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Quantification of Caspase 3 Levels in Patients with Periodontitis with or without Diabetes Mellitus

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Authors' contributions

This work was carried out in collaboration among all authors. Author SMAH. designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft manuscript. Authors MJ and SJ managed the analyses of the study and NDJ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Diabetes mellitus is a debilitating systemic disease with several major complications affecting the quality and length of life. Periodontal disease has been considered another diabetic complication in addition to cardiovascular disease, nephropathy, neuropathy, peripheral vascular disease. Caspase-3 plays an important role in intracellular signaling pathways that regulate apoptosis. High levels of glucose could induce human periodontal ligament fibroblast apoptosis.
Aim: The aim of the study is to compare caspase 3 levels in periodontitis patients with or without diabetes mellitus.
Materials and Methods: 30 patients were included in the study and they are divided into 3 groups: Group a- Periodontal health; Group b- Periodontitis with diabetes mellitus and Group c- Periodontitis patients without diabetes mellitus. Whole unstimulated saliva was collected from 30 patients using expectoration into sterile bulbs. Caspase 3 levels in saliva samples were measured in duplicate using a commercially available Human Caspase-3 (CASP3) enzyme linked

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immunosorbent assay (ELISA) Kit. Results were analyzed statistically by a one-way analysis of variance (ANOVA) and significant differences between the mean values were measured using Newman-Keuls multiple comparison test using Graph Pad Prism version 5. The results with the $p < 0.05$ level were considered to be statistically significant.

Results: From this study, it was observed that there was a significant increase of caspase 3 levels in periodontitis patients with diabetes mellitus (86.29±24.25 pmol/L) when compared to periodontitis patients without diabetes (55.06±12.90 pmol/L). The results showed a positive correlation and high level of significance when compared between periodontally healthy patients and periodontitis patients along with diabetes mellitus (86.29±24.25 pmol/L). Also, relatively significant results were observed in comparison between periodontally healthy patients (43.37±15.35 pmol/L) and patients with periodontitis without diabetes mellitus (55.06±12.90 pmol/L). Please add the most important statistical values.

Conclusion: The present study showed that caspase-3 concentrations in saliva increases in patients with periodontitis complicated along with diabetes mellitus. Moreover, saliva concentrations of caspase-3 increase with periodontal disease and caspase-3 plays a role as a biomarker of periodontal disease and its progression.

Keywords: Periodontitis; caspase- 3; diabetes mellitus; innovative technology.

1. INTRODUCTION

Diabetes mellitus is a debilitating systemic disease with several major complications affecting the quality and length of life. Periodontal disease has been considered another diabetic complication in addition to cardiovascular disease, nephropathy, neuropathy, peripheral vascular disease [1–3]. High glucose or hyperglycemia can trigger apoptosis in many tissues and cells [4–6].

Periodontitis, a common infectious disease characterized by inflammation and destruction of periodontal tissue and the major cause of tooth loss in adults, is considered one of the main complications of diabetes mellitus [7]. Apoptosis is an important biological process which is involved in regulating many physiological and pathologic pathways [8,9]. It is a highly regulated form of programmed cell death, defined by distinct morphological and biochemical features and plays a pivotal role in tissue homeostasis in multicellular organisms. Its perturbation has been associated with several disorders, which include cancer, rheumatoid arthritis, and periodontal diseases [10–13]. Various stimuli like hormones, cytokines, and growth factors can modulate the apoptotic process. Hyperglycemia and the accompanying production of excess amounts of advanced glycation end products (AGEs), contributes to reactive oxygen species (ROS) generation leading to oxidative stress and eventually cell death or apoptosis.

Recent literature demonstrates that apoptosis is essentially mediated by a family of cysteine proteases, called caspases, which can be divided into initiator and effector caspases [14]. Initiator caspases, such as caspase-8 or -9, activate downstream effector caspases, such as caspase 3, 6, or 7, which cleave various cellular substrates [15].Caspases play an important role in modulating apoptosis, necrosis, and inflammation [16–18]. Caspase activation can lead to initiation of irreversible protein degradation [18]. Caspase-3 plays an important role in intracellular signaling pathways that regulate apoptosis [13,19]. Caspase-3, a member of the *CED-3* subfamily of caspases, initially exists as a 32 kDa inactive proenzyme known as procaspase-3.Caspase-3 modulates either partial or total proteolytic cleavage of many important key proteins, such as nuclear enzyme poly ADP ribose polymerase, which are cleaved during apoptosis. Increased expression of Caspase-3 in cell lines of lymphocytic origin suggests that it is an important mediator of apoptosis in the immune system [20].

