Acute Dental Pain and Salivary Biomarkers for Stress and Inflammation in Patients with Pulpal or Periapical Inflammation

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Aims: To investigate whether acute dental pain due to pulpal or periapical inflammation is associated with increased expression of cortisol and inflammatory markers and mediators in the saliva, as well as changes in salivary flow rate. **Methods:** Patients experiencing pain (n = 42) were recruited when seeking emergency dental treatment. A 0 to 10 numeric rating scale (NRS) was used as a measure of the severity of pain, and the number of days with pain sensation was also recorded. Unstimulated saliva was collected for 3 minutes (salivary flow measured in mL/minute) and stored at –80°C. Saliva was analyzed for the biomarkers cortisol, C-reactive protein (CRP), and cytokines interleukin-1β (IL-1β) and interleukin-6. In addition, the participants completed a simple questionnaire about stress-inducing factors such as insomnia, dental anxiety, or home/workplace stress. Patients received a dental examination and diagnosis (eg, symptomatic pulpitis/apical periodontitis or acute apical abscess), which was confirmed during dental treatment. The control group (n = 39) consisted of participants without any pain and no known medical or dental problems. **Results:** Patients experiencing acute pain due to pulpal or periapical inflammation had a mean NRS score of 7.0 ± 2.59. The mean duration of pain was 6.5 ± 7.9 days. There was no significant difference in pain level between male and female subjects, tooth type affected, or diagnosis. Higher levels of cortisol, IL-1β, and IL-6 and increased salivary flow were detected in patients with pain when compared to controls ($P < .05$). CRP was higher in patients with acute pain compared to control participants without pain, but this difference was not statistically significant. Stress at home or the workplace was reported by 79% of patients experiencing pain and by 28% of control participants. **Conclusion:** Acute dental pain due to pulpal or periapical inflammation was associated with an increase in salivary cortisol, IL-1β, and IL-6 levels and in salivary flow rate. Stress arising from home or the workplace may aggravate a symptom-free pulpal or periapical inflammation to an acute phase. Inflammation in the pulp and periapical region can have effects in regions remote from the disease site.
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Pain of endodontic origin can reach the maximum intensity on any pain rating scale due to the dense innervation of the dental pulp by nociceptive fibers.[1,2] The most frequent cause of activation of these nociceptive fibers is inflammation of the dental pulp (ie, pulpitis) due to untreated dental caries. Pain symptoms localized to teeth are among the most frequently experienced orofacial pain complaints.[3] Since the dental pulp lacks collateral blood circulation, inflammation due to untreated caries or other factors often progresses to partial and then complete necrosis without healing. Inflammatory mediators and bacterial toxins released from the necrotic dental pulp exit via the apical foramen into the alveolar bone, creating periapical inflammation (ie, apical periodontitis). In both pulpal and periapical inflammation, a wide range of cytokines and neuropeptides are released locally.[4–6] Pro-inflammatory cytokines interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) are released in the inflamed dental pulp and are involved in the progression of endodontic disease.[5,7]

Interestingly, endodontic diseases can progress without any symptoms. Experiencing acute dental pain of sudden onset is a potentially stressful event that results in patients seeking emergency dental treatment. Stress activates the sympathoadrenal axis to secrete catecholamines and the hypothalamic-pituitary-adrenocortical (HPA) axis to secrete cortisol. Because the sympathetic nervous system is an important modulator of immune reactions,[8] its activation can affect inflammatory reactions, which are also shown in the dental pulp and the periapical region.[8] Cortisol, a steroid hormone released from the adrenal glands in response to stress, is responsible for activating the body's "fight-or-flight" response and also modulates inflammation.[9–11]

Diseases of dental origin have in recent years been shown to increase systemic levels of C-reactive protein (CRP).[12,13] CRP is an acute-phase protein produced by the liver in response to most forms of inflammation, infection, and tissue damage. CRP synthesis starts about 6 hours after stimuli and peaks around 48 hours, with a half-life of about 19 hours. CRP levels are monitored in clinical practice to assess the extent and intensity of many infections.[14–16] Dental diseases causing increased CRP levels are also associated with increased risk of cardiovascular diseases, atherosclerosis, and stroke.[17,18] Furthermore, a recent study on patients with chronic kidney disease and necrosis of the dental pulp tissue reported high systemic levels of CRP and IL-6.[19]

Cortisol, CRP, and cytokines are secreted in the saliva, along with most compounds found in blood.[20–22] Cortisol, IL-6, and salivary flow rate are influenced by circadian rhythm, which means their physiologic release varies during the course of a day.[23,24] Salivary flow is controlled by the brain and is affected by sympathetic and parasympathetic activation. There is a paucity of information on salivary flow rate in healthy individuals with acute dental pain.

