Estimation of Salivary pH, Salivary Amylase in Patients with Oral cancer, Pre and Postoperatively - A Comparative Study

ABSTRACT

Introduction: Saliva has been studied for so long and it almost stands hand in hand with other diagnostic tests which could possibly replace blood in disease diagnosis and monitoring. Salivary pH and salivary amylase levels are an excellent predictor of the oral environment and salivary gland health, hence, estimating these characteristics is a reasonable method of assessing a patient's risk of disease onset, severity, and prognosis.
Aims: The aim of the study was to compare salivary pH, salivary amylase, pre and postoperatively in oral cancer patients.
Study design: The study is a comparative study.
Place and Duration of Study: The study was conducted in the Department of Oral and Maxillofacial Surgery, Yenepoya Dental College, Mangalore between October 2020 and October 2021.
Methodology: We included 24 patients (20 men, 4 women; age range 40-70 years) who were histopathologically confirmed oral cancer patients. For every patient saliva was collected preoperatively on the day of surgery and postoperatively 5 days after the surgery. Salivary pH was measured using pH meter and salivary amylase was measured using color spectrophotometry.
Results: A significant increase in salivary pH was observed post operatively(7.07) as compared to the preoperative values(6.63). A significant decrease in salivary amylase value was observed post operatively(147.13) as compared to preoperative values(147.88) both salivary pH and salivary amylase values were statistically significant.
Conclusion: There was an increase in salivary pH postoperatively and decrease in salivary amylase levels in postoperatively. However , an increase in sample size with further research should be carried out to further authenticate the study.

Keywords: Salivary pH, Salivary amylase, Oral Cancer, Saliva.

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3. Research Papers and Short Notes should follow the structure of Abstract, Introduction, Methodology, Results and Discussion, Conclusion, Acknowledgements, Competing Interests, Authors' Contributions, Consent (where applicable), Ethical approval (where applicable), and References plus figures and/or tables.)

1. INTRODUCTION

Saliva plays a very important role in the oral cavity by its antimicrobial, digestive, and lubricative action. Submandibular glands contribute to about 65% of unstimulated salivary secretion whereas parotid contributes to 25% and sublingual and other minor salivary glands constitute about 4% and 8% of unstimulated saliva simultaneously [1-2].

In the past decade, there had been an increase in the number of research that had been carried out on the diagnostic potential of saliva. The non-invasive method of collection and the close relationship of the oral fluid concentration to plasma made it one of the most frequently used diagnostic tests [3-5].

The major constituent of saliva is water- around 99%, it also contains many organic and inorganic components which are responsible for the properties exhibited by saliva. In healthy individuals, salivary pH ranges within 6.7 -7.3 whereas the normal salivary amylase range varies from 40 to 140 units per liter (U/L) [6].

The study was aimed at measuring and comparing the salivary pH, salivary amylase pre and post-operatively in oral cancer patients.

2. MATERIAL AND METHODS

The study was carried out in the Department of Oral and Maxillofacial surgery for a span of 12 months (between October 2020 and October 2021). The sample comprised of 24 patients who are histopathologically confirmed with oral cancer. All patients were verbally explained the nature of the study and informed written consent was obtained prior to the study and was approved by The Ethics Committee of the institution.

Inclusion Criteria

- Biopsy proven cases of OSCC undergoing surgical treatment were included in the study
- Patients undergoing surgical management for oral cancer.
- Age 4th decade to 7th decades of life.
- Both male and female patients.

Exclusion Criteria

- Patients who have a previous history of chemotherapy and Irradiated patients.
- Patients with systemic conditions, where the salivary pH become acidic.
- Patients under medications that can make salivary pH acidic and altered amylase levels.
- Psychologically unstable patients.
- Patients who weren't willing to give consent

Saliva sampling

Saliva collection was done on the day of surgery preoperatively and 5 days after the surgery postoperatively. The subjects were instructed to rinse their oral cavity with water 5 minutes before the saliva sample was collected. After five minutes of oral rinse, the patients were asked to dribble unstimulated saliva into a sterile saliva collection container about 5 mL volume of saliva was collected. The samples were stored at 3°C for further pH meter and amylase analysis.

Salivary pH analysis

The pH meter is initially standardized and calibrated using distilled water. The 2 ml of samples are diluted up to 10 ml, and the calibrated electrode of the pH meter was dipped into the sample and the pH was measured.

