Clinical Correlation of Salivary Alpha Amylase Activity versus Dental Anxiety Levels in Patients with Chronic Endodontic Pain

Immadi Laxmi, Sujith Kumar, and Sindhu Ramesh*

Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

Corresponding author: Sindhu Ramesh, Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India, E-mail: sindhuramesh

Abstract

Introduction: The aim of this study was to investigate the correlation between dental anxiety, salivary cortisol, and salivary Alpha Amylase (sAA) levels in chronic Endodontic pain. Furthermore, the aim was to look into individual differences such as age, race, gender, any existing pain, or traumatic dental experience and their effect on dental anxiety. **Materials &Methods:** This study followed a cross-sectional design and included a convenience sample of 46. Every patient was asked to complete the Dental Anxiety Scale (DAS) and a basic demographic/dental history questionnaire. A saliva sample, utilizing the spitting method, was then collected in sterile containers. Samples were analyzed for salivary cortisol and sAA levels by Salimetrics. **Results:** Significant associations were observed between DAS scores and presence of pain and history of traumatic dental experience. However, significant correlations were observed between DAS, cortisol, and sAA levels. Our study reconfirms that dental anxiety is associated with presence of pain and a history of traumatic dental experience. **Conclusion:** Based on the results of our study, we can conclude that presence of pain and any history of traumatic dental experience are associated with patients' dental anxiety level. **Clinical Significance:** Dentists need to be trained in anxiety management and communication techniques, this consideration will help in development of specialist postgraduates courses for dentists in management of dental anxiety. Such initiatives allow interested dentists to gain more confidence, more experience and skills in this specialized in the field of dentistry.

Keywords: Stress; Dental anxiety; Salivary cortisol; Salivary alpha amylase; Dental anxiety scale

Introduction

Dental anxiety is a very common phenomenon and remains an obstacle for many patients to seek proper dental care despite all the technological advances in dentistry. Multiple etiologies have been proposed in the past. Thomson et al, suggested that even though endogenous factors (personality traits) play a role in its development, it develops mainly from exogenous (conditioning) factors. [1] Van Wijk et al., revealed that a single early traumatic experience can be the main cause of dental anxiety. [1,2] Oosterink et al, showed that a previous traumatic experience may involve pain, Negative Dentist Remarks (NDR), and strong negative emotional responses. [3] As a consequence, these variables act as predictors for cancelled/missed appointments, a decrease in pain threshold with increase in patient discomfort, poor compliance, increased number of emergency appointments, jeopardized patient/dentist relationship, high Decayed Missing and Filled Teeth (DMFT) index, poor oral health perception, decreased self-esteem, and decreased oral health–related quality of life. [4-20] Rhudy et al. suggested that the pain reactivity is modulated by emotional stress. [21] In addition, Loggia et al. revealed changes in pain pathways on neuroimaging techniques with a negative emotional state. Furthermore, Klages et al. revealed that anxiety increases expected or experienced pain where patients with higher anxiety levels predicted a higher pain experience.

How to cite this article: Laxmi, Kumar, RameshClinical Correlation of Salivary Alpha Amylase Activity versus Dental Anxiety Levels in Patients with Chronic Endodontic PainAnn Med Health Sci Res.2021;11:62-67

