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

Sherin Salim Shah a* and Joyce P. Sequeira a

a Department of Oral and Maxillofacial Surgery, Yenepoya Dental College, Mangalore, India.

Authors' contributions
This work was carried out in collaboration between both authors. Author SSS 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 JPS had guided the Author SSS and cross-checked all the statistical analyses and drafts. Both authors read and approved the final manuscript.

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.

### 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. MATERIALS 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.

#### 2.1 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.

#### 2.2 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

#### 2.3 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.

#### 2.4 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.
2.5 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].

2.6 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.

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<th>Table 1. Pre and post-operative values of salivary pH</th>
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Fig. 1. Graphical representation of preoperative and postoperative salivary pH values

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<th>Table 2. Pre and postoperative values of salivary amylase</th>
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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.

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 neuuropeptides, 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 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".

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, dciąj registration no: ecr/ 1337/ inst/ka/2020.

Protocol number yec2/495

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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9. Chitra S, Shyamal Devi C S. Effects of radiation and alpha tocopherol on saliva

