

Comparison of Salivary pH and Clinical Periodontal Status in Smokers with and without Periodontitis

V.T.Thamarai Selvi

Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, Chennai - 600077. E-mail I'd: 151801086.sdc@saveetha.com

Dr. Sankari malaiappan*

Professor, Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, Chennai - 600077. Mobile: 98402 85905 E-mail I'd: sankari@saveetha.com

ABSTRACT:

BACKGROUND: Periodontitis can be defined as an inflammatory disease of the supporting structures of the teeth which is caused by a specific group of microorganisms. Smoking and tobacco product usage is one of the major risk factors for almost 80% of systemic diseases, also for the development of Periodontal disease. The Present study aimed to assess the Salivary pH and clinical periodontal status Periodontal Probing depth (PPD), Gingival Bleeding on Probing (BOP), Clinical Attachment Loss (CAL) among smokers with periodontitis and smokers without periodontitis.

MATERIALS AND METHODS: 50 smokers were chosen for the present study, they were divided into Smokers with periodontitis and smokers without periodontitis and divided equally. From all the subjects, 5ml of Saliva sample was collected which was used to assess salivary pH and the periodontal status was assessed clinically. The data were collected and SPSS software was used for statistical analysis.

RESULTS: The mean salivary pH in the present study of Group A and Group B were 6.56 ± 0.9 and 6.9 ± 0.4 respectively. Among the Smokers with Periodontitis, the Periodontal Probing Depth was 1.1 - 2, attributed to 25% of the Smokers whereas Smokers without Periodontitis had a Periodontal Probing Depth of 0 - 1.0 which was attributed to 19% of the Smokers. 26.5% of the Smokers with Periodontitis had Gingival Bleeding on Probing in the range between 0.1 - 1.0 and 24.5% of Smokers without Periodontitis had Gingival Bleeding on Probing in the range of 1.1 - 2.0, with a statistical insignificance $p > 0.05$. 18.3% of the Smokers with Periodontitis had Clinical Attachment Loss in the range between 1.1 - 2.0 whereas 51.02% of Smokers without Periodontitis had no Clinical Attachment Loss, p Value < 0.05 which is statistically significant.

CONCLUSION: in the present study smokers have low salivary pH which increases the risk of periodontitis. The oral mucosa may become susceptible to several oral and dental ailments as a consequence of this pH change brought on by the long-term effects of smoking. Further studies have to be conducted to evaluate the salivary pH among current smokers, passive smokers and patients quitted smoking.

KEYWORDS: Smokers, Litmus paper, Salivary pH, novel study, innovative technique, Clinical loss of attachment, bleeding on probing, Eco friendly.

INTRODUCTION:

Periodontitis can be defined as an inflammatory disease of the supporting structures of the tooth which is caused by a specific group of microbes that destroys periodontal ligament and alveolar bone. Periodontitis may further lead to pocket formation, gingival recession, or both. Periodontitis is one of the major causes of loss of tooth and also has a major contribution to systemic disease progression. The bacteria which are associated with chronic periodontitis include *Campylobacter* species, *Eubacterium nodatum*, *Fusobacterium nucleatum*, *Prevotella intermedia/nigrescens*, *Streptococcus* species, *Treponema denticola*, and *spirochetes* (1,2). Periodontal diseases are mostly caused due to plaque accumulation which eventually leads to the formation and accumulation of calculus. As periodontitis is a multifactorial disease, chronic periodontitis has a wide array of risk factors which can be divided into

risk factors that can be modified and Non-Modified. environmental or behavioral factors like Smoking, Poor control of Diabetes, Obesity, and Chronic inflammation are risk factors that can be modified, whereas Osteoporosis, Hematological disorders, Age, Genetic disorder, History of Periodontitis, Host response with IL-1 genotype are risk factors that cannot be modified (3,4)

Smoking or usage of Tobacco products is one of the major risk factors for almost 80% of systemic diseases like Myocardial infarction, Chronic Lung diseases, Renal failure, Malignancies, etc.,(5) One of the primary and most significant environmental risk factors for the onset of periodontal disease is smoking(6). Severity of periodontal destruction depends on the smoking habit depending on the host bacterial interaction in chronic periodontitis is altered resulting in extensive periodontal destruction. Some studies report that Smoking affects the immune response, fibroblast activity, etc (7).

