Original Article

Correlation Between Salivary Amylase A Biomarker And Postoperative Pain After Third Molar Surgery

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Abstract

Objective – This paper investigates and analyses the relationship of postoperative pain with that of salivary alpha amylase a salivary biomarker after third molar surgery.

Study design – A prospective study was performed using 10 patients who had undergone surgical extraction of impacted mandibular third molars. All extractions were performed under local anesthesia. Swelling, maximum inter-incisal distance and mean visual pain analogue scale were determined accordingly.

Results – A total of 20 extractions was performed. 2 samples of saliva were taken from each patient that is before extraction and immediately after extraction. The average value of the marker was compared in the study. Statistical analysis revealed that salivary alpha amylase was not related to the inflammatory response after third molar surgery.

Conclusion - this study indicate salivary amylase is not a reliable marker and the need for further research on this to identify those factors that are linked to inflammation after lower third molar extraction and to the betterment of how they are affected during the postoperative period.

INTRODUCTION

Saliva being a biological fluid has an extensive diagnostic potential. It can be collected non-invasively, and provides a viable alternative to blood, serum, or plasma. Most compounds found in the blood are also present in saliva like proteins, hormones, antibodies, drugs and cytokines. These proteins are useful for the disernment and treatment of oral and systemic diseases. As the composition of saliva can be influenced by systemic changes, their specific biomarkers could help identify certain disease conditions. Recent studies have shown that how saliva can aid in the diagnosis of cardiovascular disease, systemic and local inflammation, hepatic damage, autoimmune disease, and insulin resistance. Analysis of recent data supports the use of salivary biomarkers associated with inflammation, the host response, and bone turnover in the diagnosis of disease progression.

One such metalloenzyme known as Salivary alpha-amylase (SAA) produced by the parotid, submandibular, and sublingual glands for the purpose of hydrolysing alpha-1,4 linkages of starch into glucose and maltose. The biomarkers have received special awareness as they are readily accessible and easily obtained. As a result, they have been used as an important indicator to detect post-operative pain in relation to extraction of impacted lower 3rd molar which is the most common surgical procedure performed in oral cavity. Salivary Alpha Amylase has emerged as a authentic biomarker for assessing patients pain which is released from preformed granules located within acinar cells that are innervated by both parasympathetic and sympathetic branches of the autonomic nervous system. Although it does not demonstrate a 1:1 relationship with plasma catecholamine levels, SAA secretion is regulated by alpha- and beta-adrenergic receptors and is still directly correlated with catecholamine release[1].

SALIVARY ALPHA-AMYLASE: -

It has been proposed as an important biomarker of stress in terms of autonomic dysregulation, as it increases in response to both physical and psychological stress via interactions with the autonomic nervous system. One of the important antibodies present in saliva is immunoglobulin A (IgA), which is normally synthesized by plasma cells in the salivary glands and then transported by an epithelial receptor-mediated mechanism. Secretory IgA levels in the saliva is also considered a potentially useful immunological marker of stress. After consecutive surgery, there is a systemic reaction that comprises of endocrine, immunological, and haematological changes. Studies have been investigated on the value of IgA, alpha-amylase, and the total protein in saliva as positive indicators of dental anxiety during the extraction of retained lower third molars.

The procedure of removal of impacted 3rd molar may result in postoperative pain, inflammation, and/or trismus. The inflammatory process is usually characterised by soft tissue swelling with subsequent facial deformity, and a degree of trismus is also sometimes observed. The postoperative recovery process generally takes around 7 days .Postoperative symptom management has improved considerably over the past few years due to a better understanding of not only the various causes of pain and inflammation, but also the mechanisms of action and pharmacodynamics behind analgesics and anti-inflammatory agents used to treat them. Therefore, the goal is now placed on the importance of preventing both pain and inflammation. Pain and inflammation are typically brief and peak in intensity during the early postoperative period, within the first 24 hrs post extraction; swelling generally appears within 48–72 hrs after the surgery[2].