Anxiety is regarded as a form of stress and, thus, has a physiological impact on the body. Stressors can cause the activation of the Autonomic Nervous System (ANS), which prepares the body for the fight-or-flight reaction, and the Hypothalamic-Pituitary-Adrenal (HPA) axis. When the Autonomic Nervous System (ANS) gets activated, it causes the release of epinephrine and norepinephrine from the adrenal medulla. [22,23] Norepinephrine was shown to increase the secretion of salivary Alpha Amylase (sAA) from the acinar cells of the parotid and submandibular salivary glands. [24] It was suggested that the level of alpha amylase in the saliva reflects the Autonomic Nervous System (ANS) activity and that measuring it presents an easy, noninvasive measure of ANS activity compared to measuring the actual catecholamines in serum [25] sAA levels were shown to increase in response to various stressors like exercise, cold exposure, and hypertension, in addition to psychological stress. [26] Nator et al,also demonstrated that sAA has a definite circadian rhythm wherein its levels fluctuate during the day in a definite pattern. [27,28] Because the ANS is considered a rapid response, it was suggested that it may be a better measure of stress compared to measuring the hypothalamic-pituitary-adrenal axis response. [29,30] Upon activation of the hypothalamic-pituitary-adrenal axis, cortisol gets secreted from the adrenal cortex to all body fluids, including saliva. It was demonstrated in the past that salivary cortisol increases in response to stress and anxiety, and that it also presents an easy, noninvasive way of measuring stress. [31] Cortisol levels in the saliva have been shown to be higher in patients with oral lichen planus. [32,33] In addition, they were higher in patients undergoing wisdom teeth extractions and prior to urgent dental care. [34] Similar to alpha amylase, cortisol has a definite circadian rhythm. The Dental Anxiety Scale (DAS), devised by Norman Corah in 1969, is the most commonly used scale to measure dental anxiety. [35] We have numerous highly cited publications on well designed clinical trials and lab studies. [36-50] Previously our team has a rich experience in working on various research projects across multiple disciplines. [51-56] Now the growing trend in this area motivated us to pursue this project.

This has provided the right platforms for us to pursue the current study. Our aim is to investigate the correlation between dental anxiety, salivary cortisol, and salivary Alpha Amylase (sAA) levels in chronic endodontic pain.

Methodology

Study design

It is a single centered, parallel randomized triple blinded controlled trial (patients, examiner, statistician were blinded to the allocation of mouthwash in respective groups)

Ethical approval

Approval for the project was obtained from the Institutional Human Ethical Committee and Review Board of Saveetha Institute of Medical and Technical Sciences, Chennai, India on Date 9/08/2019. IHEC/SDC-ENDO-1703/19/168.

Eligibility Criteria

Inclusion criteria

Patients under the age group of 20-30 years, patients with baseline plaque score greater than 1.5 and a baseline Decayed Missing and Filled Teeth (DMFT) index of 3 to 5, patients with no systemic disorders, and patients with no salivary gland disorders. In addition, the participants in the study were required to have a normal unstimulated (resting) whole saliva flow rate (0.5 ml/min).

Exclusion criteria

The exclusion criteria were subjects with medical disorders such as diabetes mellitus, renal disease, gastrointestinal disorders, respiratory diseases, evidence of recent bronchitis, sinusitis, or tonsillitis, patients undergoing antibiotic or other antimicrobial therapy, smokers, those who, on pre study clinical screening, presented a probing depth ≥ 4 mm, subjects with naso-pharyngeal alterations, mouth breathers, and patients with prostheses, orthodontic or dental appliances.

Sample size

Sample size estimation was calculated using a priori by G*Power 3.1.2 software. The minimum sample size of each group was calculated, following these input conditions: power of 0.95 and $P \leq 0.05$ and sample size arrived at 25 per group.

This study took place at Saveetha dental college and hospital. It followed an observational, cross sectional design. Informed consent was given by all participants. From prior literature [54] a Pearson correlation coefficient equal to 0.535 yielded statistically significant results. Using the same value, with the aid of G power, aiming for 80% power and a type I error rate of 5% showed that a sample size of 23 would be required. In order to increase the power, we used a sample size of 50, which resulted in a power of over 90% while maintaining a type I error rate of 5%. Inclusion criteria for the study entailed that participants had no prior history and were presenting as new patients. Adult patients, age range 18–80, who were in good systemic health, were considered. Exclusion criteria included: first, patients with any preexisting conditions affecting cortisol levels, such as adrenal gland insufficiency; second, pregnant women, or women taking oral contraceptives; third, asthmatic patients who were on steroid inhalers; fourth, patients with xerostomia and those taking B-blockers, because of their effect on salivary flow; lastly, patients on antidepressants or antipsychotic medication, because of their effect on mood and mental status. Sample collection was performed between the hours of 9 am and noon to account for the diurnal rhythm for both cortisol and alpha amylase.Upon participants' approval, and their meeting all inclusion/ exclusion criteria and signing informed consent, they were asked to complete Corah's DAS, as well as a basic demographic/dental history questionnaire. A saliva sample was then collected utilizing the method of spitting, using sterile containers provided by Salimetrics. Participants were asked to

allow the saliva to pool at the floor of the mouth first, and asked them to spit into the containers.