The aim of this study was to investigate whether acute dental pain due to pulpal or periapical inflammation is associated with increased expression of the stress hormone cortisol and inflammatory markers and mediators in the saliva, as well as changes in salivary flow rate. The hypothesis was that acute pain symptoms due to pulpal or periapical inflammation cause an increase in salivary biomarkers for stress and inflammation, as well as changes in salivary flow rate.

Materials and Methods

Participants

This research was approved by the Regional Committees for Medical and Health Research Ethics West, Norway, and was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All participants were over 20 years of age and gave written informed consent. Patients suffering from systemic diseases; taking medications such as hydrocortisone and others known or suspected to cause hyposalivation; and/or experiencing pain of nonendodontic origin were excluded.

Experimental Protocol

A total of 92 participants were recruited in this study: 53 were patients seeking emergency dental treatment for acute dental pain due to pulpal or periapical inflammation at either the Department of Clinical Dentistry (Faculty of Medicine, University of Bergen, Norway) or Tannlegevakten in Bergen (The Public Dental Services in Hordaland County, Norway), and 39 were control participants. Eleven of the patients were excluded because of the inability to diagnose whether pain was of an acute or chronic nature; chronic infections in other organs; use of cortisol cream; or an incomplete questionnaire.

To minimize potential effects of circadian rhythm, procedures were performed between 13:00 and 18:00 hours. Baseline pain levels were recorded on a numeric rating scale (NRS) from 0 (no pain) to 10 (worst possible pain). Patients received both clinical and radiographic examinations. Once a diagnosis was established, unstimulated saliva was collected over a 3-minute period, during which patients were asked to sit upright with the head tilted down and to drool into a funnel attached to a 0.1-mL graded sterile glass measuring cylinder. Salivary flow was calculated in mL/minute by dividing the value in the measuring cylinder by three, since it was a 3-minute unstimulated drool. While waiting for treatment, the participants completed a simple questionnaire on food intake in the past 12 hours; use of medications; experience of dental anxiety; and possible social events at the home or workplace that the participant considered stressful at the time of sample collection. The volume of collected saliva was measured, and then the saliva was transferred to smaller aliquots, transported on ice, and stored at −80°C until analysis. Diagnosis was confirmed during treatment and categorized as symptomatic (ie, painful) irreversible pulpitis; symptomatic apical periodontitis; or acute apical abscess (swelling). Emergency treatment was comprised of one of the following procedures: pulpotomy; root canal debridement; or incision and drainage.

Control participants (n = 39) were age- and sex-matched as closely as possible. Control participants did not experience pain of any origin; did not have any previous endodontic treatment; had no known dental or medical problems; and were not on any medication.

Table 1 Patient Characteristics

Characteristics	Patients (n = 42)	Controls (n = 39)
Gender, n		
Male	25	17
Female	17	22
Age (y), mean (SD)	37.26 (11.37)	36.36 (10.86)
Pain on 0–10 NRS, mean (SD)	7.0 (2.59)	0
Days with pain, mean (SD)	6.5 (7.9)	0
Salivary flow (mL/min), mean (SD)	0.87 (0.45)**	0.70 (0.29)
CRP (pg/mL), mean (SD)	6,126.58 (6,802.77)	5,117.38 (4,188.66)
IL-6 (pg/mL), mean (SD)	34.95 (50.43)**	7.36 (8.38)
IL-1β (pg/mL), mean (SD)	495.86 (798.04)*	257.71 (248.59)
Cortisol (μg/dL)		
Mean (SD)	0.39 (0.88)*	0.14 (0.11)
In male subjects, mean (SD)	0.55 (1.13)	0.19 (0.15)**
In female subjects, mean (SD)	0.15 (0.08)	0.10 (0.03)

SD = standard deviation; NRS = numeric rating scale; CRP = C-reactive protein; IL-6 = interleukin-6; IL-1β = interleukin 1 beta.
*$P < .05$; **$P < .01$.