A study by Liu et al, hypothesized that high levels of glucose could induce human periodontal ligament fibroblast apoptosis by quantitatively detecting the extent of apoptosis by flow cytometry to determine how the duration of high glucose levels affected human periodontal ligament fibroblasts apoptosis and investigating the role of the caspase-3/PARP apoptotic signaling pathway on human periodontal ligament fibroblasts apoptosis. They concluded that increased glucose levels and human periodontal ligament fibroblasts apoptosis were directly proportional to time and that caspase-

3/PARP apoptotic signaling pathway played an important role in this process [21].

Despite recent progress in scientific research, a great deal is still unknown about apoptosis [22,23]. Our team has extensive knowledge and research experience that has translated into high quality publications [24–43]. Therefore, elucidating the mechanism of apoptosis in response to high glucose is essential in order to better understand the etiopathogenesis and pathophysiology of high glucose induced periodontitis and to develop novel medical treatments against this debilitating condition. to establish a significant relationship between increased glucose levels and periodontal fibroblast apoptosis. Hence the aim of the study is to compare the caspase 3 levels in periodontitis patients with or without diabetes mellitus. Please ameliorate.

2. MATERIALS AND METHODS

2.1 Patient Population and Study Design

Patients aged 30 to 60 years, visiting the Department of Periodontics, Saveetha dental college and hospitals, Chennai, India from December 2020 to February 2021 were examined. 30 patients were included in the study and divided into three groups, 10 in each group: Periodontally healthy patients (Group a), patients with periodontitis and diabetes mellitus (Group b) and patients with periodontitis only (Group c).

The enrollment criteria for the periodontitis cases are as follows: Not more than two teeth missing in each quadrant; more than or equal to 30% periodontal sites with probing depth more than or equal to 4 mm; More or equal to 20% of periodontology sites periodontal sites with interproximal clinical attachment loss more than equal to 2mm; More than or equal to 30% of sites showing bleeding on probing and radiographic evidence of bone loss visible in posterior vertical bitewing films. 24 individuals with clinically healthy periodontium of similar age, race, ethnicity and sex, who had less than 10% sites with bleeding on probing, no sites with probing depth more than or equal to 4mm, no clinical attachment loss of more than 2 mm and no radiographic evidence of bone loss visible in posterior bite wing. Exclusion criteria included individuals who had undergone periodontal treatment in the last 6 months, smoking or use of any form of tobacco, history of alcoholism and any acute periodontal conditions.

2.2 Saliva Collection

Participants were instructed to refrain from eating, drinking and practicing oral hygiene procedures 12 hours before saliva collection, Whole unstimulated saliva was collected from all patients using expectoration into sterile containers and the samples were immediately transported to the laboratory, where they were centrifuged at 5,000 rpm for 10 minutes and the clear supernatants were stored in aliquots at -70°C. The samples were thawed and the assay was performed.

2.3 Caspase 3 Analysis in Saliva

Caspase 3 levels in saliva samples were measured in duplicate using a commercially available Human Caspase-3 (CASP3) enzyme linked immunosorbent assay (ELISA) Kit procured from Abbkine Scientific Co., Ltd, China as per the manufacturer protocols. This assay is used to quantitatively analyse using sandwich enzyme immunoassay technique. The samples were diluted with calibrator diluent provided with a ratio of 1:4 and the assay was performed according to the instructions. Standards were included and all results were read as the value of optical density set to 450 nm. The intra and inter assay coefficient variance (CV) was found to be $<$11% and <9%.

2.4 Statistical Analysis

The triplicate analysis results of the experiments performed on control and treated rats were expressed as mean ± standard deviation. Results were analyzed statistically by a one-way analysis of variance (ANOVA) and significant differences between the mean values were measured using Newman-Keuls multiple comparison test using Graph Pad Prism version 5. The results with the $p < 0.05$ level were considered to be statistically significant. Please improve.

