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Salivary Amylase as a Predictive Marker for Radiation-Induced Salivary Dysfunction in Head and Neck Cancer: A Pilot Study

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Abstract

Background: Reports correlating changes in salivary flow rate and amylase with radiation dose to parotid glands and development of salivary dysfunction for Head and Neck cancers (HNC) are lacking. In the current study, an attempt was made at understanding this.

Method: This was a prospective study carried out on people newly diagnosed with HNC requiring curative radiotherapy of more than 60 Gy. The salivary flow rate and levels of salivary α-amylase were evaluated before the start of radiation [day 1, before exposure to the first fraction of 2 Gy radiation], after 2 Gy [24 hours after the 1st fraction of 2 Gy, before exposure to 2nd fraction of 2 Gy on day 2 of the treatment], and on the completion of 30 Gy [(15 fraction of 2 Gy), before start of the 16th fraction, at the start of the fourth week on day 22] of radiation and development of salivary dysfunction was evaluated on a weekly basis. The demographic data were subjected to frequency and percentage, while biochemical data were stratified depending on dose to parotids and subjected to unpaired "t-test". We also employed chi square/Fishers exact test to ascertain changes in the number of patients developing various degrees of salivary dysfunction on a weekly basis. A *P* value of <0.05 was considered significant.

Results: Radiation decreased salivary flow rate from 0.29 ± 0.02 to 0.20 ± 0.04 (*P* = 0.0001) and amylase from 147.69 ± 11.15 to 109.07 ± 23.21 U/L (*P* = 0.0005). Both salivary flow rate and amylase was less in patients with severe salivary gland dysfunction (*P* = 0.014) and cumulative dose of radiation to the parotid glands (*P* = 0.014). The number of patients with a severe degree of salivary dysfunction was seen in people exposed to more than 25 Gy to the parotids (*P* = 0.04).

Conclusion: The results suggested that the evaluation of salivary amylase on day 22 could be a useful predictive marker to understand the development of radiation-induced dysfunction in patients with curative radiotherapy for their head and neck cancer.

Keywords: Radiation, Saliva, Amylases, Salivary glands, Xerostomia

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Introduction

Radiation therapy is an important treatment option in head and neck cancers (HNC) and plays a vital role when the treatment objectives are curative or palliative.[1] However, the use of radiation is associated with side-effects. Salivary dysfunction (dry mouth or xerostomia) increases morbidity and decreases the patient's quality of life.[2] Conservative estimates are that approximately 70% of the patients receiving HNC radiotherapy (RT) develop salivary dysfunction.[2] The time of presentation and severity is proportional to the dose the salivary gland receive. In this regard the radiation dose the parotid glands, which are the principal salivary secretory apparatus receive is very important.[1, 3, 4] Clinically, efforts are made to see that the parotid glands do not receive cumulative radiation dose of more than 25 Gy to prevent development of salivary dysfunction and xerostomia.[1, 2] From a mechanistic view point during the course of the curative RT, the major salivary glands invariably get exposed to a fraction of the radiation used, consequentially leading to damage of the glands, which will subsequently alter the volume, consistency, and pH of secreted saliva.[3, 4] Xerostomia can occur early and may persist for six months to several years after the completion of the curative treatment.[3, 4]

From a physiological and functional view point, saliva is an integral part of our defence system against infectious organisms and has a number of beneficial functions in the oral cavity, namely lubrication, protection of mucosal integrity, and antimicrobial activity. The protective functions of saliva are altered in HNC and radiation therapy can further cause gross changes.[3, 4] In the curative treatment of HNC, significant salivary flow reduction develops, if the parotid glands are exposed to doses above 25 Gy.[1] Reports indicate that the average salivary flow rate decreases by 57% after one week of radiation, by 67% after six weeks of radiation, and by 95% after three years of treatment.[5, 6] Generally, the damage is irreversible in patients receiving doses ≥6000 cGy.[5] Physiologically, the parotid glands are more radiosensitive than the submandibular or sublingual glands.[7, 8] Radiation effects on parotid gland tissue are mainly responsible for xerostomia and associated side-effects. Disruption of mucosal integrity as a direct effect of radiation therapy demonstrates enhanced sensitivity to physical, chemical, and microbial insults in the mouth. Significant changes in salivary flow rate,