Salivary amylase analysis.

Salivary α-amylase was assayed by the enzymatic hydrolysis of the di-nitro salicylic acid reagent, and α-amylase levels were analyzed using a color spectrophotometer at 540 nm [6]

Statistical analysis

Statistical analysis was performed using Paired t-test. P-value < 0.05 was considered statistically significant. All data were analyzed using SPSS 21.

3. RESULTS AND DISCUSSION

Paired t Test was done for pre operative and post operative pH (Table 1) and salivary amylase (Table 2) comparison.

The mean preoperative pH value obtained was 6.63 with a standard deviation of 0.31, and a mean postoperative pH value obtained was 7.07 with a standard deviation of 0.28. A statistically significant increase in salivary pH value was observed postoperatively when compared with preoperative values with a P value less than 0.001.

Table 1. Pre and post-operative values of salivary pH

	N	Mean	Standard deviation	P value
Preoperative pH	24	6.63	0.31	
				*<0.001**
Postoperative pH	24	7.07	0.28	
P-value based on Paired-t-Test		** = Statistically Significant (P< 0.05)*		

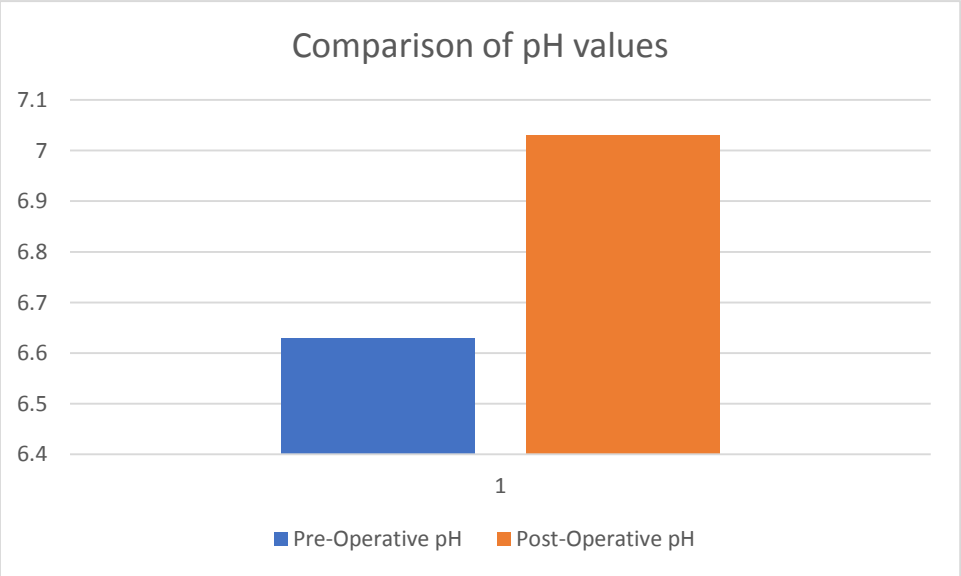

Figure 1: Graphical representation of preoperative and postoperative salivary pH values

The mean preoperative salivary α-amylase value obtained was 147.88 with a S.D of 2.49, and the mean postoperative salivary amylase value obtained was 147.13 with a standard deviation of 3.18. A

statistically significant decrease in salivary amylase levels was observed postoperatively when compared to preoperative values with a P-value of 0.046.

Table 2. Pre and postoperative values of salivary amylase.

	N	Mean	Standard deviation	P value
Preoperative Salivary Amylase	24	147.88	2.49	*0.046*
Postoperative Salivary amylase	24	147.13	3.18	
P-value based on Paired-t-Test		** = Statistically Significant (P < 0.05)*		