This study is designed so as to evaluate the salivary alpha-amylase level in the saliva as a biomarkers for inflammation after lower third molar surgery in which the unstimulated whole saliva was collected at two different time points: -

I) Before surgery

II) Immediately after surgery

MATERIALS AND METHODS

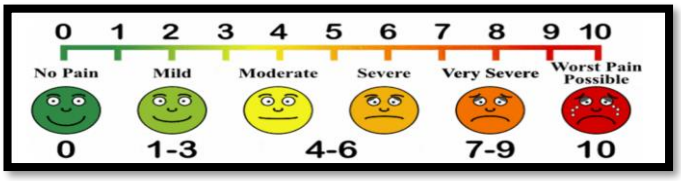
Patients and samples

The following prospective study was performed in the Department of Oral and Maxillofacial surgery, I.T.S. Centre for Dental Studies and Research, Muradnagar, Ghaziabad. A total number of 10 patients with impacted third molars, was randomly divided into 2 groups for this clinical study and the surgical extractions were performed. Case histories were taken of all the patients who agreed to participate in the study. The inclusion criteria where as follows: - patients with impacted lower third molars with a moderate degree of difficulty, no relevant systemic pathology, ASA I as per the American Society of Anesthesiologists classification; where as subjects with poor oral hygiene , medically compromised conditions like existing systemic disorder , suspected previous surgical intervention for the disease , Pregnant and lactating women were excluded.

PARAMETERS

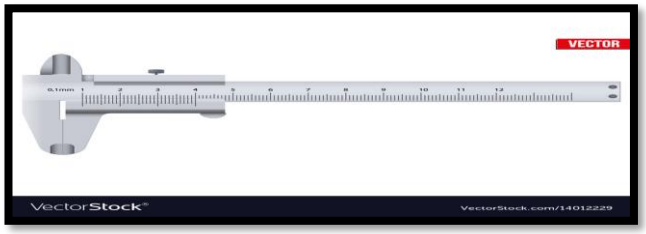
1) Pain

Pain intensity will be assessed using a 10- level visual analogue scale (VAS) with the patient placing a mark on the scale to indicate an intensity range from no pain [0] to severe / unbearable pain [10].



2) Maximum Interincisal Distance (for extent of trismus)

To assess the extent of trismus, the maximum interincisal distance will be measured using Vernier callipers, from the incisal tip of the upper central incisor to the incisal tip of the lower central incisor. Measurements will be in mm.



PROCEDURE: -

Each patient had undergone an exhaustive radiographic examination (a panoramic radio-graph was used to classify the lower third molars based on Winter's classification: mesioangular, distoangular, vertical, and horizontal impactions) and diagnosis to ensure that the surgery would be simple as possible. Complete case histories will be collected: age, sex, and smoking status (smoker or non-smoker), among others.

The patient was asked to abstain from smoking for 2 h prior to the collection of saliva. Saliva of 1ml was collected from the floor of mouth using an auto-aspiration pipette tip. Then the patient was given local anaesthetic (lignocaine 2% and epinephrine 1:80,000) and osteotomy was performed for all cases using a 20,000-rpm hand piece and saline irrigation . Some cases required tooth sectioning .
A second saliva sample was taken immediately after extraction, without any blood.
Ibuprofen postoperatively (8 hourly for 5 days) and amoxicillin/clavulanic acid (625 mg every 8 h for 5 days) are prescribed as anti-inflammatory agents during the postsurgical period. Each patient was instructed postoperatively.

Collection and assessment of sample :-

The unstimulated whole saliva was collected at two different time-points: before surgery and immediately after surgery. Samples were collected in sterile containers and kept at – 80 degree C in a vertical deep freezer until testing. Salivary Alpha-amylase levels were estimated using the kinetic method with an amylase assay kit . Fifty microlitres of each saliva sample and standard dilutions were added to a 36-well plate. Then, 100 ml of Reaction Mix was added to each well and mixed thoroughly. Salivary IgA will be measured using a Human IgA Platinum ELISA kit. A 36-well plate coated with anti-IgA was used, and two-fold serial dilutions were prepared for the standard curve. With 20 ml of each sample in duplicate, the standards and blank were added to the corresponding wells. Next, 50 ml of diluted peroxidase (HRP)-conjugated antibody were added to every well. The absorbance of each well was studied straight away on the spectrophotometer at a wavelength of 450 nm. Average absorbance values were calculated for each set of samples.