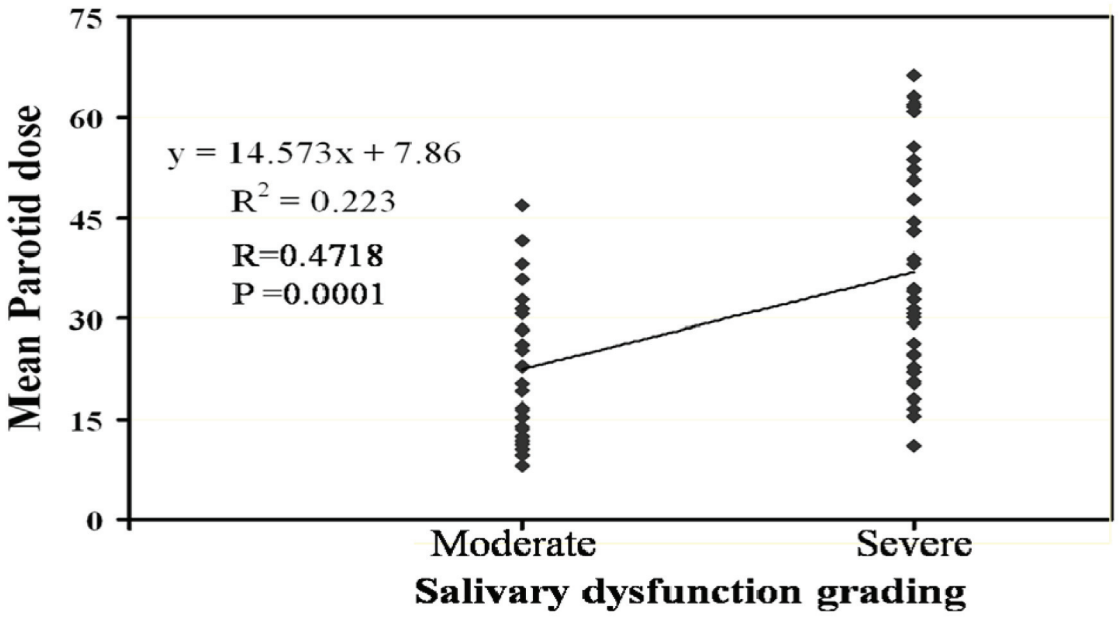

Figure 1. This figure depicts the correlation between the mean dose of the parotids and the severity of salivary dysfunction.

Table 1. Patient age, gender, and habit details

Parameter	Group	HNC (N = 60)	Percentage (%)
Age	Mean age	53.88 ± 10.89	
	Less than 30	1	1.67
	31 to 40	6	10
	41 to 50	15	25
	51 to 60	27	45
	Above 60	11	18.33
Sex	Male	46	76.67
	Female	14	23.33
Cigarette smoking	Yes	38	63.33
	No	22	36.67
Beedi smoking	Yes	14	23.33
	No	46	76.67
Alcohol	Yes	42	70
	No	18	30
Chewing	Yes	22	36.67
	No	48	80
Snuff	Yes	11	18.33
	No	49	81.67

HNC: Head and neck cancer

electrolytes, enzymes, pH, immunoglobulins, and other chemical components have been observed in HNC patients undergoing radiation therapy.[3, 8-12]

Saliva as a diagnostic tool has distinct advantages of non-invasiveness of its collection, non-requirement of skilled personnel for collection, suitability for repeated sample collection with least compliance problems, and presence of various biomarkers mimicking plasma. Salivary flow rate and constituents have been proposed to be reliable biomarkers of oral and systemic diseases.[13, 14] Salivary amylase is mainly produced in the parotid gland and it is responsible for starch hydrolysis, initiating carbohydrate digestion in the oral cavity. Few studies have reported the altered activity of salivary amylase in HNC and during radiation therapy of HNC.[15-17] The increase in amylase activity is dependent on the volume of salivary glands included in the irradiated target volume and dose of radiation.[16] There is paucity of studies investigating the salivary amylase and flow rate on various days after the radiation therapy and comparing amylase levels in mild and severe dysfunction of salivary gland by considering the doses parotid glands incur. The present study is an attempt to ascertain the role of salivary amylase in predicting radiation-induced mucositis in HNC patients undergoing curative cisplatin-based chemo-irradiation.