The DAS score was the primary predictor for the analyses done for the above salivary biomarkers; however, it was also the outcome variable for the secondary analyses. Predictors that were included in the secondary analyses, which allowed for comparisons between basic sample characteristics and dental anxiety, were age, gender, race, presence of pain, and history of traumatic dental experience. Salivary flow rate was considered a potential confounder for sAA levels; thus, sAA output (in U/min) was calculated by multiplying sAA levels with salivary flow rate for each sample.

Results

The DAS score was the primary predictor for the analyses done for the above salivary biomarkers; however, it was also the outcome variable for the secondary analyses. Predictors that were included in the secondary analyses, which allowed for comparisons between basic sample characteristics and dental anxiety, were age, gender, race, presence of pain, and history of traumatic dental experience. Salivary flow rate was considered a potential confounder for sAA levels; thus, sAA output (in U/min) was calculated by multiplying sAA levels with salivary flow rate for each sample.

DAS and pain

Seven subjects reported pain. Their mean DAS score was 14.00 (SD ¼ 4.76). On the other hand, 39 subjects did not report pain, and their mean DAS score was 9.33 (SD ¼ 3.04). An independent-samples t test was performed to test the association between DAS and presence of pain. Equal variances were tested using Levene's test, and equal variances were assumed. A statistically significant association was observed between DAS and presence of pain.

Table 1: Sample demographics (n=46). t values represent outcomes of independent samples t tests. DAS indicates Dental Anxiety Scale; PLA: Painful Local Anesthesia; PDTF: Pain reported while Drilling Tooth for a Filling.

Variable	%in sample	Total	DAS score mean(SD)	t	P value
Gender	56.52	26	9.92(2.99)	-0.249	0.805
Males	43.48	20			
Females			10.20(4.55)		
Pain	15.22	39	9.33(3.05)	-3.411	0.001
Yes	84.78	7			
No			14.00(4.76)		
PLA	21.74	10	12.20(4.47)	-2.164	0.036
Yes	78.26	36			
No			9.44(3.26)		
PDTF	28.26	13	10.39(4.25)	-0.388	0.700
Yes	71.74	33			
No			9.91(3.53)		

DAS and painful local anesthesia injection

Ten subjects reported history of a painful local anesthesia injection. Their mean DAS score was 12.20 (SD=4.47). On the other hand, 36 subjects did not report a painful local anesthesia injection, and their mean DAS score was 9.44 (SD=3.29). Equal variances were tested using Levene's test, and equal variances were assumed (P=256). An independent-samples t test was performed and a statistically significant association was observed between DAS and history of painful local anesthesia injection (t=2.164, P=.036) [Table 1].

DAS and pain reported while drilling tooth for a filling

Thirteen subjects reported history of Pain reported while Drilling Teeth for a Filling (PDTF) on the other hand, 33 subjects did not report PDTF.

Table 2: Continuous variables in sample(n=46). sAA: salivary Alpha Amylase.

Variable	Mean(SD)	Range	r	P value
Age	46.07(16.22)	19-76	-0.193	0.199
sAA(U/mL)	73.73(46.194)	3.000-171.5000	-0.045	0.766
Cortisol(ug/dl)	0.28(0.27)	0.001-1.7779	0.126	0.403

DAS and sAAlevel and sAA output

The means for sAA level and sAA output were 73.73 U/mL (SD=46.19) and 47.11 U/min (SD=45.79) respectively. The mean DAS score was 10.04 (SD=3.71). Pearson's correlation coefficient was used to test the correlation between these variables; however, no statistically significant correlations were observed (r=0.045, P=.766, r=0.162, P=.282 respectively) [Table 2].

DAS and salivary cortisol levels

The mean salivary cortisol level was 0.277 lg/dL (SD=0.273) and the mean DAS score was 10.04 (SD=3.71). Pearson's correlation coefficient was performed to test the correlation between DAS and salivary cortisol levels; however, no significant correlation was observed (r=0.126, P=.403) [Table 2].