3. RESULTS AND DISCUSSION

Apoptosis is a tightly regulated cellular suicidal program that plays a central role in the homeostasis of multicellular organisms by eliminating cells with defects during normal metabolism. In addition, apoptosis is also

considered to be essential for the health of periodontal ligament cells and tissues [23]. Diabetes mellitus is a very common systemic disease, and periodontitis is considered to be one of the main oral complication associated with it [24,25]. Therefore, it is important to evaluate how increased glucose levels could lead to human periodontal ligament fibroblasts apoptosis and cell death.Only a few studies have incorporated the caspase 3 using salivary samples. An inflammatory exudate derived from the periodontal tissues called gingival crevicular fluid, is composed of serum and locally generated materials such as tissue breakdown products, inflammatory mediators, and antibodies directed against dental plaque bacteria [44]. When compared to collection of saliva, collection of GCF is found to be more difficult.

The amount of caspase-3 was measured by the ELISA method. On comparing the three groups, the p value was found to be p=0.001 which was statistically significant. The significance was considered at the levels of $p<0.05$. The present study describes the association of caspase 3 level in periodontitis patients with and without diabetes mellitus and comparing the levels with periodontally healthy patients. The test done with ELISA showed that the caspase 3 level was increased in periodontitis patients who were also affected with diabetes mellitus. From this study, it was observed that there was a significant increase of caspase 3 levels in periodontitis patients with diabetes mellitus (86.29±24.25 pmol/L) when compared to periodontitis patients without diabetes (55.06±12.90 pmol/L). The results showed a positive correlation and high level of significance when compared between periodontally healthy patients and periodontitis patients along with diabetes mellitus (86.29±24.25 pmol/L). Also, relatively significant results were observed in comparison between periodontally healthy patients (43.37±15.35 pmol/L) and patients with periodontitis without diabetes mellitus (55.06±12.90 pmol/L). The results obtained were statistically significant with a p value of $p<0.0001$ ($p<0.05$ level of significance) (Fig. 1 and Table 1).

Previous literature studies have emphasized the association of diabetes mellitus in relation to periodontitis [45]. Results obtained in this study are in concordance with previous data reported by Pradeep et al., who reported that GCF concentration of caspase-3 proportionally increases with the progression of periodontal disease [46]. A study by Malak et al, demonstrated the increase in caspase 3 level in GCF samples collected from periodontitis patients with diabetes mellitus [47]. Results from a study by Liu et al, show that high glucose could induce human periodontal ligament fibroblasts apoptosis in a time dependent manner and caspase-3 apoptotic signaling pathway plays an important role in this process [21].

This high level of caspase 3 in poorly controlled patient could be attributed to accumulation of advanced glycation end products (AGE) as shown by Takeda et al, who reported that increased AGE in the gingival crevicular fluid from diabetic patients compared with non-diabetic controls are significantly associated with deterioration of periodontitis [48]. Thus it can be assumed that worsening of glycemic control may lead to more accumulation of AGE and hyper responsive monocyte and this results in the increased release of cytokines, hence the increase in the level of caspase 3 concentration. This provides a plausible explanation for the increased incidence and severity of periodontal destruction in patients with diabetes mellitus [49].

Hence, this study has ensured the strong association of caspase 3 in periodontitis patients and its impact with diabetes mellitus using salivary sample collection. Thus, caspase 3 activity can be used as a novel biomarker to predict periodontitis in diabetic patients, and can be used to diagnose and elicit a comprehensive periodontal therapy with reduction of blood glucose levels.

The present study also has certain limitations, as the study population was restricted within the geographical limit and can be established in large scale population. Due to the use of restrictive inclusion and exclusion criteria, in an attempt to minimize the occurrence of confounding factors, the small sample size is one limitation of this study. Further multicenter, longitudinal, prospective studies with larger sample sizes are required for the validation of the results of the present study.