STATISTICAL ANALYSIS OF DATA

Statistical Methods: The data will enter into Microsoft Excel XP software program. Statistical analysis will be done by Statistical Package for the Social Sciences (SPSS) software package
(SPSS 16 Inc, Chicago IL, USA). The normality of data will be tested by Shapiro Wilk's test. The descriptive statistics mean, S.d. will be calculated To compare the parameters between groups and with in groups for normal data parametric test Independent t- test will be used among the study population. The level of significance and confidence interval will be 5% and 95% respectively, i.e. $p< 0.05$.
*Significant $p<0.05$
** Highly significant $p<0.01$
*** Very highly significant $p<0.001$

Figure 1.

Figure 2.

RESULTS

Demographic data

The study group had a mean age range of 34.8±2.147 with a majority of female patients.
A total of 20 mandibular third molar extractions were performed of which 20 patients fulfilled the study. There were 8 female (80%) patients and 2 male (20%) patients ; their age ranged from between 20 to 50 years. The patients completed their treatment fully without any complication in the postoperative period.
The parameters were of the salivary alpha amylase both preoperatively and postoperatively in the study.
Statistical analysis revealed that the salivary biomarker was found to have no correlation with the inflammatory response after the third molar surgery ,which was not significant .Thus, the salivary alpha amylase concentration after the removal of third molar was irrelevant.
Since, there were no statistically significant results obtained and there was no correlation between post-surgical inflammation with that of concentration of salivary alpha amylase.

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pre-op Salivary amylase	95.733	10	34.6889	10.9696
	Post-op Salivary amylase	78.431	10	41.2580	13.0469

TABLE 1. Group Statistics

There was a significant decrease in the level of salivary amylase post-operatively after third molar extraction.

Paired Samples Test

	Paired Differences					T	df	P value(2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 Pre-op SalivaryamylaSe - Post-op Salivaryamylase	17.3020	14.4627	4.5735	6.9560	27.6480	3.783	9	.004

TABLE 2. Paired Sample Tests

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pre-op Pain	7.40	10	1.174	.371
	Post-op Pain	6.40	10	1.265	.400

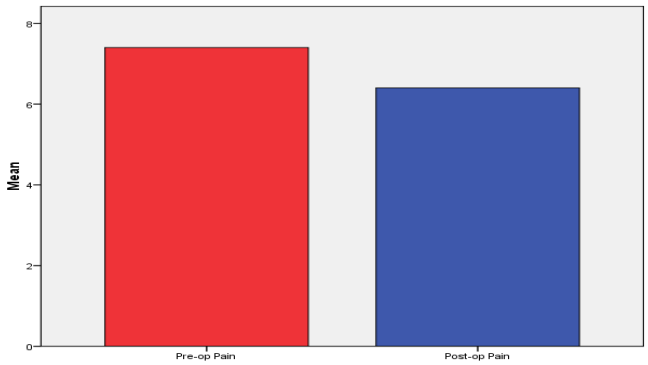
There is significant difference between the pre-operative and post-operative pain in which the post-operative pain is less after third molar extraction.

TABLE 3. –Paired Sample Test

Paired Samples Test									
		Paired Differences					t	df	P value(2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Pre-op Pain - Post-op Pain	1.000	1.247	.394	.108	1.892	2.535	9	.032

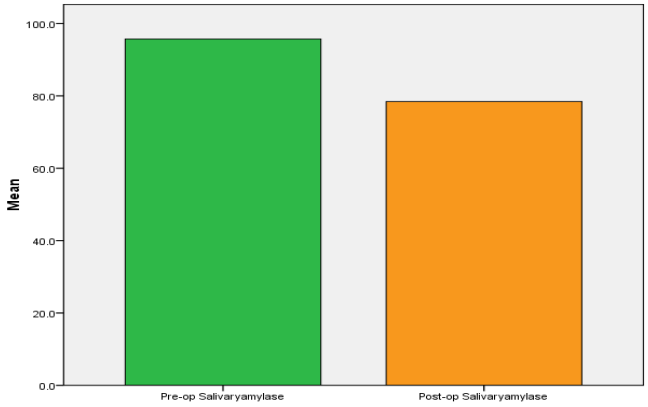
GRAPH 1

According To The Visual Pain Analogue Scale There Was Increase In Intensity Of Pain Pre- Operatively As Compared To Post-Operative.