Salivary Data Analysis

Saliva samples were stored at −80°C until assayed commercially using Salimetrics high-sensitivity salivary enzyme immunoassay kits for quantitative determination of salivary cortisol, CRP, IL-1β, and IL-6, without modification to the manufacturer's recommended protocol (Salimetrics LLC). The test volume was 15 μL. Samples were thawed to room temperature, centrifuged at 3,000 rpm for 15 minutes to remove mucins, and diluted 1:10 prior to assay. All samples were assayed in duplicates, and the mean of the duplicates was used in the statistical analyses. The intra- and inter-assay coefficients of variation were between 10% and 15%.

Statistical Analyses

Clinical parameters are presented as means ± standard deviation (SD). Student *t* test and Mann-Whitney rank sum test were used to determine statistically significant differences between gender groups; experimental and control groups; patients with self-reported stress vs no self-reported stress; dental anxiety vs no dental anxiety; and insomnia vs no insomnia. A step-wise multiple regression analysis sequentially removing the least significant variable was performed. One-way analysis of variance (ANOVA) was used to test differences in NRS pain scores with regard to gender, tooth type affected, and diagnosis. A P value ≤ .05 was considered significant. All analyses were performed using Statistical Package for the Social Sciences (SPSS, version 22.0, IBM).

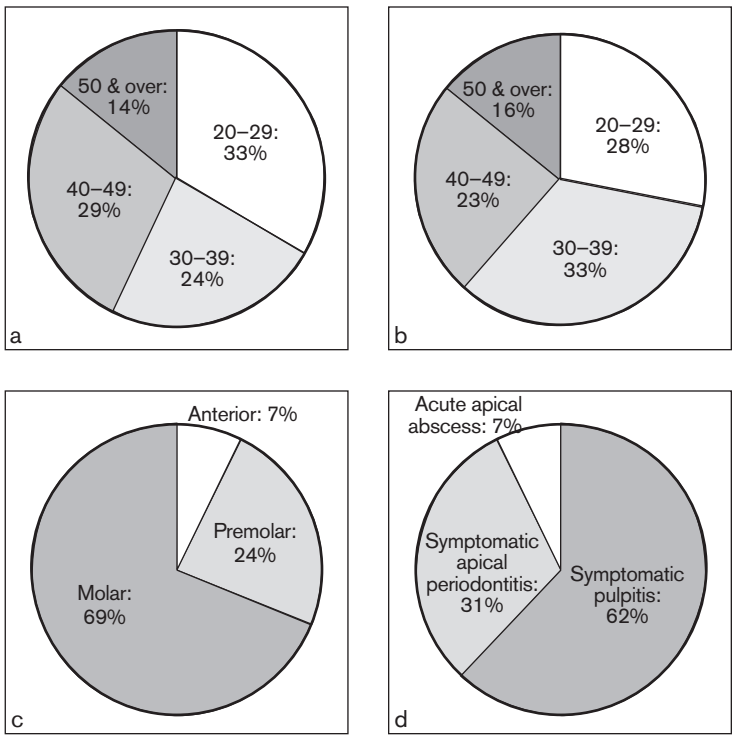

Fig 1 Distribution of **(a)** age of patients experiencing acute pain and **(b)** controls; of **(c)** tooth affected; and of **(d)** diagnosis.

Results

Clinical parameters of the sample are listed in Table 1. After applying exclusion criteria, a total of 81 participants were included: 42 patients seeking treatment for acute dental pain, and 39 age- and sex-matched controls. Distribution of patients by age, tooth type, and diagnosis is presented in Fig 1. A majority of patients with acute pain (85%) had taken medications for pain relief. These medications were mainly over-the-counter analgesics, such as paracetamol (acetaminophen), ibuprofen (nonsteroidal anti-inflammatory drug [NSAID]), or a combination of

