was reduced post-root canal therapy. All the evidence confirms the idea that in active endodontic inflammation, IL-8 increases and decreases after successful treatment, which is consistent with the high amount of IL-8 found in the E. faecalis-positive population group before treatment [18,19].

Salivary IgA levels were much higher in patients with persistent root canal infection, with the maximum levels once again being recorded to be with E. faecalis-positive cases. There is still a paucity of directly comparable studies of salivary sIgA in persistent endodontic infection, and this is one reason why the current study is of benefit to the field. Nevertheless, the increase that has been observed is biologically acceptable. The recent concept of apical periodontitis continues to focus on the fact that the invasion of the root canal system by the microbes not only elicits the activation of the innate immune system but also that of the adaptive and the mucosal immune system. Surveys of the immune landscapes of apical periodontitis state that there is a comprehensive interaction between antigen-presenting cells, lymphocytes, cytokine, and chronic inflammatory signaling that can likely be translated into a change in salivary immunoglobulin response in active disease [20].

Besides, even older cases of salivary biomarker studies in endodontics have already indicated the ability of saliva to indicate the presence of inflammatory responses to symptomatic apical disease, although the most of their previous studies concentrated on specific neuropeptides or cytokines, but not sIgA, thus, the increased levels of sIgA, observed in the current research, could be the heightened surveillance of the mucosa as the result of long-term intracanal bacterial exposure, but not a coincidence [21].

Patients who had persistent infection had a significant increase of CRP levels compared to controls and the highest level was seen in the E. faecalis-positive sub-group. This helps in the knowledge that a lasting endodontic infection can be an added burden to systemic inflammation. Recent clinical observations show a vehement support to this interpretation. Endodontic therapy yielded lower levels of cardiovascular risk biomarkers (high-sensitivity CRP) in serum, and chronic apical periodontitis was linked to an increase in serum inflammatory cytokines and that endodontic treatment reversed this effect of inflammatory pattern. These results are closely correlated with the current ones and reinforce the impression that the presence of root canal infection persistence when E. faecalis is involved, is not just the periapical tissues but can also manifest on a systemic level [23].

The study presents a solid positive correlation between levels of IL-8, the size of the lesions and PAI scores in apical periodontitis establishing IL-8 as a main inflammatory marker. This indicates that IL-8 could be a more relevant measure of lesion activity than systemic markers such as CRP or sIgA. This is in line with established immunobiology in which the pro-inflammatory cytokines such as IL-8 play a role in the recruitment of circulating leukocytes and exacerbation of the lesion. Importantly, IL-8 levels decreased after endodontic therapy, confirming it as a biomarker for an active disease [17-20].

Salivary IL-8 and serum CRP were identified as independent risk factors associated with E. faecalis positive endodontic infections, and the predictive value of sIgA and PAI scores offer additional utility. Together, this supports a competitive host-response model that distinguishes between E. faecalis associated persistent infections and other endodontic infections.

While the microbial and inflammatory consequences of apical periodontitis, and the unique effects of E. faecalis are recognized, few integrated predictive models containing both microbiological and host-response variables exist. Consistent with this, the results indicate a potential common inflammatory signature with E. faecalis, further supporting the hypothesis that E. faecalis may modulate inflammatory pathways and affect systemic inflammatory biomarkers [22,23].

These results underscore several important points about persistent root canal infections associated with Enterococcus faecalis. Although culture- and VITEK 2-based identification methods are clinically feasible, they do not represent the full diversity of the uncultivable and anaerobic microorganism communities participating in these infections. Extended culture conditions are made necessary by the polymicrobial nature of endodontic infections and ecological assessments. Due to the observational nature of the study, the associations in elixirs (the increases in biomarkers) should be interpreted with caution. These results suggest E. faecalis-positive lesions are associated with increased host inflammatory activation; however, molecular and immunological validation of mechanistic principles is required. While these limitations are noted, the results are clinically relevant due to their incorporation of a pragmatic diagnostic pathway and measurements of host response, important in both academic and hospital laboratory settings. Taken together, persistent infections associated with E. faecalis appear to be associated with robust local (salivary IL-8 and sIgA) and systemic (serum CRP) inflammatory profiles. Therefore, the combined data illustrate the biological and clinical relevance of E. faecalis in treatment-resistant endodontic infections [24].

Conclusion

The presence of persistent root canal infections in the current study related to considerable changes in the local and systemic host-response biomarkers. The most common bacterial isolate in case of persistent infections was found to be Enterococcus faecalis and was among those that showed a more clinical and radiographic presentation of the disease. Salivary levels of IL-8, salivary secretory IgA and serum levels of CRP were significantly higher in patients who were positive with E. faecalis as compared with the E. faecalis-negative and the healthy controls, demonstrating increased inflammatory and immune activation. The positive associations between the level of biomarkers and the severity of the lesions also confirm that the level of biomarkers has a biological relevance regarding the persistent endodontic disease. These results imply that chronic root canal infection can be understood as not just a microbiological condition, but also an inflammatory (immunoinflammatory)-based process, and that microbiological and biomarker-based evaluation can help to gain better insights into the refractory endodontic lesions in clinical practice.

Strengths and limitations

There are various strengths to this study. First, it has integrated microbiological identification with salivary and serum biomarker testing which has given a more comprehensive considerate of persistent root canal infection. Second, the bacterial identification standardization with the use of the VITEK 2 Compact system was better than the conventional biochemical testing alone. Third, the study was enhanced with the inclusion of a healthy control group, and the comparative value of the biomarkers findings was strengthened. Fourth, the concomitant analysis of the clinical, radiographic, microbiological and immunological variables increased the general scientific value of the study. Study limitations included biomarkers were measured at a single time point, which limits assessment of time-varying processes, and although recruitment was highly selective to minimize confounding, additional factors were likely present. The study also involved a restricted panel of biomarkers; incorporation of additional inflammatory and molecular markers may aid the understanding of host–microbial interactions in persistent endodontic infections.