Saliva is a sophisticated oral fluid that incorporates water, cells, organic and inorganic elements, and other substances. Saliva represents an individual's equilibrium. The salivary glands produce between 500 - 700 ml of saliva every day, of which 90% of the secretion is contributed by the 3 major salivary glands, and the remaining is secreted by the minor salivary glands (8). The actions of saliva include cleaning, antibacterial, buffering, pH maintenance, lubricating the mouth cavity, and protection from different microorganisms, among other things. To diagnose conditions such as cancer, heart disease, etc biomarkers are present in the secretion (9). The pH of saliva is usually 6.8 - 7.2, and the pH of saliva is altered in systemic conditions, Xerostomia, malignancy, etc, Buffering capacity of the saliva is associated with the salivary flow rate, when the salivary flow rate is decreased the buffering capacity will also be reduced resulting in reduced salivary pH, making the saliva acidic (10). The growth of the periodontal microbes facilitates decreased salivary pH. smokers have comparatively higher oral pH than non-smokers. Therefore, the decreased salivary pH can extract the calcium ions from the scales deposited on the tooth surfaces which might result in elevated levels of salivary calcium levels (11,12). The present study aimed to assess the Salivary pH and clinical periodontal status among smokers with periodontitis and smokers without periodontitis.

MATERIALS AND METHODS:

The present study was conducted on the premises of the university. The age group of the sample population in the present study was 20 - 70 years with smoking habits included in the present study. 50 subjects with Smoking habits were selected from the patients who visited Saveetha Dental College & Hospital from November 2020 to February 2021. Subjects with systemic ailments, those using medicines for systemic disorders, and individuals who consume alcohol are excluded from the study. The selected participants were grouped into 2 equally depending on the periodontal status as

GROUP - A: Smokers with Periodontitis

GROUP - B: Smokers without Periodontitis

The study was conducted between 8 a.m - 10 a.m to avoid diurnal variation in the pH of saliva. A total of 5 mL of saliva was collected in a sterile container from each subject and the salivary pH was checked with the help of a pH strip within 5 minutes of collecting the saliva from the subjects. The pH was estimated by comparing the color change in the pH strip against the standard and the pH value was noted.

In The clinical periodontal status, the parameters evaluated were Probing depth of the Periodontium (PD), Gingival Bleeding on Probing (BOP), Clinical Attachment Loss (CAL) was assessed using William's Probe clinically on all 6 surfaces of the tooth and the values were noted.

The collected data were tabulated and imported into SPSS software version 23 for further statistical analysis. Chi-square test and Pearson correlation analysis were used for the data analysis, with a p-value

RESULTS:

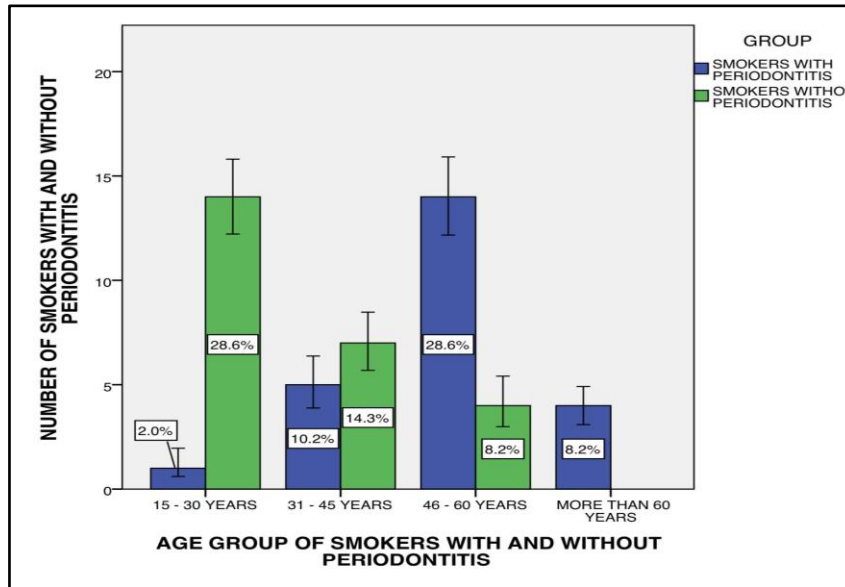


Figure 1 represents the association between the Number of Smokers with and without Periodontitis and the Age group of Smokers with and without Periodontitis. The X-axis represents the Age group of Smokers with and without Periodontitis, and Y-axis represents the Number of Smokers with and without Periodontitis. The blue color represents Smokers with Periodontitis and the Green color represents Smokers without Periodontitis. 29% of the Smokers with Periodontitis were in the Age Group of 46 - 60 years of age and 29% of Smokers without Periodontitis were in the Age Group of 20 - 30 years of age. However this is statistically not significant with chi-square value - 21.42 and p-value = 0.3 (p-value > 0.05) hence insignificant.

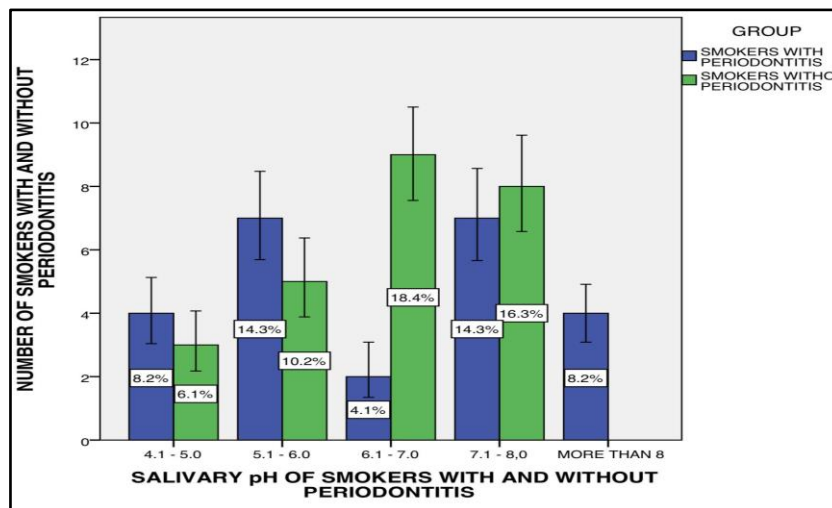


Figure 2 represents the association between Salivary pH among Smokers with and without Periodontitis and the Number of Smokers with and without Periodontitis. The X-axis represents the Salivary pH among Smokers with and without Periodontitis and Y-axis represents the Number of Smokers with and

without Periodontitis. The blue color represents Smokers with Periodontitis and Green color represents Smokers without Periodontitis. 14.2% of the Smokers with Periodontitis had salivary pH in the range between 5.1 - 6.0 and 7.1 - 8.0 and 18.3% of Smokers without Periodontitis had salivary pH in the range of 6.1 - 7.0, with a statistical significance of chi-square value - 8.98 and p-value = 0.03 (p-value < 0.05) hence significant.