Discussion

Anxiety and pain

In agreement with previous literature, our study results do confirm a relationship between dental anxiety and pain. In their study about perceived pain in relation to a dental hygienist treatment, Hakeberg et al. demonstrated that even though patients generally report higher anxiety towards dental treatment, it was shown that perceived pain is correlated with

higher anxiety towards different aspects of a dental hygiene treatment. [57] Their results also showed higher anxiety reported in women. Another review by Loggia et al. demonstrated evidence that psychological factors influence pain perception. [58] This is revealed by changes in activity in pain pathways with changes in attentional state, stress, and positive and negative emotions via neuroimaging techniques. Furthermore, Van Wijk et al. demonstrated that anxious patients report more perceived pain than non-anxious patients while receiving a local anesthesia injection, and Klageset al. 16 showed that patients with high anxiety report and anticipate more pain when exposed to a critical situation. [59,60] Our study outcomes reconfirm the results of previous literature in which higher anxiety scores were reported in patients who had pain.

Anxiety, stress, and salivary cortisol

Previous studies by Shah et al.and Hill et al. have demonstrated a positive correlation between stress, anxiety, and salivary cortisol levels. [61] A study by Krueger et al. showed that patients who have higher anxiety showed significantly higher salivary cortisol levels in an educational session compared to those who had a low dental anxiety score. In addition, a study by Koray et al. found a positive association between state/trait anxiety scores and salivary cortisol in patients with oral lichen planus. [62] Furthermore, in a study by Miller et al. it was demonstrated that salivary cortisol levels in dental treatment are highest in patients undergoing tooth extraction compared to other procedures such as prophylaxis, restorative, and examination. [63] On the contrary to all prior literature mentioned, Brand et al, 1999 attempted to correlate between DAS scale score and salivary cortisol; however, no statistically significant correlation was observed. [64,65] In comparison to all the studies mentioned, our study results did show significant correlation between the DAS score and salivary cortisol levels.Our institution is passionate about high quality evidence based research and has excelled in various fields. We hope this study adds to this rich legacy.

Conclusion

Based on the results of our study, we can conclude that presence of pain and any history of traumatic dental experience are associated with patients' dental anxiety level. As far as the type of traumatic dental experience, a painful local anesthesia injection was found to be associated with the anxiety experienced by patients compared to other types of traumatic experiences. Dental anxiety was found to be associated with an increase in salivary cortisol or sAA levels, and there were no differences between gender and age. Future research should aim at comparing baseline levels of these biomarkers with any changes due to different types of dental treatment, such as cleanings, extractions, and fillings, and correlate their levels to dental anxiety. In addition, the effects of volume and flow rate on the level of sAA will need to be looked into further in future research to determine whether the volume collected may be a potential confounder for alpha amylase.

Clinical Significance

Dentists need to be trained in anxiety management and communication techniques, this consideration will help in development of specialist postgraduates' courses for dentists in management of dental anxiety. Such initiatives allow interested dentists to gain more confidence, more experience and skills in this specialized field of dentistry.