Materials and Methods

This was a prospective study conducted from October 2012 to September 2015 in the Departments of Radiation Oncology and Biochemistry at Father Muller Medical College, Mangalore, Karnataka, India. The study was approved by the Father Muller Medical College Institutional Ethics Committee (FMMC/FMIEC/877/2012) and was carried out as per the ethical principles of Declaration of Helsinki. The subjects comprised of histopathologically confirmed adult HNC patients scheduled to receive curative chemoradiotherapy (60-70 Gy). The pretreatment staging of the tumors was performed through clinical examination, computed tomography / magnetic resonance imaging (CT/MRI), endoscopy, and biopsies taken from the primary tumor. The tumors were staged according to AJCC Cancer Staging Manual 7th Edition.[18]

The inclusion criteria included patients with a definitive diagnosis of HNC, requiring curative chemoradiation or radiation (> 60 Gy) either as primary treatment or postoperative treatment

Table 2. The tumor and the radiation details

Tumor and treatment details choice	N (%)
Cancer site	
Alveolus	2 (3.33)
Buccal mucosa	4 (6.67)
Floor of the Mouth	2 (3.33)
GBS	2 (3.33)
Cheek	1 (1.67)
Hypopharynx	6 (10)
Larynx	1 (1.67)
Maxillary Antrum	1 (1.67)
Nasopharynx	4 (6.67)
Oral cavity	9 (15)
Oropharynx	8 (13.33)
Pyriform sinus	2 (3.33)
Retromolar trigone	2 (3.33)
Tongue/Base of tongue	9 (15)
Tonsil	5 (8.33)
vallecula	2 (3.33)
Tumor size (T)	
T1	4 (6.67)
T2	20 (33.33)
T3	24 (40)
T4	10 (16.67)
TX	2 (3.33)
Regional nodes (N)	
N0	16 (26.67)
N1	9 (15)
N2	30 (50)
N3	5 (8.33)
NX	0 (0)
Metastasis (M)	
M0	59 (98.33)
MX	1 (1.67)
Radiation type and dose	
Radiation only	4(6.67)
Chemo-radiation	56 (93.33)
Radiation dose	69.13 ± 1.66
Radiation fraction	34.46 ± 0.89

(when parotids were not involved) and with a general health condition of above 80% (according to Karnosky's scale) at the start of the treatment, above the age of 18 years, and that were not pregnant or lactating. The exclusion criteria included patients who were not willing to be a part of the study, female patients who were pregnant or lactating, patients who had oral surgery within the previous six weeks, patients with pre-existing ulceration or open wound in the treatment area, patients receiving neoadjuvant chemotherapy or radiation treatment previously to the head and neck region, using high doses of non-steroidal anti-inflammatory drugs, having severe comorbid conditions (poorly controlled diabetes mellitus, hypertension), and patients with existing mental illnesses (schizophrenia, bipolar disorders).

Radiation treatment planning

In the treatment of HNC, in the recent past, 3D-CRT and intensity-modulated radiation therapy (IMRT) techniques have been used as this technique facilitates the demarcation of the site for irradiation and spares the surrounding vital tissues, such as the parotids, eyes, spinal cord,

and brain tissue. Compared to the conventional parallel opposed treatment fields, this facilitates an accurate delivery of radiation to the tumor and helps achieve higher control.[19-22] Therefore, a precise delineation of the clinical target volume and the normal tissues is required and according to ICRU-50/62, adequate margins have to be defined, accounting for internal organ motion and patient treatment set-up uncertainties. We determined the gross tumor volume (GTV) via clinical examination, endoscopy, MRI, or CT. GTV was included in the clinical target volume (CTV) where RT was conducted to 60 to 70 Gy in fractionation with 2 Gy. For head and neck RT, CTV to PTV margins of 5–10 mm were reported[19, 21] and a 3 mm margin were added to the CTVs to obtain the planning target volume (PTV). This margin was selected based on the set-up accuracy measurements performed with the localization of the tumor. The dose uniformity criteria inside the PTV were defined according to ICRU 50.[23]