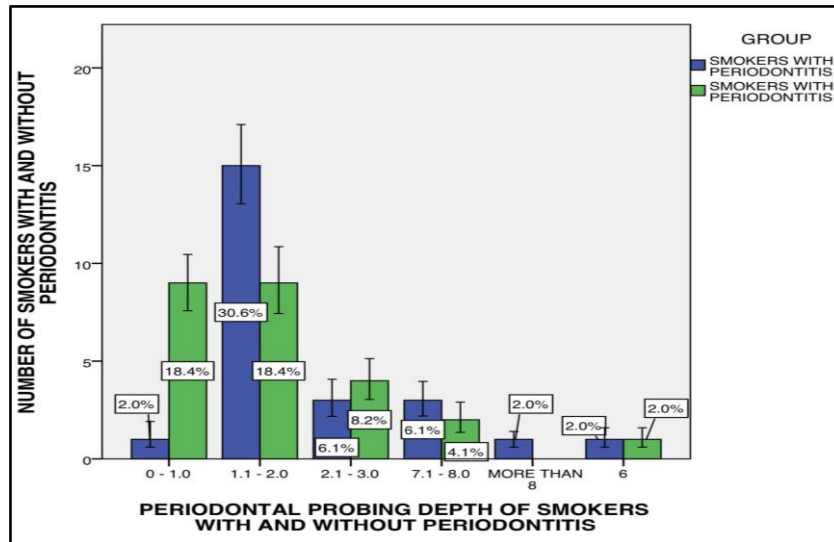


Figure 3 represents the association between Periodontal Probing Depth among Smokers with and without Periodontitis and the Number of Smokers with and without Periodontitis. The X-axis represents the Periodontal Probing Depth among Smokers with and without Periodontitis and Y-axis represents the Number of Smokers with and without Periodontitis. The blue colour represents Smokers with Periodontitis and Green colour represents Smokers without Periodontitis. Among the Smokers with Periodontitis, the Periodontal Probing Depth was 1.1 - 2, attributed to 25% of the Smokers whereas Smokers without Periodontitis had a Periodontal Probing Depth of 0 - 1.0 which was attributed to 19% of the Smokers, with a statistical significance of chi-square value - 9.23 and p-value = 0.1 (p-value > 0.05) hence insignificant.

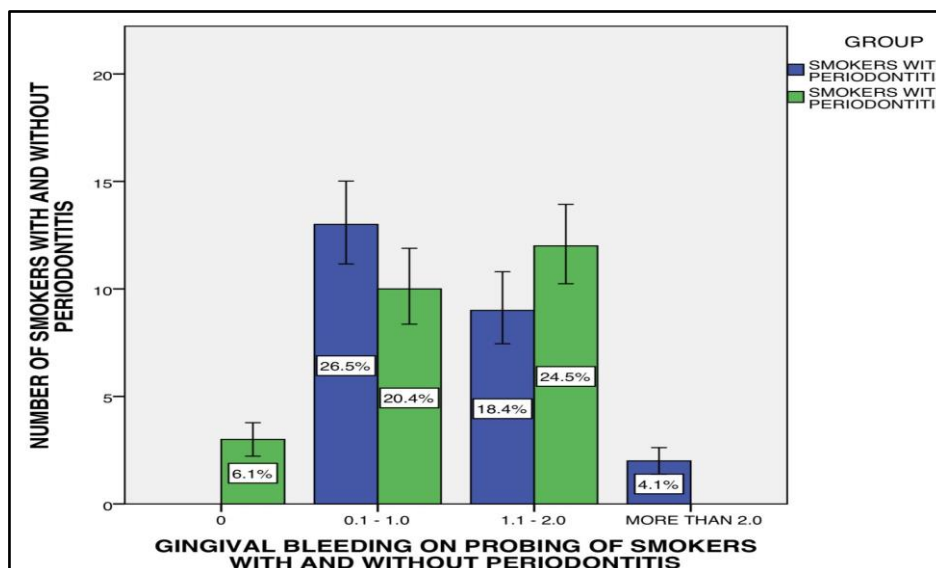


Figure 4 represents the association between Gingival Bleeding on Probing among Smokers with and without Periodontitis and the Number of Smokers with and without Periodontitis. The X-axis represents the Gingival Bleeding on Probing among Smokers with and without Periodontitis and Y-axis represents the Number of Smokers with and without Periodontitis. The blue colour represents Smokers with Periodontitis and Green colour represents Smokers without Periodontitis. 26.5% of the Smokers with Periodontitis had Gingival Bleeding on Probing in the range between 0.1 - 1.0 and 24.5% of Smokers without Periodontitis had Gingival Bleeding on Probing in the range of 1.1 - 2.0, with statistical insignificance of chi-square value - 5.9 and p-value = 0.12 (p-value > 0.05) hence insignificant.

