Awareness and Knowledge of the Role of Iron and Copper in Oral Submucous Fibrosis Patients: A Survey amongst the Dental Practitioners of Chennai

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Abstract

Oral submucous fibrosis is a precancerous condition caused by use of the areca nut. The reported prevalence of oral submucous fibrosis was 0.16%. It is extremely important to understand the level of knowledge of practitioners in not only diagnosis of oral submucous fibrosis, but even in the pathogenesis of oral submucous fibrosis. The aim of the study is to determine the knowledge and attitude of dental practitioners of presence of iron and copper in oral submucous fibrosis a sample size of 250 dental practitioners in the general public of Chennai in India was selected and an online questionnaire of 10 questions on oral submucous fibrosis was circulated. The data collected was tabulated in Microsoft Excel and the data is analyzed using IBM SPSS version 20.0 software. It can be said that the majority of clinicians are well educated about the etiological agent, malignancy rate as well as clinical features and are not aware about levels of iron and copper in oralsubmucous fibrosis.

Keywords: Copper; Iron; Malignancy rate;Oral submucous fibrosis; Survey

Introduction

Oral submucous fibrosis [1] is a progressive, chronic, debilitating disease that was first reported in 1953 from India. Initially, the disease was found only amongst people living in Indian subcontinent or in Indian migrants. Later, it was reported from many South-east Asian populations.

In this disease, the buccal mucosa loses its elasticity and fibrous bands develop over a marked duration of time. [2] The epithelium shows atrophy and there is a marked intolerance to food which is spicy.[3,4] The mouth opening capacity of the patient progressively reduces and in certain extreme cases, it may be difficult for even a straw to pass into the oral cavity. [5] The disease is a precancerous state [6] and carries a high relative risk (397.3 after controlling for tobacco use) for malignant transformation. Spontaneous regression has not been reported and there is no effective or widely accepted treatment. Several etiological hypotheses for oral submucous fibrosis have been proposed. The most prominent amongst them was its relation with the intake of chillies. This was influenced by the fact that affected individuals showed a high degree of sensitivity to chillies as well as its occurrence mainly among Indians who used chillies liberally in their daily diet. However, this and other hypotheses have not been confirmed.

Recently, it has been demonstrated that the chewing of areca nuts is the most important etiological factor for oral submucous fibrosis. [7] In a series of case-control studies from Karachi, Pakistan and Bhavnagar, Gujarat, very high levels of relative risk for areca nut chewing were reported (94 and 109.6, respectively). A hospital-based prevalence study with a sample size of 1790 patients" and a population-based prevalence study with a sample population of 11,046 individuals, diagnosed 335 and 136 oral submucous fibrosis

cases, respectively, all of whom were avid areca nut chewers. In an intervention trial, the incidence of oral submucous fibrosis was lower (although not significantly) in the intervention cohort study where there was a significant decrease in smokeless tobacco as well as areca nut use due to the health education, than in the control cohort study. [8]

The practice of chewing areca nuts in betel quid is at least two millennia old. [9] It remained as a rather personal habit in which the consumer purchased areca nuts from the market or ordered a vendor to prepare a fresh quid. Tobacco was introduced nearly four centuries back and currently, almost every regular areca nut chewer uses it with tobacco. This necessitates the frequent spitting of a red coloured juice, a scourge in almost all public places in India. This practice appeared to be slowly decreasing with increasing levels of education, urbanization as well as unacceptability of spitting behaviour.

During the last two decades, the situation has changed once again and the practice of chewing areca nuts has received a boost with the advent of 'pan masala'. These areca nut containing products are manufactured industrially and commercially marketed. They are available in convenient small sachets and are backed by high profile advertising campaigns. As a result the use of 'pan masala' and similar mixtures containing areca nuts has again become quite common.

The prevalence of oralsubmucous fibrosis was reported from population-based, house-to-house surveys in rural areas of six districts in India. The highest prevalence (0.36%) was in Ernakulam district and the next highest, in Bhavnagar district (0.16%). The annual incidence per 100,000 was 13.5 in Ernakulam and 5.5 in Bhavnagar district. Thus, oral submucous fibrosis did not appear to be a very common disease. However, there is a widespread feeling among health professionals, especially dentists and otorhinolaryngologists in northern India, that there is a marked increase in the incidence of oralsubmucous fibrosis. The hallmarks of the disease aresubmucosal fibrosis that affects most of the parts of the oral cavity, pharynx and upper third of the oesophagus. The factors that have been discussed as possible etiological factors to date are areca nuts, capsaicin in chillies and micronutrient deficiencies of iron, zinc and essential vitamins.