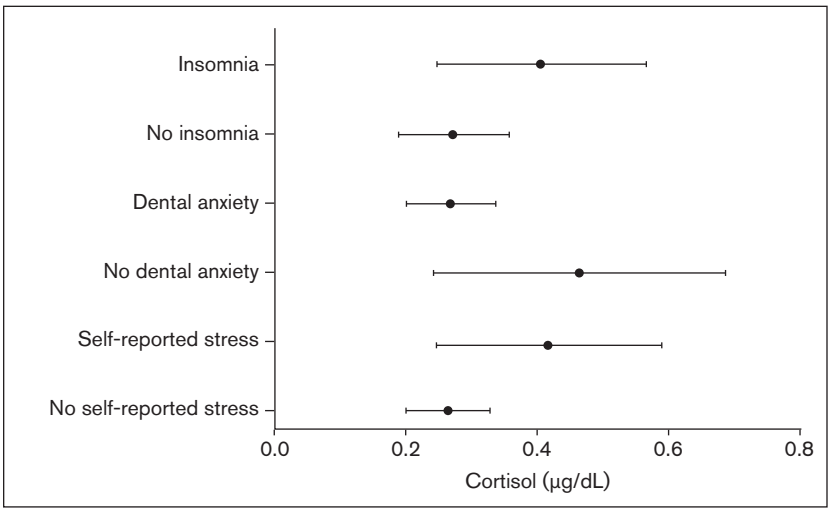

Fig 2 Scatter horizontal error bars for mean cortisol level (µg/dL). There were no significant differences within the self-reported stress, dental anxiety, or insomnia groups.

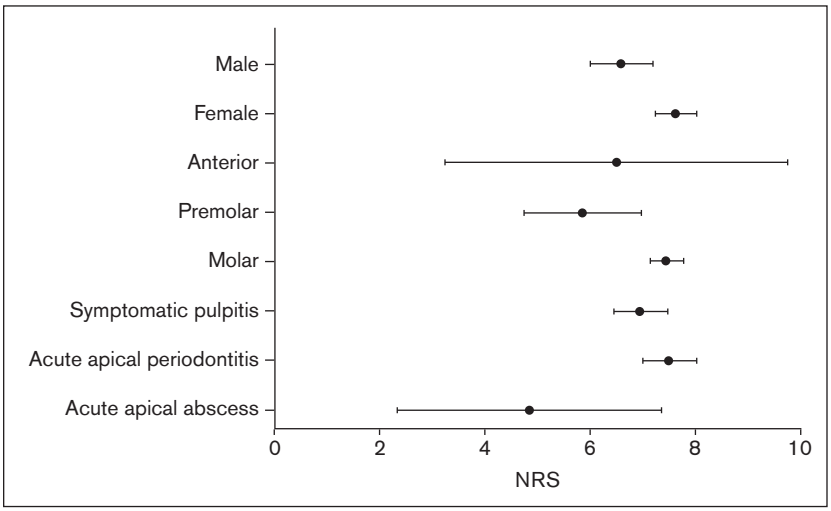

Fig 3 Scatter horizontal error bars for mean pain score on 0–10 numeric rating scale (NRS). There were no significant differences within gender, tooth affected, or diagnosis with respect to pain sensation.

both. Five patients (12%) had self-medicated with prescription drugs, such as paracetamol with codeine, ibuprofen with codeine, and antibiotics prescribed for another condition. A total of 35 patients (83%) reported insomnia because of pain even though they had taken medication, and 3 patients (7%) could sleep after taking analgesics. A total of 17 patients (40%) reported having dental anxiety, and 33 (79%) reported some kind of stress at home or the workplace. No patients in the pain group reported that the experience of acute pain was stressful. In the control group, 28% reported experiencing stress.

As evident from Table 1, cortisol ($P < .05$), IL-1β ($P < .05$), and IL-6 ($P < .01$) levels and salivary flow ($P < .01$) were significantly higher in patients experiencing pain compared to control participants. Cortisol level was significantly higher in male compared to female patients in the control group ($P < .01$), but this difference was not significant in the pain group. There were no significant differences in cortisol levels in pain patients reporting dental anxiety vs no anxiety, stress vs no stress, or insomnia vs no insomnia (Fig 2). CRP level was not significantly different between the pain and control groups. There were also no significant differences in NRS scores for pain between female and male patients, tooth type affected, or diagnosis (Fig 3).

Discussion

In the current study, healthy patients experiencing acute pain due to pulpal or periapical inflammation had higher salivary levels of cortisol, IL-6, and IL-1β, as well as higher salivary flow. Within the control group, male participants had a significantly higher cortisol level than female participants. There was no gender difference in pain intensity according to the NRS. There was no correlation between pain and the extent of pulp or periapical inflammation. There was no difference in CRP level between patients experiencing pain and controls. Self-reported stress, dental anxiety, and insomnia did not affect cortisol levels in this study.