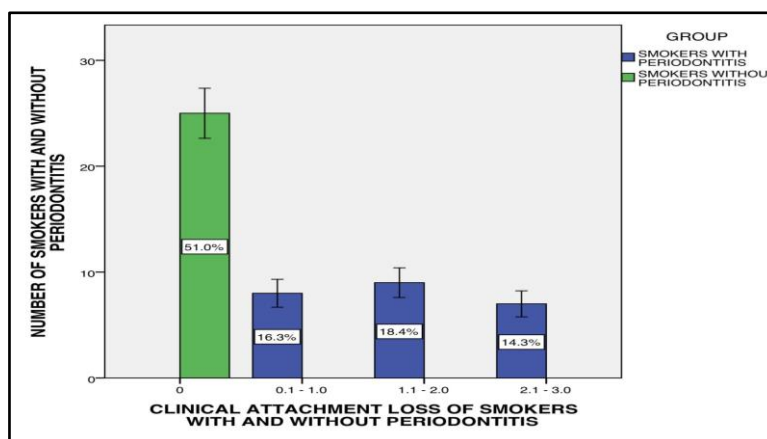


Figure 5, represents the association between Clinical Attachment Loss among Smokers with and without Periodontitis and the Number of Smokers with and without Periodontitis. The X-axis represents the Clinical Attachment Loss among Smokers with and without Periodontitis and Y-axis represents the Number of Smokers with and without Periodontitis. The blue colour represents Smokers with Periodontitis and Green colour represents Smokers without Periodontitis. 18.3% of the Smokers with Periodontitis had Clinical Attachment Loss in the range between 1.1 - 2.0 whereas 51.02% of Smokers without Periodontitis had no Clinical Attachment Loss, with a statistical significance of chi-square value - 49.2 and p-value = 0.04 (p-value < 0.05) hence significant.

DISCUSSION:

Around One-third of the world's adult population are smokers of which 60% of them are males and 43% are Females. It is predicted that in the future, the yearly death rate from tobacco use across the globe will be around 10 million a year (13). Smoking in developing countries is peaking by 3% a year. In the present study, when the Age Group and number of Smokers with and without Periodontitis are Correlated it is evident that 28.5% of the Smokers with Periodontitis were in the age group of 46-60 years of age, 28.5% of the Smokers without Periodontitis were in the age group of 15-30 years of age (Fig 1). We can assume periodontal diseases will also rise. Nevertheless, the molecular basis by which smoking worsens periodontitis is not entirely known. It is unclear if smoking has a local effect on the periodontium or if smoking has systemic effects that induce periodontal disease. (14).

Saliva is an oral fluid that has a major role in the homeostasis of the body, However, saliva is the initial physiological fluid to still be exposed to cigarette smoke, including several toxic compounds that cause structural and functional alterations in saliva, resulting in an imbalance in the oral cavity's homeostasis(15). 14.2% of the Smokers With Periodontitis had their Salivary pH in the range of 5.1-6.0, wherein 18.3% of Smokers Without Periodontitis had their Salivary pH of 6.1-7.0 (Fig 2). The

mean salivary pH in the present study of Group A and Group B were 6.56 ± 0.9 and 6.9 ± 0.4 , which is analogous to Groover et al., the salivary pH mean in smokers was 6.75 ± 0.11 and the salivary pH in tobacco chewers was 6.5 ± 0.29 (16). The salivary pH was lowered due to the loss of bicarbonate ions from the buffering system which makes the saliva acidic. Due to interactions with the buffering mechanisms of saliva, changes in electrolytes and ions modifies the salivary pH.(17). Also a study by Parmar *et al.* also observed smokers had a lower pH than non-smokers, which was in line with the findings of this study (18,19).

Depending on how long a person has smoked and how many cigarettes they smoke each day, periodontal disease can alter in severity. This association is also proved in previous studies⁽²⁰⁾. In a study by Wickholm et al., as the frequency of cigarette usage in pack years increased the severity of periodontal disease also increased (21,22).

Periodontal Probing Depth (PPD) is the distance from the soft tissue (Alveolar mucosa or Gingiva) margin to the tip of the periodontal probe during probing. It is the only technique that can accurately pinpoint periodontal pockets and their extent throughout each tooth surface. Among the Smokers with Periodontitis 25% of the Smokers had PPD the in range of 1.1 - 2, whereas Smokers without Periodontitis had PPD in the range of 0 - 1.0 in 19% of the Smokers (Fig 3). The mean Periodontal probing depth of Group A and Group B was 2.02 ± 1.05 and 1.7 ± 1.02 respectively. the results of the present study are coherent with the previous literature where the PPD was greater among the smokers compared to that of non-smokers. In a Stolenberg et al., the probing depth was 5 times greater in the proximal surfaces of the maxillary teeth compared to the other surfaces of the maxillary and mandibular teeth (23). According to Arowojolu et al., Post non surgical Periodontal therapy there was a slight reduction in the PPD and comparatively, in the anterosuperior region the PPD reduced significantly (24,25)