The daily intake of copper in an adult in developing countries is between 0.6 to 1.6 mg/day. [10]An adultIndian chewing areca nuts daily consume over 5 mg of copper per day. The copper released during chewing is brought over direct contact with oral mucosal keratinocytes for prolonged periods of time. Early studies have shown that it takes 40 minutes for raised salivary copper levels to return to its baseline value. Copper content of various constituents of quid are red areca (18.3 ppm), white areca (14.9 ppm), Betel leaf (18.5 ppm), gutkha (13.2 ppm), flavoured areca (12.2 ppm), tobacco (6.3 ppm). In institutional setups, there are various types of studies which can be carried out. Majorly conducted studies in colleges are of 4 types *in vitro* study, [11,12] *in vivo* study,

case reports [13-17] and surveys. [18,19] Apart from research studies, even reviews are published often. [20-25] Several studies show iron, vitamin, protein deficiencies in oral submucous fibrosis. Oral submucous fibrosis is basically a collagen disorder. The decrease of iron is due to utilization of iron in fibrosis. Lack of iron causes improper vascular channel formation and concomitant decrease in vascularity. This makes percolation of esters of arecoline easier. Previously our team has a rich experience in working on various research projects across multiple disciplines. [26-40] Now the growing trend in this area motivated us to pursue this project. The aim of the study was to determine the knowledge and attitude of dental practitioners of the presence of iron and copper in oral submucous fibrosis.

Materials and Methods

The study was conducted in an online setting the sample size ethnicity was verified to be Asian. The study was a survey study consisting of 10 questions which were passed on to the sample size using an online link to the sample size of 250 dental practitioners. The measure taken to minimize sampling bias was the technique of stratification and randomisation.

Ethical approval

The institutional review board of Saveetha institute of medical and technical sciences, Chennai, India on the date April, 2020, was granted the ethical permission and approval for the project.

Eligibility criteria

Dental undergraduates students during their bachelors program in the internship, Dental postgraduate students during their master's program as well as the dental practitioners all situated in Chennai were the target participants of the study.

Data collection

This cross sectional survey was conducted among general dentists and specialists from April 2020 to May 2020. A structured questionnaire consisting of 10 questions was shared online and 250 responses were obtained. The internal validity was assured by a questionnaire and external validity of the study was assured by homogenisation. The data collection was done survey conducted online and tabulation of results was done by percentage.

Sample size

Total number of participants of the survey was 250. All of the participants were ensured to have answered all 10 questions in the questionnaire and none of the participants were excluded from the study. Hence, 250 responses were analysed.

Statistical analysis

After data entry in the excel sheet, SPSS software was used to analyze the data. The descriptive statistics were used to determine the frequencies and percentage of the responses given by the participants.

Correlation analysis was performed to find the association between KAP and their application in clinical situations. Chi Square test was performed to determine the association between the knowledge about the role of iron and copper in oral submucous fibrosis patients. The results are obtained in the form of a bar graph.

Results and Discussion

Of the sample size of 250 dental practitioners, 47.6% are females and 52.4% are males. The sample size's perception of etiological agents is as follows: Areca nuts (75.2%),

smoking (6.4%), both smoking and alcohol (13.6%). The age range of the sample size is 18-25 year are of 51.6%, 26-35 years are 19.6%, 36-50 years are 9.6% and above 50 years are 18.8%.

The malignancy rate of oral submucous fibrosis was perceived as 2.0%-36%, 5.0%-17.2%, 7.6%-32.4%, 4.2%-14.4%. The sample size perception of the rate of cases of oral submucous fibrosis as: 1 per day: 19.6%, 1 every 3 days: 13.6%, 1 every week: 66.8% [Figure 1 and Figure 2].

The copper levels of oral submucous fibrosis has been perceived as-increased by 52.8%, decreased by 38.4% and remains constant in 8.8% of sample size's perception.

The iron level perception of the sample size in cases of oral submucous fibrosis-increased in 74.8%, decreased in 11.2% and remains constant in 14% [Table 1 and Table 2].