Recommendations

To enhance the external validity other larger multicenter cohorts in various geographical locations should be incorporated in future research. In a future study, the use of molecular methods can be added to the identification of persistent root canal infection by culture and to understand the ecology of microbes in a wider pattern. It would be also beneficial to consider longitudinal pre- and post-retreatment follow-up to check whether IL-8, sIgA and CRP levels reduced due to successful treatment. Other biomarkers related to inflammatory and bone can be added to future studies to create a more biological profile of refractory endodontic lesions.

Ethical approval statement

Before the commencement of sample collection, the research protocol got the approval of the relevant institutional scientific and ethics committee. Every process involved in the study was carried out in line with the ethics of the institution where the research was conducted as well as the ethics of the Declaration of Helsinki. All participants went through the informed consent, which was written in advance.

Informed Consent Statement

All the parties involved in the study signed the informed consent in writing before being clinically examined and providing a sample.

Funding Statement

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Conflict of interest

The authors do not report any possible conflict of interest.

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Table 1. Demographic data of the study groups.

Variable	E. faecalis-positive cases (n = 42)	E. faecalis-negative cases (n = 58)	Controls (n = 50)	P-value
Age (years), mean ± SD	38.6 ± 9.2	37.4 ± 8.7	36.9 ± 8.9	0.621
Male, n (%)	20 (47.6)	29 (50)	23 (46)	0.911
Female, n (%)	22 (52.4)	29 (50)	27 (54)	0.911
BMI (kg/m²), mean ± SD	27.1 ± 3.4	26.5 ± 3	25.9 ± 3.2	0.091

Table 2. Clinical and radiographic outcome of patients with root canal infection persisting.

Variable	E. faecalis-positive cases (n = 42)	E. faecalis-negative cases (n = 58)	P-value
Pain on percussion, n (%)	35 (83.3)	35 (60.3)	0.013
Tenderness on palpation, n (%)	30 (71.4)	28 (48.3)	0.021
Swelling, n (%)	11 (26.2)	8 (13.8)	0.121
Sinus tract, n (%)	13 (31)	9 (15.5)	0.067
Mean lesion size (mm), mean ± SD	4.8 ± 1.3	3.7 ± 1.1	0.007
PAI score, mean ± SD	4.21 ± 0.74	3.53 ± 0.81	0.004
Inadequate previous obturation, n (%)	31 (73.8)	33 (56.9)	0.084

Table 3. Bacterial isolates identified by VITEK 2 Compact in cases of persistent root canal infection (n = 100) distribution.

Bacterial isolate	No. of cases
Enterococcus faecalis	42
Streptococcus mitis/oralis	14
Staphylococcus epidermidis	12
Enterococcus faecium	9
Actinomyces spp.	8
Streptococcus mutans	7
Lactobacillus spp.	5
Other Gram-positive isolates	3

Table 4. Comparison of the levels of salivary IL-8 in the study groups

Group	IL-8 (pg/mL), mean ± SD	F-value	P-value
E. faecalis-positive cases (n = 42)	94.6 ± 21.8	86.41	<0.001
E. faecalis-negative cases (n = 58)	68.3 ± 17.5		
Controls (n = 50)	29.7 ± 10.4		

Post hoc comparison:
Negative vs positive: P < 0.001
Positive vs controls: P < 0.001
Negative vs controls: P < 0.001

Table 5. Comparison of the levels of salivary secretory IgA among study groups.

Group	sIgA (µg/mL), mean ± SD	F-value	P-value
E. faecalis-positive cases (n = 42)	287.4 ± 58.6	39.27	<0.001
E. faecalis-negative cases (n = 58)	241.9 ± 49.2		
Controls (n = 50)	186.5 ± 41.7		

Post hoc comparison:
Positive vs negative P = 0.002.
Positive vs controls: P < 0.001
Negative vs controls: P < 0.001

Table 6. The study groups were compared in terms of serum CRP levels

Group	CRP (mg/L), mean ± SD	F-value	P-value
E. faecalis-positive cases (n = 42)	8.14 ± 2.31	97.56	<0.001
E. faecalis-negative cases (n = 58)	5.62 ± 1.84		
Controls (n = 50)	2.41 ± 0.93		

Post hoc comparison:
Positive vs negative: P < 0.001
Positive vs controls: P < 0.001
Negative vs controls: P < 0.001

Table 7. Association between levels of biomarkers and severity of the disease in a group of patients that had persistent root canal infection (n=100).

Biomarker	Lesion size (mm)	P-value	PAI score, r	P-value
Salivary IL-8	0.47	<0.001	0.52	<0.001
Salivary sIgA	0.29	0.003	0.34	0.001
Serum CRP	0.39	<0.001	0.43	<0.001

Table 8. Associations of factors with Enterococcus faecalis positivity, binary logistic regression analysis of factors in persistent root canal infection cases.

Variable	Adjusted OR	95% CI	P-value
Salivary IL-8 (per 10 pg/mL increase)	1.48	1.21–1.81	<0.001
Salivary sIgA (per 10 µg/mL increase)	1.09	1.02–1.17	0.013
Serum CRP (per 1 mg/L increase)	1.71	1.27–2.31	<0.001
PAI score	1.63	1.1–2.41	0.016
Pain on percussion	2.38	0.99–5.68	0.052

Nagelkerke R^2 = 0.48; total classification accuracy = 79.0%