Gingival Bleeding on Probing is a marker for Periodontal disease initiation and progression. There will be a bleeding from the Gingival Sulcus during Gentle probing. 26.5% of the Smokers with Periodontitis had Gingival Bleeding on Probing in the range between 0.1 - 1.0 and 24.5% of Smokers without Periodontitis had Gingival Bleeding on Probing in the range of 1.1 - 2.0 (Fig 4). The mean Gingival Bleeding on Probing of Group A and Group B were 1.09 ± 0.6 and 0.8 ± 0.5 respectively. In concordance with this study, Previous studies conducted by Machua et al., also suggests that Smokers have lesser Gingival Bleeding on Probing than Non-Smokers (26). Contracting also Linden J et al., reported increased gingival Bleeding On Probing. The level of gingival Inflammation in smokers is comparatively lesser (27). The typically developed gingival inflammatory reaction linked to plaque provocation can be disrupted by tobacco products because they can affect the vascular inflammatory response. Smoking reduces gingival bleeding, however, the exact processes by which this occurs are yet unknown. Due to the effects of nicotine-stimulated adrenaline and noradrenaline on 1-adrenergic receptors, gingival vasoconstriction has traditionally been claimed the decrease bleeding in smokers (28).

Clinical Attachment Loss is the distance from the cemento-enamel Junction to the apical extent of the pocket. CAL is a measure of the severity of the disease in terms of loss of support from the Tooth. 18.3% of the Smokers with Periodontitis had Clinical Attachment Loss in the range between 1.1 - 2.0 whereas 51.02% of Smokers without Periodontitis had no Clinical Attachment Loss (Fig 5). The mean Clinical Attachment Loss of Group A and Group B are 1.5 ± 0.8 and 0. A study by Monten et al., In the Swedish adult population, discovered comparable results with the presence of periodontal disease and a much greater incidence of gingival recessions in wet snuff users vs non-users, which is concurrent with the present study(29). A study conducted by Varma et al., on the examination of periodontal effects

related to the length of smokeless tobacco use, discovered that smokers with a habit of more than seven years have increased gingival recession.⁽³⁰⁾.

CONCLUSION:

Based on the present study smokers have low salivary pH which increases the risk of periodontitis. The oral mucosa may become susceptible to several oral and dental ailments as a consequence of this pH change brought on by the long-term effects of smoking. Further studies have to be conducted to evaluate the salivary pH among current smokers, passive smokers and patients quitted smoking.

ACKNOWLEDGEMENT:

The authors would like to acknowledge the help and support rendered by the Department of Periodontics of Saveetha Dental College and Hospitals , Saveetha Institute of Medical and Technical Sciences, Saveetha university and the management for their constant assistance with the research.

CONFLICT OF INTEREST: The authors have none to declare

SOURCE OF FUNDING:

The study is funded by

- Saveetha Institute of Medical and Technical Sciences
- Saveetha dental college and Hospitals
- Saveetha University
- Thangavelu's Law Office (Reference Number : IJKT23RT21)

REFERENCES:

- [1] Varghese M. Quantitative Assessment of Calcium Profile in Whole Saliva From Smokers and Non-Smokers with Chronic Generalized Periodontitis [Internet]. JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH. 2015. Available from: <http://dx.doi.org/10.7860/jcdr/2015/12334.5948>
- [2] 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 [Internet]. Vol. 148, Computer Communications. 2019. p. 176–84. Available from: <http://dx.doi.org/10.1016/j.comcom.2019.09.020>
- [3] Nayyar A, Kanakdande V, Patil K. Comparative evaluation of clinical, hematological and systemic inflammatory markers in smokers and non-smokers with chronic periodontitis [Internet]. Vol. 6, Contemporary Clinical Dentistry. 2015. p. 348. Available from: <http://dx.doi.org/10.4103/0976-237x.161885>
- [4] Johnson GK, Guthmiller JM. The impact of cigarette smoking on periodontal disease and treatment [Internet]. Vol. 44, Periodontology 2000. 2007. p. 178–94. Available from: <http://dx.doi.org/10.1111/j.1600-0757.2007.00212.x>
- [5] 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 Aug 1;8(3). Available from: <http://dx.doi.org/10.3390/biom8030068>
- [6] Hyman JJ, Winn DM, Reid BC. The Role of Cigarette Smoking in the Association Between Periodontal Disease and Coronary Heart Disease [Internet]. Vol. 73, Journal of Periodontology.