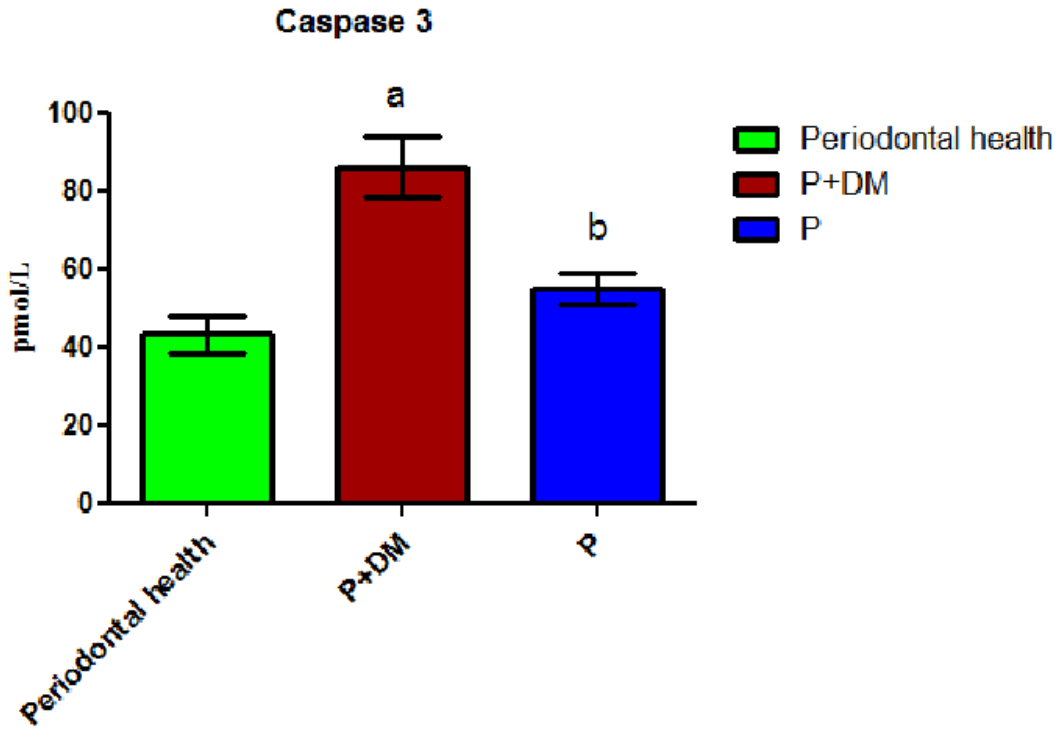

Fig. 1. Assessment of salivary caspase-3 concentration among periodontal health, periodontitis (P) and periodontitis with diabetes mellitus (P+DM). The levels of salivary caspase-3 were assessed by the Enzyme Linked Immunosorbent Assay (ELISA) method. Significance at p <0.05, a- compared with the periodontal health group. b-compared with periodontitis with diabetes mellitus

Table 1. Comparison of salivary caspase-3 levels among 3 groups, periodontal health, periodontitis (P) and periodontitis with diabetes mellitus (P+DM). The values are expressed in pmol/L. The levels of salivary caspase-3 were assessed by the Enzyme Linked Immunosorbent Assay (ELISA) method. Significance at p <0.05. Please improve

Groups	Periodontal health	P+DM	P	P value
Caspase-3 (pmol/L)	43.37±15.35	86.29±24.25	55.06±12.90	P<0.0001

4. CONCLUSION

The present study showed that caspase-3 concentrations in saliva are higher in patients with periodontitis along with diabetes mellitus when compared with periodontal disease only. Thus diabetes mellitus have an impact causing increased periodontal destruction. Therefore, caspase-3 plays a role as a biomarker of periodontal disease in diabetes mellitus and its progression.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