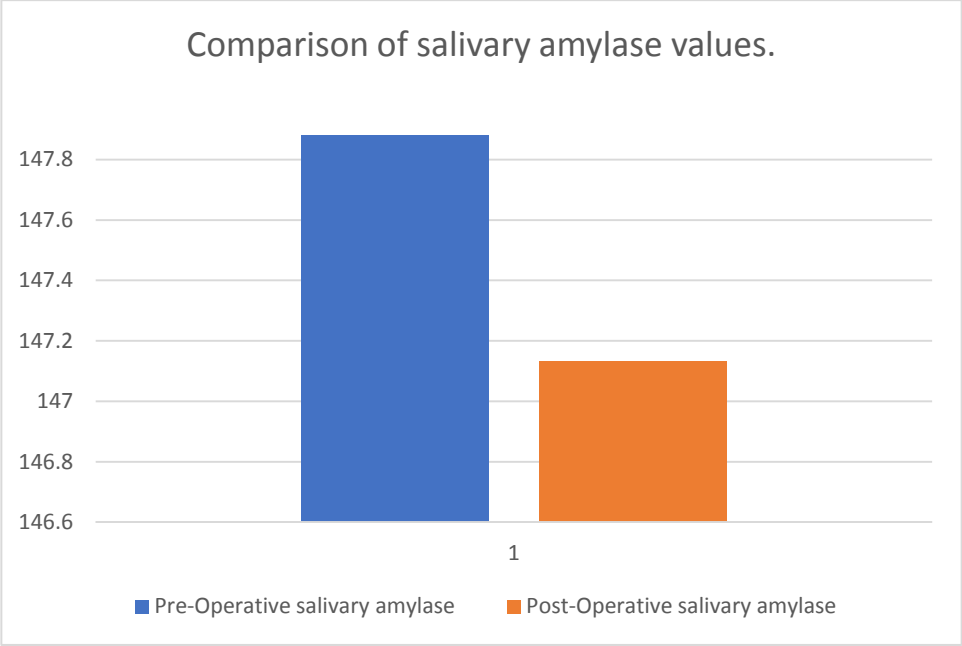

Figure 2: Graphical representation of preoperative and postoperative salivary α-amylase values.

Saliva has already proved its potential as a very good diagnostic tests due to its distinctive advantages. The present study was conducted on 24 patients who were clinically evaluated and histopathologically diagnosed and confirmed cases of oral squamous cell carcinoma. The results from the present study showed a significant decrease in salivary pH postoperatively. An increase in the utilization of glucose by the tumor cells along with the anaerobic glycolysis which in turn led to increasing in lactic acid production that further enhanced the acidity of the environment might have furthermore lowered the pH. The results in our study were in accordance with the study conducted by Chitra et al, who also found a decreased pH of saliva preoperatively in oral cancer patients[8].

The results in our study showed a decrease in salivary α-amylase levels postoperatively. This can be attributed towards the sudden shift in the pH(more basic) after the surgical removal of the tumor. The more basic environment switches off the α-amylase activity as α-amylase showed optimum activity around a pH of 6.8, thereby leading to the denaturation of the enzyme. Acinar cells and cells of the intercalated ducts, which make up more than 80% of the cells in the major salivary glands, synthesize and release salivary α-amylase. [9] Because salivary α-amylase secretion is controlled by the sympathetic nervous system, which activates salivary gland acinar cells via beta-adrenergic receptors, salivary α-amylase activity has been considered as a measure for salivary gland health.

The salivary α-amylase enzyme is regarded as a reliable enzyme for determining the functioning of serous cells. Furthermore , the collection of saliva in our study was done on the day of the surgery preoperatively and 5days postoperatively after the surgical intervention, the reduction in the oral consumption of food also might have added up towards the decrease in salivary α- amylase levels along with the reduction in saliva production.

Recent studies show that salivary α-amylase is a viable noninvasive measure of adrenergic activity. First, nonadrenergic sympathetic mediators, such as neuropeptides, cause α-amylase to be released. Second, parasympathetic stimulation stimulates α-amylase production, and parasympathetic stimulation enhances the effects of sympathetic stimulation.[10]

According to the findings of our study, cancer patients' pH levels had shown a α-statistically significant decrease in value preoperatively, which might be related to tumor cell multiplication, which in turn had led to higher lactic acid levels due to high uptake of glucose by tumor cells and which resulted in anaerobic glycolysis, lactic acid is produced, which explains the acidic environment observed in cancer patients' oral cavities.[11-12].

There had been quite a number of studies evaluating the pH and salivary α-amylase level pre and post-radiation therapy but there are very limited studies that had been conducted measuring the effects of salivary pH and salivary amylase pre and post-surgical therapy.

However, this study needs to be carried out in greater numbers to come to a definite inference that can show more information regarding the exact relation between the salivary pH and amylase on oral carcinomas.