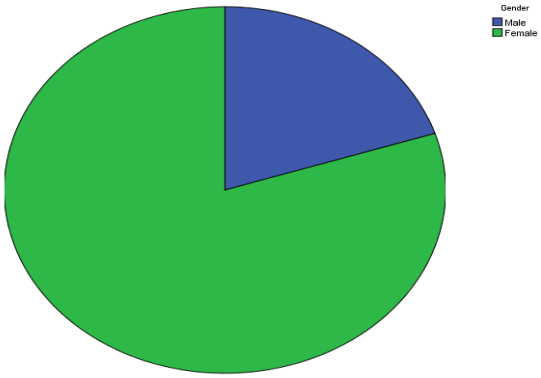
GRAPH 2

There Was Significant Increase In The Level Of Salivary Amylase In Patients Pre-Operatively As Compared To Post-Operatively.



PIE CHART

Female Prediction Was More As Compared To Males.

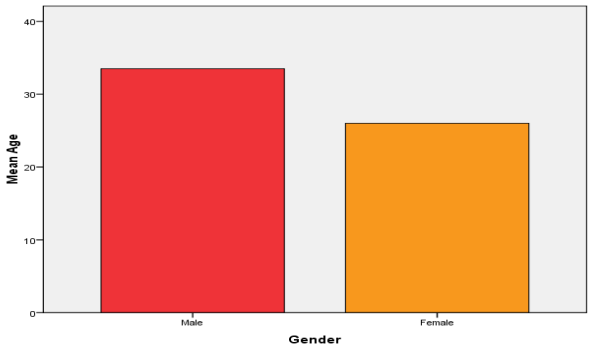


Descriptive Statistics

	N	Range	Minimum	Maximum	Mean	Std. Deviation
Age	10	18	20	38	27.50	5.169
Valid N (listwise)	10					

GRAPH 3

Minimum Age Taken Was 20 Years And Maximum Age Taken Was 50 Years Out Of Which The Mean Age Of The Study Was 35.



DISCUSSION

The surgical removal of mandibular third molars is the most common surgeries performed in the oral cavity. Extraction of impacted lower third molar is usually followed by postoperative swelling, pain, restricted mouth opening and in some cases, there are complications such as alveolar osteitis, infection, nerve injury, abscesses, or mandible fracture[1].

The mandibular third molars extraction is the most common surgical procedure. Thus, despite of differentiated demands of practice, dental surgeons often face problems during the removal of impacted mandibular third molars . So its necessary for both the patient and dentist to have a scientific evidence-based information about the estimated degree of surgical difficulty in each case. Macgregor was the one who did the first attempt to establish a model on assessing surgical difficulty. The classic Pell and Gregory classification has somehow considered inappropriate on the examination of surgical difficulty. There are a number of studies done to evaluate surgical difficulty during the extraction of impacted mandibular third molars. However, those studies are mostly based on dental factors evaluated through radiologic assessments. Being a topic of debate, most authors agreed to the fact that radiologic factors play quite some important role in assessing difficulty, whereas some believe it is difficult to estimate difficulty through radiologic methods alone. Some authors have also stated that clinical variables, such as patient age, gender and weight, play a significant role. Few authors have proposed indexes like Pederson, but it is seldom used due to reports that it does not correlate with actual surgical difficulty[2].