Stress and Cortisol

Stress is a disease of the 21st century. Stress activates the sympathetic nervous system, which has an influence on dental inflammation.[8,10,11,25–27] A high cortisol level in the saliva is an indication of stress.[20,28] Cortisol release by the adrenal gland follows the circadian rhythm: It is highest during early morning and falls during the day. In order to avoid circadian variations, all procedures were undertaken between 13:00 and 18:00 hours, when the cortisol level is stable. In the control group, male and female participants showed a significant difference in cortisol level, which is consistent with previous studies.[29,30] Patients experiencing pain had a significantly higher cortisol level compared

to the control participants. A high percentage of patients experiencing pain also reported having home and workplace stress, insomnia, and dental anxiety. Patients experiencing insomnia and dental anxiety did not show significant differences in cortisol levels compared to patients with no insomnia or dental anxiety in this study. This finding is in accordance with a recent study on patients with dental phobia who did not have significant differences in cortisol level.[31]

It may be possible that the acute dental pain experienced by the patients may have increased the cortisol level in this study; however, none of the patients reported that experiencing pain was stressful. In addition, a recent clinical study on intensive care patients after a major surgery concluded that cortisol was not a useful marker for pain and that its use as a marker for pain is questionable.[32] The high cortisol level in patients experiencing pain could be due to the self-reported stress, since a large number of patients (79%) in this study also reported having stress at the workplace, home, or both.[33] The control participants in this study were selected for not having any previous endodontic treatment or known endodontic diseases; however, a small number (28%) also reported experiencing stress. The severity of the stress among the two groups was not investigated in detail in this study, but increased salivary cortisol as a result of stress has been shown to be associated with aggravation of diseases such as periodontal disease, atopic dermatitis, and asthma.[28,34–37]

Neuropeptides and Stress

Stress has also been shown to cause the release of the neuropeptide substance P, enhance neuronal plasticity, and cause neurogenic inflammation, thereby aggravating an inflammatory response.[10,11,38–40] Substance P is a neuropeptide found extensively in the dental pulp that is involved in pain mechanisms and is upregulated during inflammation of the dental pulp.[4,25,41]

Acute Dental Pain

Although the dental pulp is densely innervated, the inflammation can be symptomatic or asymptomatic.[42] The incidence of a tooth presenting with pulp necrosis and periapical inflammation with no history of pain is reported to be up to 60%.[43,44] Mechanisms causing pain in the dental pulp and periapical region are poorly understood. One theory for asymptomatic irreversible pulpitis is that pulpal inflammatory cells release opiates, which can negate painful symptoms.[45] However, this does not explain why a symptom-free tooth may become symptomatic, as it has been clinically observed that the dental pulp undergoes different stages of degeneration, and there is no specific stage in the inflammatory process that can be pinpointed as acute or painful during inflammation of the dental pulp. Recent advances in pain studies indicate that there is a psychosocial component to orofacial pain[46]; it can therefore be speculated that, in this study, stress may have triggered an underlying inflammatory state or a subclinical infection to an acute phase, turning asymptomatic irreversible pulpitis to symptomatic irreversible pulpitis or asymptomatic/chronic apical periodontitis to symptomatic apical periodontitis or acute apical abscess. This theory is supported by the recent proposition that stress around the time of an inflammatory challenge enhances the inflammatory response, with deleterious effects on chronic inflammatory diseases.[40] The authors speculate that stress experienced at home or the workplace may have triggered the acute pain sensation.

Cytokines

Pro-inflammatory cytokines IL-1β and IL-6 have a broad spectrum of biologic events and play a key role in regulating inflammatory responses. Cytokines are produced by different types of cells, including macrophages, monocytes, T-cells, and fibroblasts. IL-6, originally described to differentiate B-cell into immunoglobulin-producing plasma cells, also activates osteoclasts to induce bone resorption, like IL-1β.[47] As pulpal inflammation progresses toward the alveolar bone to cause periapical inflammation, it is not surprising that the expressions of bone-resorbing cytokines such as IL-1β and IL-6 were elevated. Cytokines have been reported to be increased locally in inflamed dental pulp and periapical tissue,[6,7] but this is the first time an increase in secretion has been reported in a remote region, such as the salivary glands.