REFERENCES

1. Agdal, Maren L, Magne R, Erik S,Gerd K. Oral health and its influence on cognitive behavioral therapy in patients fulfilling the diagnostic and statistical manual of mental disorders-iv criteria for intra-oral injection phobia. ActaOdontol Scand. 2010;68:98-105
2. Azeem, Rubeena A,Nivedhitha M. Clinical performance of direct versus indirect composite restorations in posterior teeth: A systematic review. J Conserv Dent. 2018;21:2-9.
3. Brand HS. Anxiety and cortisol excretion correlate prior to dental treatment. Int Dent J. 1999;49:330-6.
4. Chen F, Ying T, Yujia S, Vishnu PV, Surapaneni KM, Chuanxin C. 6-Shogaol, a active constiuents of ginger prevents UVB radiation mediated inflammation and oxidative stress through modulating NrF2 signaling in human epidermal keratinocytes (HaCaT Cells). J PhotochemPhotobiol B. 2019;197:111518.
5. Corah NL. Development of a dental anxiety scale. J Dent Res. 1969;48(4):596.
6. Crofts NP, Brough E, Wilson KE, Beddis AJ, Girdler NM. Anxiety and quality of life in phobic dental patients. J Dent Res. 2010;89:302-6.
7. Dinesh SP, Kumaran S, Vijay R, Singaravelu D, Vinod A, Sanjay MR, Suchart S, Subrahmanya B. Influence of wood dust fillers on the mechanical, thermal, water absorption and biodegradation characteristics of jute fiber epoxy composites. J Pol Res. 2020; 27.
8. Eitner S, Wichmann M, Paulsen A, Holst S. Dental anxiety - an epidemiological study on its clinical correlation and effects on oral health. J Oral Rehabil. 2006;33:588-93.
9. Ezhilarasan D, Velluru S, Nandhigam V. SyzygiumCuminiextract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. J Oral Pathol Med. 2019;48:115-121.
10. Girija S, Esaki S, Marie L. Could SARS-CoV-2-Induced hyperinflammation magnify the severity of Coronavirus Disease (CoViD-19) leading to acute respiratory distress syndrome? Front immunol. 2020; 27:11-1206
11. Govindaraju L, Prasanna N, James L. Effect of root canal irrigating solutions on the compressive strength of tricalcium silicate cements. Clin Oral Investig. 2017; 21:567-571.
12. Guzeldemir E, Hilal T, Ulkem C. Pain perception and anxiety during scaling in periodontally healthy subjects. J Periodontol. 2008;79:2247-55.
13. Hakeberg M, Luciette C. Dental anxiety and pain related to dental hygienist treatment. ActaOdontol Scand. 2008;66:374-9.
14. Hill C, Walker R. Salivary cortisol determinations and self-rating scales in the assessment of stress in patients undergoing the extraction of wisdom teeth. Br Dent J. 2001;10:513-5.
15. Janani K, Raghu S. A survey on skills for cone beam computed tomography interpretation among endodontists for endodontic treatment procedure. Indian J Dent Res. 2019;30:834-838.
16. Jenarthanan S, Chandana S. Comparative evaluation of the efficacy of diclofenac sodium administered using different delivery routes in the management of endodontic pain: A randomized controlled clinical trial. Journal of Conservative Dentistry: JCD; 2018;21:297–301.
17. Khandelwal A, Ajitha P. Correlation between dental caries and salivary albumin in adult population in chennai: An in vivo study. Braz Dent Sci. 2019; 22:228–33.
18. Klages U, Simin K, Ozlem U, Heinrich W. Anxiety sensitivity as predictor of pain in patients undergoing restorative dental procedures. Community Dent Oral Epidemiol. 2006;34:139-45.