2002. p. 988–94. Available from: <http://dx.doi.org/10.1902/jop.2002.73.9.988>
- [7] 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* [Internet]. 2018 Oct;89(10):1241–8. Available from: <http://dx.doi.org/10.1002/JPER.17-0445>
- [8] ThamaraiSelvi VT, Brundha MP. Salivaomics - A Review. *European Journal of Molecular & Clinical Medicine* [Internet]. 2020 Dec 1 [cited 2021 Apr 11];7(1):2914–31. Available from: https://ejmcm.com/article_3336.html
- [9] Agarwal R, Lakshmi T. Salivary Enzymes as Biomarkers for Periodontitis--An Update. *Research Journal of Pharmacy and Technology* [Internet]. 2014;7(1):98–100. Available from: <http://www.indianjournals.com/ijor.aspx?target=ijor:rjpt&volume=7&issue=1&article=020>
- [10] Petrušić N, Posavac M, Sabol I, Mravak-Stipetić M. The Effect of Tobacco Smoking on Salivation. *Acta Stomatol Croat* [Internet]. 2015 Dec;49(4):309–15. Available from: <http://dx.doi.org/10.15644/asc49/4/6>
- [11] Singh M, Yadav P, Ingle N, Ingle E, Kaur N. Effect of long-term smoking on salivary flow rate and salivary pH [Internet]. Vol. 13, *Journal of Indian Association of Public Health Dentistry*. 2015. p. 11. Available from: <http://dx.doi.org/10.4103/2319-5932.153549>
- [12] Rad M, Kakoie S, Niliye Brojeni F, Pourdanghan N. Effect of Long-term Smoking on Whole-mouth Salivary Flow Rate and Oral Health. *J Dent Res Dent Clin Dent Prospects* [Internet]. 2010 Dec 21;4(4):110–4. Available from: <http://dx.doi.org/10.5681/joddd.2010.028>
- [13] 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 Jun 21; Available from: <http://dx.doi.org/10.1111/odi.13937>
- [14] Haber J, Wattles J, Crowley M, Mandell R, Joshipura K, Kent RL. Evidence for cigarette smoking as a major risk factor for periodontitis. *J Periodontol* [Internet]. 1993 Jan;64(1):16–23. Available from: <http://dx.doi.org/10.1902/jop.1993.64.1.16>
- [15] Chakrabarty S, Patil S, Bandalore SH, Kempegowda R, Shivu, Pewa S. A comparative study of long-term effect of tobacco on resting whole mouth salivary flow rate and pH [Internet]. Vol. 27, *Journal of Indian Academy of Oral Medicine and Radiology*. 2015. p. 549. Available from: <http://dx.doi.org/10.4103/0972-1363.188759>
- [16] Grover N, Sharma J, Sengupta S, Singh S, Singh N, Kaur H. Long-term effect of tobacco on unstimulated salivary pH. *J Oral Maxillofac Pathol* [Internet]. 2016 Jan;20(1):16–9. Available from: <http://dx.doi.org/10.4103/0973-029X.180907>
- [17] Rooban T, Mishra G, Elizabeth J, Ranganathan K, Saraswathi TR. Effect of habitual arecanut chewing on resting whole mouth salivary flow rate and pH [Internet]. Vol. 60, *Indian Journal of Medical Sciences*. 2006. p. 95. Available from: <http://dx.doi.org/10.4103/0019-5359.22760>
- [18] Parmar P, Radha G, Rekha R, Pallavi S. Assessing Salivary Flow Rate, Salivary pH and Oral Candidiasis among Tobacco Chewers, Smokers and Healthy Controls- A Cross Sectional Study [Internet]. Vol. 7, *Asian Journal of Medicine and Health*. 2017. p. 1–8. Available from: <http://dx.doi.org/10.9734/ajmah/2017/36522>
- [19] Vellappally S, Al-Kheraif AA, Anil S, Basavarajappa S, Hassanein AS. Maintaining patient oral health by using a xeno-genetic spiking neural network [Internet]. *Journal of Ambient Intelligence and Humanized Computing*. 2018. Available from: <http://dx.doi.org/10.1007/s12652-018-1166-8>
- [20] Kamath KP, Mishra S, Anand PS. Smokeless Tobacco Use as a Risk Factor for Periodontal