IL-6 is also involved in the acute phase response, being the main inducer for CRP production by hepatocytes. Acute phase response is a rapidly induced inflammation associated with infection, tissue damage, or injury that results in fever and CRP production.[15] CRP levels are elevated in other oral diseases, such as periodontal diseases.[12,13,48] IL-6 is significantly higher in patients with acute dental pain, but CRP is not. This is in accordance with previous studies on plasma CRP levels in patients with endodontic problems reporting that IL-6, rather than CRP, is a better indicator of odontogenic infections.[19] The utility of salivary CRP in monitoring endodontic pain is currently unknown; however, in severe odontogenic infections, CRP is used as a measure for severity of disease before hospitalization.[14,16] The patients with acute dental pain in this study were in good general health when they sought emergency treatment; however, a majority had taken medication to alleviate pain symptoms prior to the dental visit, which may have affected the levels of cortisol and inflammatory markers and could

therefore be considered a limitation. Despite this, no significant differences were found in the analysis of salivary biomarkers.

Salivary Flow Rate

An interesting finding in this study is the high salivary flow in patients experiencing pain. Nociception has been shown to be modulated by vagal afferents, which have a parasympathetic component.[49–51] Therefore, pain and activation of the HPA axis may have increased salivary flow via parasympathetic stimulation of the salivary glands. On the other hand, substance P has also been reported to increase salivary flow.[52–54] As substance P, a sensory neuropeptide commonly found in the dental pulp, is increased and released during pulpal inflammation,[4,25,41] it is possible that this may have stimulated the salivary flow observed in patients with acute pain. Further investigations are needed to elucidate this observation.

One of the limitations of this study is that saliva was used as a diagnostic tool rather than blood, which has a well-established normal reference range. However, most compounds found in blood are also present in saliva,[21] and technical advances in recent years have made it possible to quantify very small quantities of compounds secreted in saliva.[55,56] Salivary cortisol and CRP levels are closely correlated with blood levels.[20,57] Collection of saliva samples is noninvasive, safe, inexpensive, and a reliable method of studying systemic levels of cytokines and inflammatory factors.[58] It is therefore an ethical method to use among patients experiencing pain. Another limitation of this study is that stress and dental anxiety were self-reported by patients, and no validated questionnaires were used. Since this is a preliminary study suggesting a correlation between acute dental pain and stress, further studies are needed to address this correlation in detail.

To the present authors' knowledge, this is the first study that implies a potential psychosocial component to acute dental pain of endodontic origin. Further studies are needed to explore this aspect when studying pain mechanisms in the dental pulp.

Conclusions

Acute dental pain due to pulpal and periapical inflammation was associated with an increase in salivary cortisol, IL-1β, IL-6, and salivary flow rate. Stress arising from the home or workplace may aggravate asymptomatic pulpal and periapical inflammation to an acute phase. Salivary flow is increased during acute pain in the oral cavity. Inflammation in the pulp and periapical region can have effects in regions remote from the disease site.

Acknowledgments

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References

1. Byers MR. Dental sensory receptors. Int Rev Neurobiol 1984; 25:39–94.
2. Gibbs JL, Melnyk JL, Basbaum AI. Differential TRPV1 and TRPV2 channel expression in dental pulp. J Dent Res 2011; 90:765–770.
3. Lipton JA, Ship JA, Larach-Robinson D. Estimated prevalence and distribution of reported orofacial pain in the United States. J Am Dent Assoc 1993;124:115–121.
4. Awawdeh L, Lundy FT, Shaw C, Lamey PJ, Linden GJ, Kennedy JG. Quantitative analysis of substance P, neurokinin A and calcitonin gene-related peptide in pulp tissue from painful and healthy human teeth. Int Endod J 2002;35:30–36.
5. Zehnder M, Delaleu N, Du Y, Bickel M. Cytokine gene expression—Part of host defence in pulpitis. Cytokine 2003;22:84–88.
6. Azuma MM, Samuel RO, Gomes-Filho JE, Dezan-Junior E, Cintra LT. The role of IL-6 on apical periodontitis: A systematic review. Int Endod J 2014;47:615–621.
7. Barkhordar RA, Hussain MZ, Hayashi C. Detection of interleukin-1 beta in human periapical lesions. Oral Surg Oral Med Oral Pathol 1992;73:334–336.
8. Haug SR, Heyeraas KJ. Modulation of dental inflammation by the sympathetic nervous system. J Dent Res 2006;85:488–495.
9. Yeager MP, Pioli PA, Guyre PM. Cortisol exerts bi-phasic regulation of inflammation in humans. Dose Response 2011;9: 332–347.
10. Strausbaugh HJ, Dallman MF, Levine JD. Repeated, but not acute, stress suppresses inflammatory plasma extravasation. Proc Natl Acad Sci U S A 1999;96:14629–14634.
11. Strausbaugh HJ, Green PG, Dallman MF, Levine JD. Repeated, non-habituating stress suppresses inflammatory plasma extravasation by a novel, sympathoadrenal dependent mechanism. Eur J Neurosci 2003;17:805–812.
12. Mattila K, Vesanen M, Valtonen V, et al. Effect of treating periodontitis on C-reactive protein levels: A pilot study. BMC Infect Dis 2002;2:30.
13. Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. J Clin Periodontol 2008;35:277–290.
14. Alotaibi N, Cloutier L, Khaldoun E, Bois E, Chirat M, Salvan D. Criteria for admission of odontogenic infections at high risk of deep neck space infection. Eur Ann Otorhinolaryngol Head Neck Dis 2015;132:261–264.
15. Pepys MB. C-reactive protein fifty years on. Lancet 1981;1:653–657.
16. Ylijoki S, Suuronen R, Jousimies-Somer H, Meurman JH, Lindqvist C. Differences between patients with or without the need for intensive care due to severe odontogenic infections. J Oral Maxillofac Surg 2001;59:867–872.