19. Koray M, Dülger O, Horasanli S, Ucok A, Tanyeri H, Badur S. The evaluation of anxiety and salivary cortisol levels in patients with oral lichen planus. Oral Dis. 2003;9:298-301.
20. Krueger T, Heinz H, Berthold PH, Philip H, Mike S, Manfred S. The dental anxiety scale and effects of dental fear on salivary cortisol. perceptual and motor skills. Percept Mot Skills. 2005;100:109-17.
21. Lin, Kevin C. Behavior-Associated self-report items in patient charts as predictors of dental appointment avoidance. J Dent Educ. 2009;73:218-24.
22. Locker D, Shapiro D, Liddell A. Who is dentally anxious? Concordance between measures of dental anxiety. Community dentistry and oral epidemiology. Community Dent Oral Epidemiol. 1996;24:346-50.
23. Loggia, Marco L, Petra S, Chantal V, Catherine B. Effects of psychological state on pain perception in the dental environment. J Can Dent Assoc. 2008;74:651-6.
24. Malli S, Nivedhitha, Kathiravan S, Jayanth KV, Mahalakshmi N, Deepak S. Concentrated growth factors as an ingenious biomaterial in regeneration of bony defects after periapical surgery: A report of two cases. Case Rep Dent. 2019;22:7046203.
25. Manickam A, Ezhilmaran D, Gunasekaran M, Malarvizhi KP, Varatharajan R, Ching-HH, Raja K. Score level based latent fingerprint enhancement and matching using SIFT feature. Multi Tools App. 2019;78:3065–85.
26. Manohar MP, Subash S. A survey of the knowledge, attitude, and awareness about the principal choice of intracanal medicaments among the general dental practitioners and nonendodontic specialists. Indian J Dent Res. 2018;29:716-720.
27. Mathew, Mebin G, Samuel SR, Ashu JS, Korishettar BR. Evaluation of adhesion of streptococcus mutans, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: Randomized controlled trial. Clin Oral Investig. 2020;24:3275-3280.
28. Ma, Yunhai, Thiruventhan K, Vishnu PV, Surapaneni KM, Shuling L. Sesame inhibits cell proliferation and induces apoptosis through inhibition of STAT-3 translocation in thyroid cancer cell lines (FTC-133). BBE. 2019;24:646–52.
29. Miller, Craig S, Jeffrey B, Donald A, Alan LK. Salivary cortisol response to dental treatment of varying stress. Oral Surg Oral Med Oral Pathol Oral RadiolEndod. 1995;79:436-41.
30. Murugan, Arul M, Jayaseelan V, Jayabalakrishnan D, Maridurai T, Selva K, Ramesh G, Arun P. Low velocity impact and mechanical behaviour of shot blasted sic wire-mesh and silane-treated aloevera/hemp/flax-reinforced sic whisker modified epoxy resin composites. Sil Chem. 2020;12:1847–56.
31. Nandakumar, Mahalakshmi, Iffat N. Comparative evaluation of grape seed and cranberry extracts in preventing enamel erosion: An optical emission spectrometric analysis. J Conserv Dent. 2018;21:516–520.
32. Nater, UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. Psychoneuroendocrinology. 2009;34:486-96.
33. Nater, Urs M, Nicolas R, Jens G, Simona B, Andreas J, Clemens K, Ulrike E. Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. Int J Psychophysiol. 2005;55:333-42.
34. Nater, Urs M., Nicolas R, Wolff S, Ulrike E, Clemens K. Determinants of the diurnal course of salivary alpha-amylase. Psychoneuroendocrinology. 2007;32:392-401.
35. Ng, Sam KS, Keung LW. A community study on the relationship of dental anxiety with oral health status and oral health-related quality of life. Community Dent Oral Epidemiol. 2008;36:347-56.
36. Oosterink, Floor MD, Ad J, Johan H. Prevalence of dental fear and phobia relative to other fear and phobia subtypes. Eur J Oral Sci. 2009;117:135-43.
37. Oosterink, Floor MD, Ad J, Johan H, Irene HA. The level of exposure-dental experiences questionnaire (LOE-DEQ): A measure of severity of exposure to distressing dental events. Eur J Oral Sci. 2008;116:353-61.
38. Paramasivam, Arumugam, Jayaseelan VP, Subramanian R. N6-Adenosine Methylation (m6A): A promising new molecular target in hypertension and cardiovascular diseases. Hypertens Res. 2020;43:153-154.
39. Pc, Marimuthu JT, Devadoss P. Prevalence and measurement of anterior loop of the mandibular canal using CBCT: A cross sectional study. Clin Implant Dent Relat Res. 2018;20:531-534.
40. Pohjola, Vesa, Satu L, Miira MV, Mimmi T, Hannu H. Age-Specific associations between dental fear and dental condition among adults in finland. ActaOdontol Scand. 2008;66:278-85.