Carvalho and do Egito Vasconcelos reported a prospective study of 285 patients undergoing extraction of impacted lower third molars, with a total of 473 surgeries performed and concluded that surgical difficulty must be taken into account when complications are anticipated. There are certain variables being identified as influencing surgical difficulty such as root number and morphology, tooth position, periodontal space, and the relationship to the second molar; whereas sex, body mass index, related pathologies, and the relationship to the mandibular canal were not significant predictors. The majority of patients undergoing third molar surgery in the present study were between 20 and 50 years old. On the basis of data regarding the age of patients undergoing third molar extractions, it clearly states that the procedure is most frequently performed on patients belonging to the age group of 18 and 30 years. Reason is that younger people have better circulation and elasticity, and the jaw bone is more resilient as compared to older patients, making bone removal less complicated and resulting in reduced postoperative pain and inflammation, with greater potential of the jaw to heal.

Nevertheless, current publications fail to agree on the link between the difficulty of extraction and postoperative complications. However, different studies have linked many factors about the varying outcomes of the surgery. This is mainly suggested during the use of different diagnostic criteria to identify postoperative inflammation (which are mostly evaluated through clinical observation). Thus, objective procedures to evaluate inflammation, through the concentration of salivary components, have been published. Due to the prevalence of this type of surgical intervention, it was necessary for a clinical study requiring third molar extraction in comparison to the concentrations of salivary biomarkers during the period of post-surgical oral inflammation. Referring to current evidence, the results indicate that inflammation is high after surgery due to an increase in the production of inflammatory mediators[1,3].

Presently, saliva has been widely recognized as a biological fluid useful for the diagnosis of oral diseases such as caries and periodontitis. Inflammatory biomarkers in the oral cavity such as proteins and derivatives that can be measured to evaluate the inflammation. Studies in which the levels of alpha amylase, IgA, and total protein in the saliva have been related to dental anxiety have been published. However, it is still not clear as to whether the levels of these biomarkers are influenced by the acute inflammatory process post extraction.

Contrary to the authors' initial phase of sAA levels did not show the increase during the surgery visit compared to the consult or follow-up. Subjective ratings of anxiety, pain, and calmness changed during each visit; however, salivary amylase levels were lower during the surgery visit in comparison to the initial consult visit and during the follow-up visit. Notably, salivary amylase levels did not show significant changes during the course of the consult or follow-up visit. In comparison to the authors' previous investigations of sAA levels in dental students, found that sAA levels were related to self-reported anxiety in relation to the academic stress, sAA levels regarding the surgical procedure were not related to self-reported anxiety. At the same time, higher pain ratings at the follow-up visit were related to higher sAA levels[1,4].

There are studies which have shown that levels of total protein, IgA, and alpha amylase are altered when inflammation occurs as a result of infectious processes inside the oral cavity.

The main objective of this study was to identify differences in the salivary concentrations of the biomarkers before and after surgery and to identify the inflammatory biomarkers that are affected by the processes within the surgical area during the postoperative period. This study, was done to analyze inflammation after third molar extraction objectively through the measurement of salivary biomarkers that have been studied previously for this type of surgery. Therefore, alpha-amylase levels were recorded throughout the perioperative period and were found that alpha amylase was showed distinctly different salivary concentrations in the preoperative and postoperative periods.

In another study, Nater et al. tried to identify a link between salivary alpha-amylase and stress markers. They found significant differences in salivary alpha-amylase levels under conditions of stress; and thus considered as a valid marker.

CONCLUSION

The results of this study suggests that the concentration of salivary alpha-amylase is not related to the surgery and the inflammatory processes occurring after the extraction of impacted lower third molars.

So, this study indicate the need for further research on this to identify those factors that are linked to inflammation after lower third molar extraction and to the betterment of how they affect the concentration of salivary alpha amylase during the postoperative period.