Table 1: Demographic variables of the survey with the number of respondents.			
Demographic variables	Categories	Number of respondents	Percentage
Gender	Males	131	52.40%
	Females	119	47.60%
Age	18-25 years	129	51.60%
	26-35 years	49	19.60%
	36-50 years	25	10%
	Above 50 years	47	18.80%

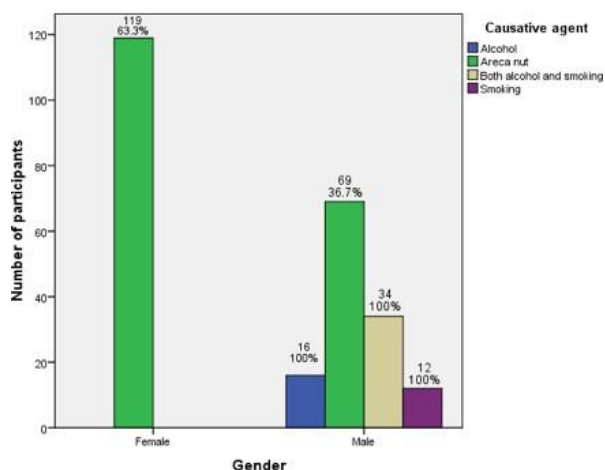| Table 2: Parameters of the study with the number of respondents.			
Parameters of the study	Categories	Number of respondents	Percentage
Malignancy rate	2	129	51.60%
	4.2	49	19.60%
	5	25	10%
	7.6	47	18.80%
Copper levels	Decreased	96	38.40%
	Increased	132	52.80%
	Remains constant	22	8.80%
Iron levels	Decreased	28	11.20%
	Increased	187	74.80%
	Remains constant	35	14%

Figure 1: Bar graph represents the relation between oral submucous fibrosis causative agent and gender of the sample size. X axis of the graph denotes the gender of the sample size and Y axis denotes the number of participants. Alcohol (blue) was found to be 100% within males. Areca nut (green) was found to be majorly within females (63.3%), both alcohol and smoking (beige) was found to be maximum within males (100%) and smoking (purple) was found to be majority in males (100%). (Pearson's chi square test, P=0.001, p<0.05).

It was found that the difference was of statistical significance. In females, only areca nuts were most prominent to be the causative agent of oral submucous fibrosis. In males, Areca nuts was the most predominant causative agent followed by both alcohol and smoking, alcohol and least predominant causative agent was seen to be smoking.
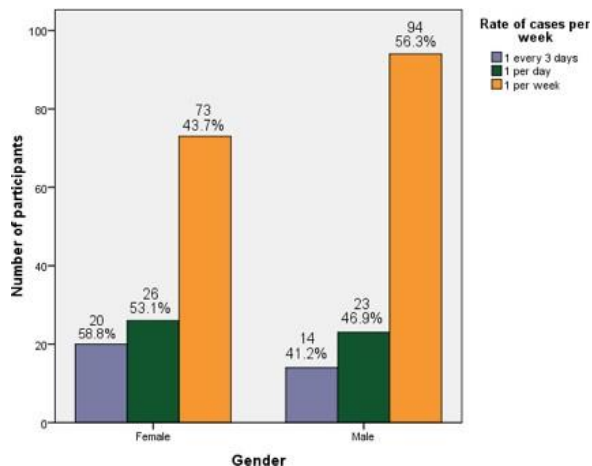
It was found that the difference was not of statistical significance in both gender groups, it was found that 1 case of oral submucous fibrosis was majorly seen per week, followed by at least 1 case per day and least seen in 1 case every 3 days.
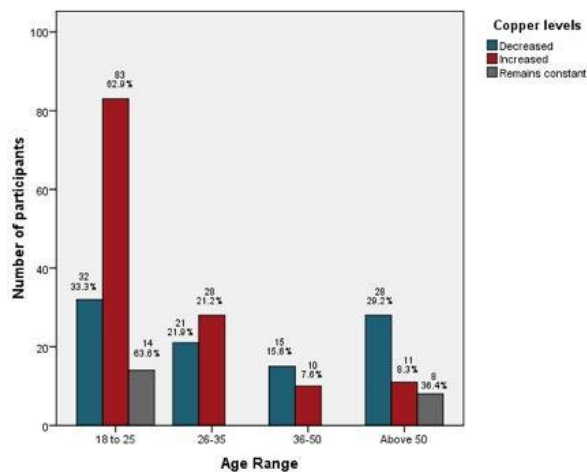

Figure 3: Bar Graph represents the relation between copper levels and age of the sample size. X axis of the graph denotes the age of the sample size and Y axis denotes the number of participants. (Pearson's chi square test, P value=0.001, P value<0.05).