Dose-volume data extraction

All the patients scheduled for HN RT were seen in the Department of Oral Oncology to review the oral complications of therapy and any needed dental extractions. Investigations, such as complete blood count, renal function tests, and liver function test, were carried out. Radiological examination included chest x-ray, ultrasound abdomen and pelvis, and CT or MRI scan of the head and neck region. Contrast enhanced CT scan (planning CT) was performed with 2.5 mm slice thickness from the base of skull to the upper mediastinum. Delineation of the tumor and critical organs was performed by manually drawing on the slices of the CT. The beam arrangement was performed by a physicist. IMRT technique typically uses seven beams placed in different angles around the patient. The intensity and shape of the beams were altered during the treatment with the help of a multi-leaf collimator (MLC) of 40 pair with width of 1 cm at isocenter. Once beam geometry was designed, optimization was performed. This was done prior to the calculation

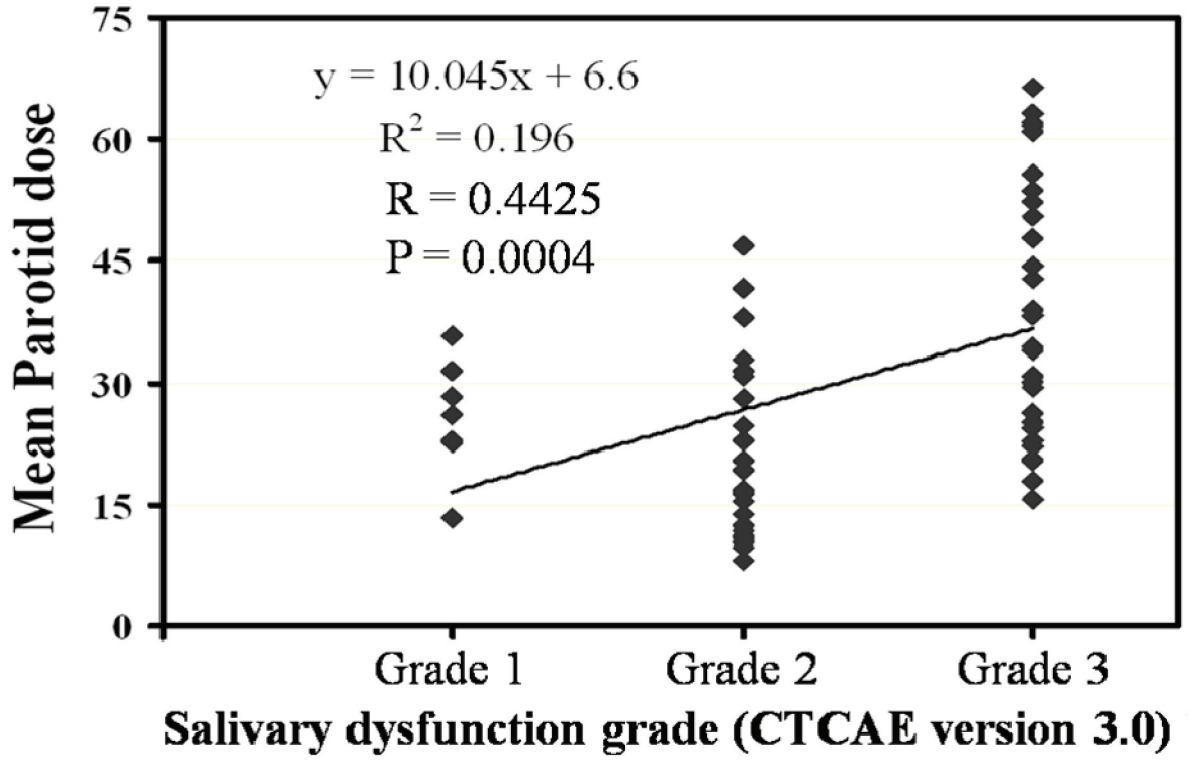

Figure 2. This figure illustrates the correlation between the mean dose to the parotids and salivary dysfunction grade according to CTCAE version 3.0.