REFERENCES

1. Seymour RA, Meechan JG, Blair GS. An investigation into post-operative pain after third molar surgery under local analgesia. British Journal of Oral and Maxillofacial Surgery. 1985 Dec 1;23(6):410-8.
2. Desborough JP. The stress response to trauma and surgery. British journal of anaesthesia. 2000 Jul 1;85(1):109-17.
3. Yuasa H, Sugiura M. Clinical postoperative findings after removal of impacted mandibular third molars: prediction of postoperative facial swelling and pain based on preoperative variables. British Journal of Oral and Maxillofacial Surgery. 2004 Jun 1;42(3):209-14.
4. Benediktsdóttir IS, Wenzel A, Petersen JK, Hintze H. Mandibular third molar removal: risk indicators for extended operation time, postoperative pain, and complications. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2004 Apr 1;97(4):438-46.
5. Hu S, Loo JA, Wong DT. Human body fluid proteome analysis. Proteomics. 2006 Dec;6(23):6326-53.
6. Nater UM, La Marca R, Florin L, Moses A, Langhans W, Koller MM, Ehlert U. Stress-induced changes in human salivary alpha-amylase activity—associations with adrenergic activity. Psychoneuroendocrinology. 2006 Jan 1;31(1):49-58.
7. Granger DA, Kivlighan KT, Fortunato C, Harmon AG, Hibel LC, Schwartz EB, Whembolua GL. Integration of salivary biomarkers into developmental and behaviorally-oriented research: problems and solutions for collecting specimens. Physiology & behavior. 2007 Nov 23;92(4):583-90.
8. Shirasaki S, Fujii H, Takahashi M, Sato T, Ebina M, Noto Y, Hirota K. Correlation between salivary α-amylase activity and pain scale in patients with chronic pain. Regional Anesthesia & Pain Medicine. 2007 Mar 1;32(2):120-3.
9. Yoon AJ, Cheng B, Philipone E, Turner R, Lamster IB. Inflammatory biomarkers in saliva: assessing the strength of association of diabetes mellitus and periodontal status with the oral inflammatory burden. Journal of clinical periodontology. 2012 May;39(5):434-40.
10. de Santana-Santos T, de Souza-Santos JA, Martins-Filho PR, da Silva LC, e Silva ED, Gomes AC. Prediction of postoperative facial swelling, pain and trismus following third molar surgery based on preoperative variables. Medicina oral, patologia oral y cirugia bucal. 2013 Jan;18(1):e65.
11. Laskin DM, Abubaker AO, Strauss RA. Accuracy of predicting the duration of a surgical operation. Journal of Oral and Maxillofacial Surgery. 2013 Feb 1;71(2):446-7.
12. Pereira DL, Vilela VS, dos-Santos TC, Pires FR. Clinical and laboratorial profile and histological features on minor salivary glands from patients under investigation for Sjögren's syndrome. Medicina oral, patologia oral y cirugia bucal. 2014 May;19(3):e237.
13. Torres-Lagares D, Recio-Lora C, Castillo-Dalí G, Ruiz-de-León-Hernández G, Hita-Iglesias P, Serrera-Figallo MA, Segura-Egea JJ, Gutiérrez-Pérez JL. Influence of state anxiety and trate anxiety in postoperative in oral surgery. Medicina oral, patología oral y cirugía bucal. 2014 Jul;19(4):e403.
14. Aznar-Arasa L, Figueiredo R, Valmaseda-Castellón E, Gay-Escoda C. Patient anxiety and surgical difficulty in impacted lower third molar extractions: a prospective cohort study. International journal of oral and maxillofacial surgery. 2014 Sep 1;43(9):1131-6.