4. CONCLUSION

Because of advancements in detection technologies combined with clinically relevant combinations of biomolecules, saliva has the potential to become a first-line diagnostic aid of choice.

To conclude the present study, an increase in salivary pH was observed postoperatively and when compared with preoperative values; also, there was a significant decrease in salivary α-amylase levels observed postoperatively when compared to preoperative values.

Variation in salivary pH and salivary α-amylase levels in cancer patients during treatment suggests an emphasis on a very important direction for future research with an increased sample size, which may in turn help in opening the doors for a new dimension in cancer which is "Non-Invasive Diagnostic Indicators".

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COMPETING INTERESTS

Conflict of interest -NIL
Funding and Sponsorship -NIL

AUTHORS CONTRIBUTIONS

'AUTHOR A' DESIGNED THE STUDY, PERFORMED THE STATISTICAL ANALYSIS, WROTE THE PROTOCOL, MANAGED THE LITERATURE SEARCHES, MANAGED THE ANALYSES OF THE STUDY, AND WROTE THE FIRST DRAFT OF THE MANUSCRIPT.
'AUTHOR B' HAD GUIDED THE 'AUTHOR A' AND CROSS-CHECKED ALL THE STATISTICAL ANALYSES AND DRAFTS.
ALL AUTHORS READ AND APPROVED THE FINAL MANUSCRIPT.

CONSENT

ALL AUTHORS DECLARE THAT 'WRITTEN INFORMED CONSENT WAS OBTAINED FROM THE PATIENT (OR OTHER APPROVED PARTIES) FOR PUBLICATION OF THIS CASE REPORT AND ACCOMPANYING IMAGES.

ETHICAL APPROVAL

ETHICAL CLEARANCE OBTAINED FROM YENEPOYA ETHICS COMMITTEE 2, DCGI REGISTRATION NO: ECR/ 1337/ INST/KA/2020.
PROTOCOL NUMBER YEC2/495

REFERENCES

1. Castagnola M, Picciotti PM, Messana I, Fanali C, Fiorita A, Cabras T. Potential applications of human saliva as diagnostic fluid. Acta Otorhinolaryngol Ital. 2011;31:347e357.
2. Ramya AS, Uppala D, Majumdar S, Surekha Ch, Deepak KG. Are salivary amylase and pH - Prognostic indicators of cancers? J Oral Biol Craniofac Res. 2015 May-Aug;5(2):81-5
3. Krishna Prasad Ramnarayan Belur, Sharma Akhilesh, Babu Harsha Mysore. An insight into salivary markers in oral cancer. Dent Res J (Isfahan). 2013 MayeJun;10:287e295.
4. Saxena V, Yadav NS, Juneja V, Singh A, Tiwari V, Santha B. Saliva: a miraculous biofluid for early detection of disease. J Oral Health Community Dent. 2013;7:64e68.
5. Cheng YS, Rees T, Wright J. A review of research on salivary biomarkers for oral cancer detection. Clin Transl Med. 2014 Feb 24;3(1):3.
6. Nater UM, Rohleder Nicolas, Schlotz Wolff, Ehlert Ulrike, Kirschbaum Clemens. Determinants of the diurnal course of salivary alpha-amylase.Psychoneuroendocrinology. 2007;32:392e401.
7. Henson BS, Wong DT. Collection, storage, and processing of saliva samples for downstream molecular applications. Methods Mol Biol. 2010;666:21-30.
8. Baliga S, Muglikar S, Kale R. Salivary pH: A diagnostic biomarker. J Indian Soc Periodontol. 2013 Jul;17(4):461-5.

9. Chitra S, Shyamal Devi C S. Effects of radiation and alpha tocopherol on saliva flow rate, amylase activity, total protein and electrolyte levels in oral cavity cancer, Indian J Dent Res 2008; 19: 213-218
10. Spielmann, N., Wong, D.Saliva: Diagnostics and therapeutic perspectives (2011) *Oral Diseases*, 17 (4), pp. 345-354.
11. Herman Aihera. pH and cancer. lifegiving.ws/Cancer% 26ThePhRelationship.pdf.
12. Pfaffe T, Cooper-White J, Beyerlein P, Kostner K, Punyadeera C. Diagnostic potential of saliva: current state and future applications. Clin Chem. 2011 May;57(5):675-87.