As per international standard or university standard guideline Patient's consent and ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Davis NL, Bursell JDH, Evans WD, Warner JT, Gregory JW. Body composition in children with type 1 diabetes in the first year after diagnosis: Relationship to glycaemic control and cardiovascular risk [Internet]. Archives of Disease in Childhood. 2012;97:312–5. Available: http://dx.doi.org/10.1136/archdischild-2011-300626
2. Lewandowski KC, Banach E, Bieńkiewicz M, Lewiński A. Matrix metalloproteinases in type 2 diabetes and non-diabetic controls: effects of short-term and chronic hyperglycaemia [Internet]. Archives of Medical Science. 2011;2:294–303. Available: http://dx.doi.org/10.5114/aoms.2011.22081
3. Tosca MA, Silvestri M, Olcese R, D'Annunzio G, Pistorio A, Lorini R, et al. Allergic sensitization and symptoms, body mass index, and respiratory function in children with type 1 diabetes mellitus [Internet]. Annals of Allergy, Asthma & Immunology. 2012;108:128–9. Available: http://dx.doi.org/10.1016/j.anai.2011.12.002
4. Guan Q, Zhang Y, Yu C, Liu Y, Gao L, Zhao J. Hydrogen sulfide protects against high-glucose–induced apoptosis in endothelial cells [Internet]. Journal of Cardiovascular Pharmacology. 2012; 59:188–93. Available:http://dx.doi.org/10.1097/fjc.0b013e31823b4915
5. Trudeau K, Molina AJA, Roy S. High glucose induces mitochondrial morphology and metabolic changes in retinal pericytes. Invest Ophthalmol Vis Sci. 2011; 52(12):8657–64.
6. Sanchez-Niño MD, Sanz AB, Sanchez-Lopez E, Ruiz-Ortega M, Benito-Martin A, Saleem MA, et al. HSP27/HSPB1 as an adaptive podocyte antiapoptotic protein activated by high glucose and angiotensin II [Internet]. Laboratory Investigation. 2012;92:32–45. Available: http://dx.doi.org/10.1038/labinvest.2011.138
7. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: Summary of developments, clinical implications and future directions [Internet]. Periodontology 2000. 1997;14:216–48. Available: http://dx.doi.org/10.1111/j.1600-0757.1997.tb00199.x
8. Yemelyanov A, Bhalla P, Ugolkov A, Yang X, Budunova I. Abstract 3929: Differential targeting of androgen and glucocorticoid receptors induces ER stress and apoptosis in prostate cancer cells: A novel therapeutic modality [Internet]. Endocrinology; 2012. Available: http://dx.doi.org/10.1158/1538-7445.am2012-3929
9. Saint-Hubert MD, De Saint-Hubert M, Bauwens M, Verbruggen A, Mottaghy FM. Apoptosis Imaging to Monitor Cancer Therapy: The Road to Fast Treatment Evaluation? [Internet]. Current Pharmaceutical Biotechnology. 2012; 13:571–83. Available: http://dx.doi.org/10.2174/138920112799436320
10. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science. 1995;267(5203):1456–62.
11. Tonetti MS, Cortellini D, Lang NP. In situ detection of apoptosis at sites of chronic bacterially induced inflammation in human gingiva. Infect Immun. 1998;66(11):5190–5.
12. Jarnbring F, Somogyi E, Dalton J, Gustafsson A, Klinge B. Quantitative assessment of apoptotic and proliferative gingival keratinocytes in oral and sulcular epithelium in patients with gingivitis and periodontitis. J Clin Periodontol. 2002; 29(12):1065–71.
13. Bantel H, Beikler T, Flemmig TF, Schulze-Osthoff K. Caspase activation is involved in chronic periodontitis. FEBS Lett. 2005;579(25):5559–64.