It was found that the difference was of statistical significance. Decreased copper levels were seen highest in 18-25 years and least in 36-50 years. Increased copper levels were seen highest in the 18-25 years and least in 36-50 years of. Constant copper levels were seen highest in 18-25 years and least in 36-50 years and 26-35 age groups.
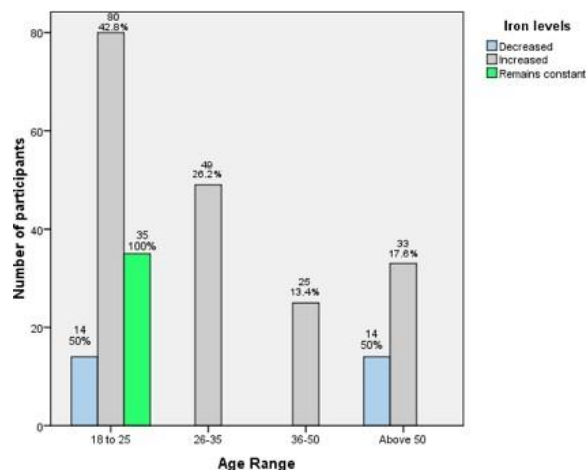

Figure 2: Bar Graph represents the relation between oral submucous fibrosis case rate seen and gender of the sample size. X axis of the graph denotes the gender of the sample size and Y axis denotes the number of participants. (Pearson's chi square test, P=0.191, p<0.05).

Figure 4: Bar Graph represents the relation between iron levels and age of the sample size. X axis of the graph denotes the age of the sample size and Y axis denotes the number of participants. (Pearson's chi square test, P value=0.001, P value<0.05).

It was found that the difference was of statistical significance. Decreased iron levels were seen highest in

18-25 years age group and least in 36-50 years of age. Increased iron levels were seen highest in the 18-25 years and above 50 age groups and least in 36-50 years and 26-35 age groups. Constant iron levels were seen highest in the 18-25 years age group (100%).

Among many trace elements, copper and iron are necessary for the functioning of numerous enzymes and therefore it is reasonable to assume that variation in the serum levels of these biochemical markers may be associated with the pathogenesis of oral cancer and precancerous states.

Iron has been recognized as an important factor for maturation of epithelium and it has been well documented that iron deficiency is associated with epithelial abnormalities along with malignancies like tumors of the pharynx and mouth as well as post cricoid carcinoma. Thus, biochemical variations of copper and iron concentrations in the serum of premalignant patients can help not only in the early diagnosis, appropriate treatment and determination of the treatment prognosis. It has been postulated that serum copper and iron levels alterations are observed among oral submucous fibrosis patients [Figure 3 and Figure 4]. Thus, the present study aimed to estimate and compare the levels of knowledge of practitioners on the topic of serum copper and iron levels alterations among subjects with oral submucous fibrosis of different clinical stages and healthy patients.

Every study must have some amount of supporting literature to help us understand a little more on the topic and the science behind it. Even the study conducted has such literature present. In Tadakamadla et al. study, copper levels increased and iron levels decreased in the study group in comparison to healthy controls. Copper levels increased as the clinical stage of oral submucous fibrosis increased. Another study by Hosthor et al. stated that trace elements may be associated with pathogenesis and progression of oral submucous fibrosis and oral squamous cell carcinoma. Another supporting study was given by Kamath et al. It stated that biochemical investigations are the best indicators of disease progress or intermediary pathways in pathogenesis.

Every study has limitations to be improved, including this study. In this study conducted, the one limitation that exists is observer bias. To undo the limitation, it could be suggested to have an outsider who is unaware of the topic and what results are expected to review the data at hand and provide the final result, hence helping us accurately prove the study result received. Our institution is passionate about high quality evidence based research and has excelled in various fields. [41-46] We hope this study adds to this rich legacy. The future scope of the study is to increase the knowledge and attitude of practitioners of the pathogenesis of oral submucous fibrosis, hence enabling practitioner's early detection and prevention of oral squamous cell carcinoma in the future. It is important for practitioners to be updated on commonly encountered pathologies and how to treat them.

Conclusion

It can be said that the majority of clinicians are well educated about the etiological agents, malignancy rates as well as clinical features of oral submucous fibrosis and are not aware of the levels of iron and copper in oral submucous fibrosis cases.

Author Contributions

First author, Dr. Nadhirah Faiz, performed the analysis and interception and wrote the manuscript. Second author, Dr. Manjari Chaudhary, contributed to conception, data design, analysis interpretation and critically revised manuscript as per guideline, alignments and formatting. The third author, Dr. Iffat Nasim, participated in the study and revised the manuscript. All the authors have discussed the results and contributed to the final manuscript.