17. Scannapieco FA, Bush RB, Paju S. Associations between periodontal disease and risk for atherosclerosis, cardiovascular disease, and stroke. A systematic review. Ann Periodontol 2003; 8:38–53.
18. Cotti E, Mercuro G. Apical periodontitis and cardiovascular diseases: Previous findings and ongoing research. Int Endod J 2015;48:926–932.
19. Niedzielska I, Chudek J, Kowol I, et al. The odontogenic-related microinflammation in patients with chronic kidney disease. Ren Fail 2014;36:883–888.
20. Hellhammer DH, Wüst S, Kudielka BM. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology 2009;34:163–171.
21. Lee YH, Wong DT. Saliva: An emerging biofluid for early detection of diseases. Am J Dent 2009;22:241–248.
22. Miller CS, Foley JD, Bailey AL, et al. Current developments in salivary diagnostics. Biomark Med 2010;4:171–189.
23. Dawes C. Circadian rhythms in human salivary flow rate and composition. J Physiol 1972;220:529–545.
24. Arvidson NG, Gudbjörnsson B, Elfman L, Rydén AC, Tötterman TH, Hällgren R. Circadian rhythm of serum interleukin-6 in rheumatoid arthritis. Ann Rheum Dis 1994;53:521–524.
25. Haug SR, Berggreen E, Heyeraas KJ. The effect of unilateral sympathectomy and cavity preparation on peptidergic nerves and immune cells in rat dental pulp. Exp Neurol 2001; 169:182–190.
26. Haug SR, Brudvik P, Fristad I, Heyeraas KJ. Sympathectomy causes increased root resorption after orthodontic tooth movement in rats: Immunohistochemical study. Cell Tissue Res 2003;313:167–175.
27. Haug SR, Heyeraas KJ. Effects of sympathectomy on experimentally induced pulpal inflammation and periapical lesions in rats. Neuroscience 2003;120:827–836.
28. Mizawa M, Yamaguchi M, Ueda C, Makino T, Shimizu T. Stress evaluation in adult patients with atopic dermatitis using salivary cortisol. Biomed Res Int 2013;2013:138027.
29. Laudat MH, Cerdas S, Fournier C, Guiban D, Guilhaume B, Luton JP. Salivary cortisol measurement: A practical approach to assess pituitary-adrenal function. J Clin Endocrinol Metab 1988;66:343–348.
30. Zumoff B, Fukushima DK, Weitzman ED, Kream J, Hellman L. The sex difference in plasma cortisol concentration in man. J Clin Endocrinol Metab 1974;39:805–808.
31. Naumova EA, Faber S, Lindner P, et al. Parallel study about the effects of psychotherapy on patients with dental phobia determined by anxiety scores and saliva secretion and composition. BMC Oral Health 2016;17:32.
32. van Gulik L, Ahlers S, van Dijk M, et al. Procedural pain does not raise plasma levels of cortisol or catecholamines in adult intensive care patients after cardiac surgery. Anaesth Intensive Care 2016;44:52–56.
33. Kirschbaum C, Wüst S, Hellhammer D. Consistent sex differences in cortisol responses to psychological stress. Psychosom Med 1992;54:648–657.
34. Vig RS, Forsythe P, Vliagoftis H. The role of stress in asthma: Insight from studies on the effect of acute and chronic stressors in models of airway inflammation. Ann N Y Acad Sci 2006;1088:65–77.
35. Koray M, Dülger O, Ak G, et al. The evaluation of anxiety and salivary cortisol levels in patients with oral lichen planus. Oral Dis 2003;9:298–301.