15. Skrinjar I, Brailo V, Vidovic-Juras D, Vucicevic-Boras V, Milenovic A. Evaluation of pretreatment serum interleukin-6 and tumour necrosis factor alpha as a potential biomarker for recurrence in patients with oral squamous cell carcinoma. Medicina oral, patologia oral y cirugia bucal. 2015 Jul;20(4):e402.
16. Wei P, Li C, Qiang L, He J, Li Z, Hua H. Role of salivary anti-SSA/B antibodies for diagnosing primary Sjögren's syndrome. Medicina oral, patologia oral y cirugia bucal. 2015 Mar;20(2):e156.
17. Agha-Hosseini F, Shirzad N, Moosavi MS. Evaluation of Xerostomia and salivary flow rate in Hashimoto's Thyroiditis. Medicina oral, patologia oral y cirugia bucal. 2016 Jan;21(1):e1.
18. Gutiérrez-Corrales A, Campano-Cuevas E, Castillo-Dalí G, Serrera-Figallo MÁ, Torres-Lagares D, Gutiérrez-Pérez JL. Relationship between salivary biomarkers and postoperative swelling after the extraction of impacted lower third molars. International journal of oral and maxillofacial surgery. 2017 Feb 1;46(2):243-9.
19. Lee KC, Bassiur JP. Salivary alpha amylase, dental anxiety, and extraction pain: A pilot study. Anesthesia progress. 2017 Mar 1;64(1):22-8.
20. Robles TF, Sharma R, Park KS, Harrell L, Yamaguchi M, Shetty V. Utility of a salivary biosensor for objective assessment of surgery-related stress. Journal of oral and maxillofacial surgery. 2012 Oct 1;70(10):2256-63.
21. Bachmeier E, Mazzeo MA, López MM, Linares JA, Jarchum G, Wietz FM, Finkelberg AB. Mucositis and salivary antioxidants in patients undergoing bone marrow transplantation (BMT). Medicina oral, patologia oral y cirugia bucal. 2014 Sep;19(5):e444.
22. Rahim MA, Rahim ZH, Ahmad WA, Hashim OH. Can saliva proteins be used to predict the onset of acute myocardial infarction among high-risk patients?. International journal of medical sciences. 2015;12(4):329.
23. Powell J, DiLeo T, Roberge R, Coca A, Kim JH. Salivary and serum cortisol levels during recovery from intense exercise and prolonged, moderate exercise. Biology of sport. 2015 Jun;32(2):91.
24. Krishnan R, Thayalan DK, Padmanaban R, Ramadas R, Annasamy RK, Anandan N. Association of serum and salivary tumor necrosis factor-α with histological grading in oral cancer and its role in differentiating premalignant and malignant oral disease. Asian Pacific Journal of Cancer Prevention. 2014;15(17):7141-8.
25. Kuehl MN, Rodriguez H, Burkhardt BR, Alman AC. Tumor necrosis factor-α, matrix-metalloproteinases 8 and 9 levels in the saliva are associated with increased hemoglobin A1c in type 1 diabetes subjects. PLoS One. 2015 Apr 27;10(4):e0125320.
26. Pfaffe T, Cooper-White J, Beyerlein P, Kostner K, Punyadeera C. Diagnostic potential of saliva: current state and future applications. Clinical chemistry. 2011 May 1;57(5):675-87.
27. Gapski R, Hasturk H, Van Dyke TE, Oringer RJ, Wang S, Braun TM, Giannobile WV. Systemic MMP inhibition for periodontal wound repair: results of a multi-centre randomized-controlled clinical trial. Journal of clinical periodontology. 2009 Feb;36(2):149-56.
28. Kinney JS, Morelli T, Braun T, Ramseier CA, Herr AE, Sugai JV, Shelburne CE, Rayburn LA, Singh AK, Giannobile WV. Saliva/pathogen biomarker signatures and periodontal disease progression. Journal of dental research. 2011 Jun;90(6):752-8.
29. Brandtzaeg PE. Do salivary antibodies reliably reflect both mucosal and systemic immunity?. Annals of the New York Academy of Sciences. 2007 Mar;1098(1):288-311.
30. Pink R, Simek J, Vondrakova J. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub.: Saliva as a diagnostic medium. Journal of Continuing Education Topics & Issues. 2010 Jan 1;12(1):50-1.
31. Fábián TK, Hermann P, Beck A, Fejérdy P, Fábián G. Salivary defense proteins: their network and role in innate and acquired oral immunity. International journal of molecular sciences. 2012 Apr;13(4):4295-320.
32. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry. 1976 May 7;72(1-2):248-54.
33. Haug RH, Abdul-Majid J, Blakey GH, White RP. Evidenced-based decision making: the third molar. Dental Clinics of North America. 2009 Jan 1;53(1):77-96.
34. Bulut E, Bulut S, Etikan Í, Koseoglu O. The value of routine antibiotic prophylaxis in mandibular third molar surgery: acute-phase protein levels as indicators of infection. Journal of oral science. 2001;43(2):117-22.
35. Vibhakar AP, Patankar RS, Yadav RM, Vibbhakar AP. Salivary Total Protein Levels and their correlation to Dental Caries. International Journal of Oral & Maxillofacial Pathology. 2013 Jul 1;4(3).