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Original Article

Are salivary amylase and pH – Prognostic indicators of cancers?

Atmakuri Shanmukha Ramya [a,*], *Divya Uppala* [b], *Sumit Majumdar* [c], *Ch. Surekha* [d], *K.G.K. Deepak* [e]

[a] *Post Graduate Student, Department of Oral Pathology and Microbiology, Gitam Dental College, Rushikonda, Vishakhapatnam, 530045, India*

[b] *Assistant Professor, Department of Oral Pathology and Microbiology, Gitam Dental College, Rushikonda, Vishakhapatnam, 530045, India*

[c] *Professor and Head of the Department, Department of Oral Pathology and Microbiology, Gitam Dental College, Rushikonda, Vishakhapatnam, 530045, India*

[d] *Associate Professor, Department of Biochemistry, Gitam Institute of Sciences, GITAM Dental College, Rushikonda, Vishakhapatnam, 530045, India*

[e] *Research Scholar, Department of Biochemistry, Gitam Institute of Sciences, GITAM Dental College, Rushikonda, Vishakhapatnam, 530045, India*

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ABSTRACT

Background: Saliva, "Mirror of body's health" has long been of particular interest as a substitute for blood for disease diagnosis and monitoring. The radiation effects on salivary glands are of particular interest in which salivary amylase is a good indicator of salivary glands function. Thus, estimation of these parameters represents a reasonable approach in evaluation of patient's risk for disease occurrence, intensity and prognosis.

Aim of study: To evaluate and compare the pH and amylase levels in saliva of cancer patients prior to treatment, patients during treatment.

Materials and methods: Saliva samples of 90 individuals were taken which were divided into 3 groups - 30 individuals without cancer, 30 cancer patients prior treatment and 30 cancer patients during treatment. Materials used were pH strips and pH meter, Salivary Amylase assay.

Results: Statistical analysis – ANOVA with post-hoc Tukey's test.

1) Significant decrease in salivary amylase levels – in cancer patients, during treatment when compared to others.

2) Significant decrease in salivary pH levels in newly diagnosed cancer patients prior to treatment.

Conclusion: To conclude, pH strips and pH meter showed to be a useful tool in the measurement of pH of saliva in individuals with and without cancer. This study showed that cancer patients without treatment have a lower pH of saliva. Treatment increased the pH of the saliva to a more alkaline level whereas amylase levels decreased in those subjects.

* *Corresponding author.*
E-mail address: rams.cutiepie@gmail.com (A.S. Ramya).

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Therefore those parameters can be an area of further research with an increased sample size, which in-turn may help in opening the doors for new dimension in non invasive prognostic markers.

1. Introduction

Cancer is one of the most common causes of morbidity and mortality today with about 6 million deaths reported each year worldwide.[1] Oral cancer, the sixth most common human cancer, is a serious and growing problem in many parts of the globe with a five year mortality rate of approximately 50%.[2] The high morbidity rate in oral cancer can be attributed to the delay in the diagnosis of the disease.[3] This underscores the importance of understanding the biological process of cancer for early detection of cancer risk and to predict its prognosis. In responding to the call for early detection of cancer, several studies have led to discovery of many cancer biomarkers, including salivary biomarkers.

Since time immemorial, saliva has been portrayed as a unique yet complex body fluid.[4] Saliva, the so called 'mirror of the body' is a non invasive and an easily accessible oral fluid, that reflects virtually the entire spectrum of normal and disease states of the body.[5] It has long been of particular interest as a substitute for blood for disease diagnosis and monitoring.[4] Saliva is an ideal translational research tool and diagnostic medium which is being used in novel ways to provide molecular biomarkers for a variety of oral and systemic diseases and conditions.[5]

Saliva is made up water (99%), many organic and inorganic components which are responsible for the roles exhibited by itself. Most of the potential salivary biomarkers include salivary pH levels and salivary proteins like amylase & lysozyme.[6]

Saliva has a pH range of 6.7–7.3 in healthy individuals.[7] A saliva pH below 7.0 usually indicates acidemia (increase acid production in the body). In tumor conditions there is a shift in pH towards acidic, this is due to anaerobic metabolism of glucose in hypoxic conditions created by the tumor burden.[8] This acidic condition acts as a favorable factor for the tumor cells to survive and flourish.[7]

Salivary α-Amylase (sAA) is one of the most plentiful components in saliva, accounting for 10–20% of the total protein content which is known to facilitate digestion.[9,10] Saliva is responsible for the initial digestion of starch, mainly by the presence of salivary amylase (or ptyalin).[11]

Many patients with cancer undergo radiation therapy as a main or adjuvant treatment. All or part of the major and minor salivary glands are often included within the radiation field owing to the site and extension of primary tumors.[12] Exposing the salivary glands to radiation often results in severe salivary gland hypo function and changes in saliva composition, leading to a number of acute and long-term oral complications like decreased salivary flow rates.[12] The radiation effects on salivary glands are of particular interest as the fluctuation of the salivary flow rate coincides with the fluctuation in the amylase concentration.[10,13]

This study is aimed at evaluating and comparing the salivary pH and amylase levels in cancer patients prior to treatment and of patients during the treatment (mainly radiotherapy).

2. Materials and methods

The study was conducted in the outpatient department of GITAM Dental College, Vishakhapatnam for a span of 18 months. Histopathologically diagnosed cancer patients were included in the study. Study population comprised of 90 subjects within age group of 10–50 years, in which 51 were females and 39 were males (Fig. 1).

All patients were verbally explained the nature of the study and an informed written consent was obtained prior to the study. The study protocol was approved by the Ethical committee of the institution.

2.1. *Saliva sampling*

(1) The subject is informed about the early morning collection of saliva sample and is ask to refrain from eating or drinking for at least 1 h prior to the collection.
(2) The subject is asked to rinse their mouth with water 5 min before the collection of saliva sample.
(3) After Five minutes of oral rinse, the subject is asked to spit into a sterile saliva collection tube and about 5 mL volume of saliva is collected.
(4) Immediately after the collection of saliva sample pH of the sample is measured using pH strip by placing 2–3 drops of sample on the test zone of indicator strip. (MERCK SERENO – PH INDICATOR STRIPS, USA).
(5) The samples are stored at 3 °C for further pH meter and amylase analysis.[5]

2.2. *Salivary pH analysis*

The pH meter (ELICO- pH meter, Hyderabad, India) is initially standardized and calibrated using distilled water. The 5 ml samples are diluted upto 30 ml and pH of the sample is measured using the pH meter. The pH meter readings of the diluted samples were tabulated.

2.3. *Salivary amylase analysis*

Saliva samples are stored at 3 °C for the amylase assay using calorimetric method. Salivary α-amylase was assayed by the enzymatic hydrolysis of the Di-nitro salicylic acid reagent and amylase levels were analyzed using calorimeter or spectrophotometer at 520 nm.[10]