36. Wright RJ, Cohen RT, Cohen S. The impact of stress on the development and expression of atopy. Curr Opin Allergy Clin Immunol 2005;5:23–29.
37. Chen E, Miller GE. Stress and inflammation in exacerbations of asthma. Brain Behav Immun 2007;21:993–999.
38. Katayama I, Bae SJ, Hamasaki Y, et al. Stress response, tachykinin, and cutaneous inflammation. J Investig Dermatol Symp Proc 2001;6:81–86.
39. Liezmann C, Klapp B, Peters EM. Stress, atopy and allergy: A re-evaluation from a psychoneuroimmunologic perspective. Dermatoendocrinol 2011;3:37–40.
40. Pavlovic S, Daniltchenko M, Tobin DJ, et al. Further exploring the brain-skin connection: Stress worsens dermatitis via substance P-dependent neurogenic inflammation in mice. J Invest Dermatol 2008;128:434–446.
41. Olgart L, Gazelius B, Brodin E, Nilsson G. Release of substance P-like immunoreactivity from the dental pulp. Acta Physiol Scand 1977;101:510–512.
42. Hasler JE, Mitchell DF. Painless pulpitis. J Am Dent Assoc 1970;81:671–677.
43. Michaelson PL, Holland GR. Is pulpitis painful? Int Endod J 2002;35:829–832.
44. Bender IB. Pulpal pain diagnosis—A review. J Endod 2000; 26:175–179.
45. Mudie AS, Holland GR. Local opioids in the inflamed dental pulp. J Endod 2006;32:319–323.
46. Sessle BJ. Relevance of psychosocial factors in oral and facial pain and headache [editorial]. J Oral Facial Pain Headache 2017;31:197–198.
47. Tamura T, Udagawa N, Takahashi N, et al. Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6. Proc Natl Acad Sci U S A 1993;90:11924–11928.
48. Yamazaki K, Honda T, Oda T, et al. Effect of periodontal treatment on the C-reactive protein and proinflammatory cytokine levels in Japanese periodontitis patients. J Periodontal Res 2005;40:53–58.
49. Randich A, Gebhart GF. Vagal afferent modulation of nociception. Brain Res Brain Res Rev 1992;17:77-99.
50. Khasar SG, Green PG, Miao FJ, Levine JD. Vagal modulation of nociception is mediated by adrenomedullary epinephrine in the rat. Eur J Neurosci 2003;17:909–915.
51. Green PG, Miao FJ, Strausbaugh H, Heller P, Janig W, Levine JD. Endocrine and vagal controls of sympathetically dependent neurogenic inflammation. Ann N Y Acad Sci 1998;840:282–288.
52. Martinez JR, Martinez AM. Stimulatory and inhibitory effects of substance P on rat submandibular secretion. J Dent Res 1981;60:1031–1038.
53. Bobyock E, Barbieri EJ, Chernick WS. Effects of substance P and substance P antagonists on rat salivary secretion. J Dent Res 1986;65:1427–1431.
54. Ueha T, Nemoto A, Kurihara K. Studies on the salivary secretion induced by substance P in perfused submandibular gland of rat. Proc Finn Dent Soc 1989;85:345–353.
55. Robles TF, Sharma R, Park KS, Harrell L, Yamaguchi M, Shetty V. Utility of a salivary biosensor for objective assessment of surgery-related stress. J Oral Maxillofac Surg 2012;70:2256–2263.
56. Wang A, Wang CP, Tu M, Wong DT. Oral biofluid biomarker research: Current status and emerging frontiers. Diagnostics (Basel) 2016;6(4). pii: E45.
57. Punyadeera C, Dimeski G, Kostner K, Beyerlein P, Cooper-White J. One-step homogeneous C-reactive protein assay for saliva. J Immunol Methods 2011;373:19–25.
58. Malamud D. Saliva as a diagnostic fluid. Dent Clin North Am 2011;55:159–178.