14. Los M, Wesselborg S, Schulze-Osthoff K. The role of caspases in development, immunity, and apoptotic signal transduction: lessons from knockout mice. Immunity. 1999;10(6):629–39.
15. Fischer U, Jänicke RU, Schulze-Osthoff K. Many cuts to ruin: A comprehensive update of caspase substrates [Internet]. Cell Death & Differentiation. 2003;10:76–100. Available: http://dx.doi.org/10.1038/sj.cdd.4401160
16. Liu J, Liu J, Mao J, Yuan X, Lin Z, Li Y. Caspase-3-mediated cyclic stretch-induced myoblast apoptosis via a Fas/FasL-independent signaling pathway during myogenesis [Internet]. Journal of Cellular Biochemistry. 2009;107:834–44. Available:http://dx.doi.org/10.1002/jcb.22182
17. Kurita-Ochiai T, Fukushima K, Ochiai K. Butyric acid-induced apoptosis of murine thymocytes, splenic T cells, and human Jurkat T cells [Internet]. Infection and Immunity. 1997;65:35–41. Available:http://dx.doi.org/10.1128/iai.65.1.35-41.1997
18. Geatch DR, Harris JI, Heasman PA, Taylor JJ. In vitro studies of lymphocyte apoptosis induced by the periodontal pathogen Porphyromonas gingivalis. J Periodontal Res. 1999;34(2):70–8.
19. Peter ME, Heufelder AE, Hengartner MO. Advances in apoptosis research. Proc Natl Acad Sci U S A. 1997;94(24):12736–7.
20. Fernandes-Alnemri T, Litwack G, Alnemri ES. CPP32, a novel human apoptotic protein with homology to Caenorhabditis elegans cell death protein Ced-3 and mammalian interleukin-1 beta-converting enzyme. J Biol Chem. 1994;269(49):30761–4.
21. Liu J, Wu Y, Wang B, Yuan X, Fang B. High levels of glucose induced the caspase-3/PARP signaling pathway, leading to apoptosis in human periodontal ligament fibroblasts. Cell Biochem Biophys. 2013;66(2):229–37.
22. Meadows SA, Vega F, Kashishian A, Johnson D, Diehl V, Miller LL, et al. PI3Kδ inhibitor, GS-1101 (CAL-101), attenuates pathway signaling, induces apoptosis, and overcomes signals from the microenvironment in cellular models of Hodgkin lymphoma [Internet]. Blood. 2012; 119:1897–900. Available:http://dx.doi.org/10.1182/blood-2011-10-386763
23. Lau GJ, Godin N, Maachi H, Lo C-S, Wu S-J, Zhu J-X, et al. Bcl-2-modifying factor induces renal proximal tubular cell apoptosis in diabetic mice. Diabetes. 2012;61(2):474–84.
24. Ramesh A, Varghese S, Jayakumar ND, Malaiappan S. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. J Periodontol. 2018; 89(10):1241–8.
25. Paramasivam A, Priyadharsini JV, Raghunandhakumar S, Elumalai P. A novel COVID-19 and its effects on cardiovascular disease. Hypertens Res. 2020;43(7):729–30.
26. S G, T G, K V, Faleh A A, Sukumaran A, P N S. Development of 3D scaffolds using nanochitosan/silk-fibroin/hyaluronic acid biomaterials for tissue engineering applications. Int J Biol Macromol. 2018; 120(Pt A):876–85.
27. Del Fabbro M, Karanxha L, Panda S, Bucchi C, Nadathur Doraiswamy J, Sankari M, et al. Autologous platelet concentrates for treating periodontal infrabony defects. Cochrane Database Syst Rev. 2018;11:CD011423.
28. Paramasivam A, Vijayashree Priyadharsini J. MitomiRs: New emerging microRNAs in mitochondrial dysfunction and cardiovascular disease. Hypertens Res. 2020 Aug;43(8):851–3.
29. Jayaseelan VP, Arumugam P. Dissecting the theranostic potential of exosomes in autoimmune disorders. Cell Mol Immunol. 2019;16(12):935–6.
30. Vellappally S, Al Kheraif AA, Divakar DD, Basavarajappa S, Anil S, Fouad H. Tooth implant prosthesis using ultra low power and low cost crystalline carbon bio-tooth sensor with hybridized data acquisition algorithm. Comput Commun. 2019;148:176–84.
31. Vellappally S, Al Kheraif AA, Anil S, Assery MK, Kumar KA, Divakar DD. Analyzing relationship between patient and doctor in public dental health using particle memetic multivariable logistic regression analysis approach (MLRA2). J Med Syst. 2018;42(10):183.
32. Varghese SS, Ramesh A, Veeraiyan DN. Blended module-based teaching in

biostatistics and research methodology: A retrospective study with postgraduate dental students. J Dent Educ. 2019;83(4):445–50.