Acknowledgement

References

1. Soni NK, Bhama LK. Oral submucous fibrosis. Clinical Cases in Otolaryngology. 2016.

2. Angelakis P, Choulis N, Gagari E, Iatrou J, Melissinos C, Pangalis G, et al. Oral submucous fibrosis (treatment of oral diseases). 2005.

3. Warnakulasuriya S. PD2. Oral submucous fibrosis: Pathogenesis and presentation. Oral Oncol. 2011;47:55.

4. Memon A, Shahid H, Shafique S. Oral Submucous fibrosis. The Professional Medical Journal. 2018;25:1005-1012.

5. Kothe S, Kapre M. The process of trismus due to oral submucous fibrosis. Surgery of trismus in oral submucous fibrosis. 2018.

6. Brennan PA, Aldridge T, Dwivedi RC. Premalignant conditions of the oral cavity. Springer; 2019.276.

7. Angadi PV, Rao SS. Areca nut in pathogenesis of oral submucous fibrosis: revisited. Oral Maxillofac Surg. 2011;15:1-9.

8. Khan HM. Evaluation of Serum Copper (Cu), Serum Iron (Fe) and Serum Copper (Cu/Iron (Fe) Ratio in Oral Submucous Fibrosis in Karachi. Journal of Advances in Medicine and Medical Research. 2018;26:1-9.

9. Porter S. Strong association between areca nut use and oral submucous fibrosis. Evid Based Dent. 2006;7:79-80.

10. Pillai KG, Burde KN. Increased copper level in oral mucosal tissue of patients with submucous fibrosis and who chew areca nut products. West Indian Med J. 2005;54:270-271.

11. Patil SR, Maragathavalli G, Araki K, Al-Zoubi IA, Sghaireen MG, Gudipaneni RK, et al. Three-Rooted Mandibular First Molars in a Saudi Arabian Population: A CBCT Study. Pesquisa Brasileiraem Odontopediatria e Clínica Integrada. 2018;18:1-7.

12. Rohini S, Jayanth Kumar V. Incidence of dental caries and pericoronitis associated with impacted mandibular third molar-A radiographic study. Research Journal of Pharmacy and Technology. 2017;10:1081-1084.

13. Misra S, Shankar Y, Rastogi V, Maragathavalli G. Metastatic hepatocellular carcinoma in the maxilla and mandible, an

extremely rare presentation. Contemp Clin Dent. 2015;6:S117–S121.

14. Dharman S, Muthukrishnan A. Oral mucous membrane pemphigoid–Two case reports with varied clinical presentation. J Indian Soc Periodontol. 2016;20:630-634.

15. Muthukrishnan A, Kumar LB, Ramalingam G. Medication-related osteonecrosis of the jaw: A dentist's nightmare. BMJ Case Rep. 2016.

16. Muthukrishnan A, Kumar LB. Actinic cheilosis: Early intervention prevents malignant transformation. BMJ Case Rep. 2017;2017.

17. Choudhury P. Vanishing roots: First case reports of idiopathic multiple cervico–apical external root resorption. J Clin Diagn Res. 2015;9:ZD17–ZD19.

18. Subashri A, Uma Maheshwari TN. Knowledge and attitude of oral hygiene practice among dental students. Research Journal of Pharmacy and Technology. 2016;9:1840-1842.

19. Steele JC, Clark HJ, Hong CHL, Jurge S, Muthukrishnan A, Ross Kerr A, et al. World workshop on oral medicine VI: an international validation study of clinical competencies for advanced training in oral medicine. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015;120:143-51.

20. Venugopal A, Maheswari TNU. Expression of matrix metalloproteinase-9 in oral potentially malignant disorders: A systematic review. J Oral Maxillofac Pathol. 2016;20:474–479.

21. Chaitanya NC. Role of Vitamin E and Vitamin A in Oral Mucositis Induced by Cancer Chemo/Radiotherapy- A Meta-analysis. J Clin Diagn Res. 2017;11:ZE06–ZE09.

22. Maheswari TNU, Venugopal A, Sureshbabu N, Ramani P. Salivary micro RNA as a potential biomarker in oral potentially malignant disorders: A systematic review. Tzu Chi Med J. 2018;30:55–60.

23. Warnakulasuriya S, Muthukrishnan A. Oral health consequences of smokeless tobacco use. Indian Journal of Medical Research. 2018;148:35-40.