41. Ponnanikajamideen, Mohamedibrahim, Shanmugam R, Mahendran V, Gurusamy A. In Vivo Type 2 diabetes and wound-healing effects of antioxidant gold nanoparticles synthesized using the insulin plant chamaecostuscuspidatus in albino rats. Can J Diabetes. 2019;43:82-89.e6.
42. Ponnulakshmi R, Shyamaladevi B, Vijayalakshmi P, Selvaraj J. In silico and in vivoanalysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. ToxicolMech Methods. 2019;29:276-290.
43. Poorni, Saravanan, Manali RS, Malli SN. Probiotic strains in caries prevention: A systematic review. J Conserv Dent. 2019;22:123-128.
44. Rajakeerthi R, Nivedhitha MS. Natural product as the storage medium for an avulsed tooth – A systematic review. Cumhuriyet Dental Journal. 2019; 22:249–56.
45. Ramadurai, Neeraja, Deepa G, A. Victor S, Emg S, Steven JL. Effectiveness of 2% articaine as an anesthetic agent in children: Randomized controlled trial. Clin Oral Investig. 2019;23:3543-3550.
46. Ramarao, Sathyanarayanan, Usha S. CRA Grid - A preliminary development and calibration of a paper-based objectivization of caries risk Assessment in Undergraduate Dental Education. J Conserv Dent. 2019;22:185-190.
47. Ramesh, Asha, Sheeja V, Nadathur DJ, Sankari M. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. J Periodontol. 2018;89:1241-1248.
48. Rhudy, Jamie L, Mary W. Fear and anxiety: Divergent effects on human pain thresholds. Pain. 2000;84:65-75.
49. Shah, Bina, L. Ashok, Sujatha GP. Evaluation of salivary cortisol and psychological factors in patients with oral lichen planus. Indian Journal of Dental Research. Indian J Dent Res. 2009;20:288-92.
50. Siddique, Riluwan, Malli SN. Effectiveness of rotary and reciprocating systems on microbial reduction: a systematic review. J Conserv Dent. 2019;22:114-122.
51. Siddique, Riluwan, Malli SN, Benoy J. Quantitative analysis for detection of toxic elements in various irrigants, their combination (precipitate), and para-chloroaniline: An inductively coupled plasma mass spectrometry study. J Conserv Dent. 2019;22:344-350.
52. Siddique, Riluwan, Nivedhitha MS, Jayalakshmi S, Benoy J, Deepak S. Qualitative and quantitative analysis of precipitate formation following interaction of chlorhexidine with sodium hypochlorite. J Conserv Dent. 2019;22:40-47.
53. Sridharan, Gokul, Pratibha R, Sangeeta P, Rajagopalan V. Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma. J Oral Pathol Med. 2019;48:299-306.
54. Subramaniam, Nandhini, Arvind M. Oral mucositis and microbial colonization in oral cancer patients undergoing radiotherapy and chemotherapy: A prospective analysis in a tertiary care dental hospital. J InvestigClin Dent. 2019;10:e12454.
55. Takai, Noriyasu, Masaki Y, Toshiaki A, Kenji E, Kenji U, Yasuo N. Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults. Arch Oral Biol. 2004;49:963-8.
56. Teja, Kavalipurapu V, Sindhu R, Vishnu P. Regulation of matrix metalloproteinase-3 gene expression in inflammation: A molecular study. J Conserv Dent. 2018;21:592-596.
57. Thanikodi, S, Dinesh S, Chandramohan D, Vijayan V, Venkatesh R. Teaching learning optimization and neural network for the effective prediction of heat transfer rates in tube heat exchangers. Thermal Science. 2020; 24:575–81.
58. Thomson WM, Broadbent JM, Locker D, Poulton R. Trajectories of dental anxiety in a birth cohort. Community Dent Oral Epidemiol. 2009;37:209-19.
59. Vadivel, Jayanth K, Meera G, Elangovan S, Arvind M. Mast cell expression in oral lichen planus: A systematic review. J InvestigClin Dent. 2019;10:e12457.
60. Vairavel, Mathivadani, EzhilarasanDevaraj, Rajeshkumar S. An eco-friendly synthesis of enterococcus sp.-mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells. Environ SciPollut Res Int. 2020;27:8166-8175.
61. Vermaire, JH, Ad J, Irene HA Aartman. Dental Anxiety and Quality of Life: The Effect of Dental Treatment. Community Dent Oral Epidemiol. 2008;36:409-16.

62. VijayashreePriyadharsini, Jayaseelan. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. J Periodontol. 2019;90:1441-1448.

63. Wijk, Van AJ, Hoogstraten J. Experience with dental pain and fear of dental pain. J Dent Res. 2005;84:947-50.

64. Wijk, Van AJ, Makkes PC. Highly anxious dental patients report more pain during dental injections. Br Dent J. 2008; 9;205:E7.

65. Wu, Fenglian, Jun Z, Guoliang L, Jiaxin W, Vishnu PV, Surapaneni KM, Qingfu Z. Biologically Synthesized Green Gold Nanoparticles from Induce Growth-Inhibitory Effect on Melanoma Cells (B16). Artif Cells NanomedBiotechnol. 2019;47:3297-3305.