33. Venkatesan J, Singh SK, Anil S, Kim S-K, Shim MS. Preparation, characterization and biological applications of biosynthesized silver nanoparticles with chitosan-fucoidan coating. Molecules [Internet]. 2018;23(6). Available:http://dx.doi.org/10.3390/molecules23061429
34. Alsubait SA, Al Ajlan R, Mitwalli H, Aburaisi N, Mahmood A, Muthurangan M, et al. Cytotoxicity of different concentrations of three root canal sealers on human mesenchymal stem cells. Biomolecules [Internet]. 2018;8(3). Available:http://dx.doi.org/10.3390/biom8030068
35. Venkatesan J, Rekha PD, Anil S, Bhatnagar I, Sudha PN, Dechsakulwatana C, et al. Hydroxyapatite from cuttlefish bone: Isolation, characterizations, and applications. Biotechnol Bioprocess Eng. 2018;23(4):383–93.
36. Vellappally S, Al Kheraif AA, Anil S, Wahba AA. IoT medical tooth mounted sensor for monitoring teeth and food level using bacterial optimization along with adaptive deep learning neural network. Measurement. 2019;135:672–7.
37. PradeepKumar AR, Shemesh H, Nivedhitha MS, Hashir MMJ, Arockiam S, Uma Maheswari TN, et al. Diagnosis of vertical root fractures by cone-beam computed tomography in root-filled teeth with confirmation by direct visualization: A systematic review and meta-analysis. J Endod. 2021;47(8):1198–214.
38. R H, Ramani P, Tilakaratne WM, Sukumaran G, Ramasubramanian A, Krishnan RP. Critical appraisal of different triggering pathways for the pathobiology of pemphigus vulgaris-A review. Oral Dis [Internet]; 2021. Available:http://dx.doi.org/10.1111/odi.13937
39. Ezhilarasan D, Lakshmi T, Subha M, Deepak Nallasamy V, Raghunandhakumar S. The ambiguous role of sirtuins in head and neck squamous cell carcinoma. Oral Dis [Internet]; 2021. Available:http://dx.doi.org/10.1111/odi.13798
40. Sarode SC, Gondivkar S, Sarode GS, Gadbail A, Yuwanati M. Hybrid oral potentially malignant disorder: A neglected fact in oral submucous fibrosis. Oral Oncol. 2021;105390.
41. Kavarthapu A, Gurumoorthy K. Linking chronic periodontitis and oral cancer: A review. Oral Oncol. 2021;105375.
42. Vellappally S, Abdullah Al-Kheraif A, Anil S, Basavarajappa S, Hassanein AS. Maintaining patient oral health by using a xeno-genetic spiking neural network. J Ambient Intell Humaniz Comput [Internet]; 2018. Available:https://doi.org/10.1007/s12652-018-1166-8
43. Aldhuwayhi S, Mallineni SK, Sakhamuri S, Thakare AA, Mallineni S, Sajja R, et al. COVID-19 knowledge and perceptions among dental specialists: A cross-sectional online questionnaire survey. Risk Manag Healthc Policy. 2021;14:2851–61.
44. Pihlstrom BL. Periodontal risk assessment, diagnosis and treatment planning. Periodontol 2000. 2001;25:37–58.
45. Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, et al. Periodontitis and diabetes: A two-way relationship. Diabetologia. 2012;55(1):21–31.
46. Pradeep AR, Kathariya R, Raghavendra NM, Sharma A. Levels of pentraxin-3 in gingival crevicular fluid and plasma in periodontal health and disease. J Periodontol. 2011;82(5):734–41.
47. [No title] [Internet]. [cited 2021 May 24]. Available from: https://www.researchgate.net/profile/Malak_Shoukheba/publication/313066128_CASPASE-3_ACTIVITY_AS_A_NOVEL_BIOMARKER_IN_TYPE_2_DIABETES_MELLITUS_PATIENTS_WITH_CHRONIC_PERIODONTITIS/links/588faf1292851c9794c49c95/CASPASE-3-ACTIVITY-AS-A-NOVEL-BIOMARKER-IN-TYPE-2-DIABETES-MELLITUS-PATIENTS-WITH-CHRONIC-PERIODONTITIS.pdf
48. Takeda M, Ojima M, Yoshioka H, Inaba H, Kogo M, Shizukuishi S, et al. Relationship of serum advanced glycation end products with deterioration of periodontitis in type 2 diabetes patients. J Periodontol. 2006;77(1):15–20.

49. Engebretson SP, Hey-Hadavi J, Ehrhardt FJ, Hsu D, Celenti RS, Grbic JT, et al. Gingival crevicular fluid levels of interleukin-1β and glycemic control in patients with chronic periodontitis and type 2 diabetes [Internet]. Journal of Periodontology. 2004;75:1203–8. Available:http://dx.doi.org/10.1902/jop.2004.75.9.1203

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