24. Chaitanya N, Muthukrishnan A, Krishnaprasad CMS, Sanjuprasanna G, Pillay P, Mounika B. An insight and update on the analgesic properties of vitamin C. J Pharm Bioallied Sci. 2018;10:119–125.

25. Subha M, Arvind M. Role of magnetic resonance imaging in evaluation of trigeminal neuralgia with its anatomical correlation. Biomedical and Pharmacology Journal. 2019;12:289–296.

26. Ponnulakshmi R, Shyamaladevi B, Vijayalakshmi P, Selvaraj J. In silico and in vivo analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. Toxicol Mech Methods. 2019;29:276–290.

27. Mathew MG, Samuel SR, Soni AJ, Roopa KB. 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. Subramaniam N, Muthukrishnan A. Oral mucositis and microbial colonization in oral cancer patients undergoing radiotherapy and chemotherapy: A prospective analysis in a tertiary care dental hospital. J Investig Clin Dent. 2019;10:e12454.

29. Girija ASS, Shankar EM, Larsson M. Could SARS-CoV-2-induced hyperinflammation magnify the severity of Coronavirus Disease (CoViD-19) Leading to acute respiratory distress syndrome? Front Immunol. 2020;11:1206.

30. Dinesh S, Kumaran P, Mohanamurugan S, Vijay R, Singaravelu DL, Vinod A, et al. Influence of wood dust fillers on the mechanical, thermal, water absorption and biodegradation characteristics of jute fiber epoxy composites. J Polym Res. 2020;27.

31. Thanikodi S, Kumar SD, Devarajan C, Venkatraman V, Rathinavelu V. Teaching learning optimization and neural network for the effective prediction of heat transfer rates in tube heat exchangers. Therm Sci. 2020;24:575–581.

32. Murugan MA, Jayaseelan V, Jayabalakrishnan D, Maridurai T, Kumar SS, Ramesh G, et al. 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. Silicon Chem. 2020;12:1847–1856.

33. Vadivel JK, Govindarajan M, Somasundaram E, Muthukrishnan A. Mast cell expression in oral lichen planus: A systematic review. J Investig Clin Dent. 2019;10:e12457.

34. Chen F, Tang Y, Sun Y, Veeraraghavan VP, Mohan SK, Cui 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 Photochem Photobiol B. 2019;197:111518.

35. Manickam A, Devarasan E, Manogaran G, Priyan MK, Varatharajan R, Hsu C-H, et al. Score level based latent fingerprint enhancement and matching using SIFT feature. Multimed Tools Appl. 2019;78:3065–3085.

36. Wu F, Zhu J, Li G, Wang J, Veeraraghavan VP, Krishna Mohan S, et al. Biologically synthesized green gold nanoparticles from induce growth-inhibitory effect on melanoma cells (B16). Artif Cells Nanomed Biotechnol. 2019;47:3297–3305.

37. Ma Y, Karunakaran T, Veeraraghavan VP, Mohan SK, Li S. Sesame inhibits cell proliferation and induces apoptosis through inhibition of STAT-3 translocation in thyroid cancer cell lines (FTC-133). Biotechnol Bioprocess Eng. 2019;24:646–652.

38. Ponnanikajamideen M, Rajeshkumar S, Vanaja M, Annadurai G. In Vivo type 2 diabetes and wound-healing effects of antioxidant gold nanoparticles synthesized using the insulin plant Chamaecostus cuspidatus in albino rats. Can J Diabetes. 2019;43:82–96.

39. Vairavel M, Devaraj E, Shanmugam R. An eco-friendly synthesis of Enterococcus sp.-mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells. Environ Sci Pollut Res Int. 2020;27:8166–8175.

40. Paramasivam A, Vijayashree PJ, Raghunandhakumar S. N6-adenosine methylation (m6A): A promising new molecular target in hypertension and cardiovascular diseases. Hypertens Res. 2020;43:153–154.

41. Vijayashree PJ. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. J Periodontol. 2019;90:1441–1448.

42. Ezhilarasan D, Apoorva VS, Ashok Vardhan N. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. J Oral Pathol Med. 2019;48:115–121.

43. 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;89:1241–1248.

44. Sridharan G, Ramani P, Patankar S, Vijayaraghavan R. Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma. J Oral Pathol Med. 2019;48:299–306.

45. Pc J, Marimuthu T, 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.

46. Ramadurai N, Gurunathan D, Samuel AV, Subramanian E, Rodrigues SJL. Effectiveness of 2% articaine as an anesthetic agent in children: Randomized controlled trial. Clin Oral Investig. 2019;23:3543–3550.