- Disease [Internet]. Vol. 2, *Frontiers in Public Health*. 2014. Available from: <http://dx.doi.org/10.3389/fpubh.2014.00195>
- [21] Wickholm S, Söder P, Galanti MR, Söder B, Klinge B. Periodontal disease in a group of Swedish adult snuff and cigarette users [Internet]. Vol. 62, *Acta Odontologica Scandinavica*. 2004. p. 333–8. Available from: <http://dx.doi.org/10.1080/00016350410001801>
- [22] Kavarthapu A, Gurumoorthy K. Linking chronic periodontitis and oral cancer: A review. *Oral Oncol* [Internet]. 2021 Jun 14;105375. Available from: <http://dx.doi.org/10.1016/j.oraloncology.2021.105375>
- [23] Stoltenberg JL, Osborn JB, Pihlstrom BL, Herzberg MC, Aepli DM, Wolff LF, et al. Association between cigarette smoking, bacterial pathogens, and periodontal status. *J Periodontol* [Internet]. 1993 Dec;64(12):1225–30. Available from: <http://dx.doi.org/10.1902/jop.1993.64.12.1225>
- [24] Arowojolu MO, Fawole OI, Dosumu EB, Opeodu OI. A comparative study of the oral hygiene status of smokers and non-smokers in Ibadan, Oyo state. *Niger Med J* [Internet]. 2013 Jul;54(4):240–3. Available from: <http://dx.doi.org/10.4103/0300-1652.119627>
- [25] 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* [Internet]. 2021 Jul 7;14:2851–61. Available from: <http://dx.doi.org/10.2147/RMHP.S306880>
- [26] Machuca G, Rosales I, Lacalle JR, Machuca C, Bullón P. Effect of cigarette smoking on periodontal status of healthy young adults. *J Periodontol* [Internet]. 2000 Jan;71(1):73–8. Available from: <http://dx.doi.org/10.1902/jop.2000.71.1.73>
- [27] Linden GJ, Mullally BH. Cigarette smoking and periodontal destruction in young adults. *J Periodontol* [Internet]. 1994 Jul;65(7):718–23. Available from: <http://dx.doi.org/10.1902/jop.1994.65.7.718>
- [28] Al-Wahadni A, Linden GJ. The effects of cigarette smoking on the periodontal condition of young Jordanian adults. *J Clin Periodontol* [Internet]. 2003 Feb;30(2):132–7. Available from: <http://dx.doi.org/10.1034/j.1600-051x.2003.00279.x>
- [29] Montén U, Wennström JL, Ramberg P. Periodontal conditions in male adolescents using smokeless tobacco (moist snuff). *J Clin Periodontol* [Internet]. 2006 Dec;33(12):863–8. Available from: <http://dx.doi.org/10.1111/j.1600-051X.2006.01005.x>
- [30] Verma A, Kaur S, Singh ST. A STUDY ON THE EVALUATION OF PERIODONTAL EFFECTS ASSOCIATED WITH DURATION OF SMOKELESS TOBACCO USE. *Indian Journal of* [Internet]. 2014; Available from: <http://search.ebscohost.com/login.aspx?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=22316973&AN=95008722&h=r%2B24cezC38wYVbcjrS1KLUmyjR7FejU%2FnAJGLLCOgK%2BGfYcZX07bsjwrtcWnJcBX0xzBiCspnDAMvuMy4MHG0Q%3D%3D&crl=c>