CTCAE: Common terminology criteria for adverse events

Table 3. Comparison of the radiation planning, salivary flow rate, and salivary amylase activity based on the radiation dose to the parotids and grade of severity of salivary dysfunction

	Dose of radiation to the parotid gland			Grade of salivary dysfunction		
	Less than 25	Above 25 Gy	*P* value	Minimum	Severe	*P* value
Age (years)	54.79 ± 12.97	53.29 ± 9.40		54.86 ± 11.27	53.06 ± 10.67	
GTV	74.86 ± 65.35	57.32 ± 64.33		72.00 ± 69.66	57.62 ± 60.48	
CTV	348.82 ± 148.04	302.36 ± 127.7		355.85 ± 133.4	290.4 ± 134.54	0.03
Right parotid	15.75 ± 4.73	38.5 ± 16.08	0.0001	22.57 ± 12.04	35.37 ± 18.54	0.008
Left parotid	16.48 ± 4.93	40.71 ± 15.01	0.0001	24.97 ± 12.43	36.31 ± 18.67	0.05
Both parotid	32.22 ± 9.10	79.2 ± 25.80	0.0001	47.53 ± 22.43	71.68 ± 33.45	< 0.0001
Day 0 salivary flow rate ml/min	0.29 ± 0.04	0.29 ± 0.04		0.29 ± 0.03	0.29 ± 0.04	
Day 1 salivary flow rate ml/min	0.28 ± 0.04	0.29 ± 0.05		0.28 ± 0.05	0.30 ± 0.05	
Day 22 salivary flow rate ml/min	0.22 ± 0.03	0.17 ± 0.04		0.20 ± 0.04	0.18 ± 0.05	
Change from baseline in salivary flow rate day 22- day 0	76.99 ± 7.96	58.37 ± 12.66	0.0001	69.88 ± 13.61	62.27 ± 14.13	0.04
Percentage decrease in Salivary flow rate day 22- day 0	23.01 ± 7.96	41.63 ± 12.66	0.0001	30.12 ± 13.61	37.73 ± 14.13	0.04
Amylase day 0	145.04 ± 10.51	147.31 ± 12.58		148.04 ± 10.82	144.97 ± 12.51	
Amylase day 1	154.13 ± 13.12	161.06 ± 13.15	0.05	157.68 ± 11.61	158.81 ± 15.07	
Amylase day 22	116.08 ± 19.48	91.42 ± 22.37	0.0005	107.11 ± 22.6	96.19 ± 25.04	
Decrease in amylase 22	75.59 ± 12.84	56.81 ± 13.35	0.0001	72.62 ± 15.9	66.28 ± 16.25	
Percent decrease amylase on day 22	24.41 ± 12.84	43.19 ± 13.35	0.0001	27.38 ± 15.9	33.72 ± 16.25	

GTV: Gross tumor volume; CTV: Clinical target volume; Salivary amylase activity in units/Liter (one U/L is the activity of enzyme which converts one micromole of the substrate to product in one minute under standard assay conditions)

of the 3D-dose distribution.

The dose calculation was based on PBC algorithm, all with the intent of predicting the delivered dose to the patient. The optimization is based on dose constrains as per RTOG guidelines, with respect to the tumor coverage and minimization of dose to organs at risk (OAR). For each patient the RT plans were generated using the Eclipse version 8.6 planning system (Varian Medical Systems, Palo Alto, CA). The patients were planned to receive the curative target dose of 60-70 Gy, five days a week without any intended gap and no more than one fraction per day of 2 Gy for six to seven consecutive weeks. The patients received cisplatin once weekly as an intravenous infusion at 50 mg/m^2/day (IV) with appropriate hydration and anti-emetic prophylaxis.[24, 25]

Saliva collection

During the first visit, the nature and purpose of the study was introduced to eligible patients satisfying the inclusion criteria, in either English or their mother tongue (Kannada, Tulu, or Malayalam) by the undergraduate student investigator (PS). The subjects were informed that they had the right to withdraw from the study at any time during the course of the study and that their non-willingness to be a part of the study will not deprive them of the necessary planned treatment. The willing patients were then included in the study and a written informed consent was collected. Unstimulated saliva was collected in accordance to the method suggested by Navazesh[26] and at three time points:

1. Before the start of radiation treatment (day 1; before exposure to the first fraction of 2 Gy);
2. The next day after 24 h (day 2; before being exposed to the second fraction of 2 Gy); and
3. On day 22 (after three weeks, after having received 30 Gy that is 15 fractions of 2 Gy, and before the start of the 16th fraction on the start of the fourth week).

Every subject was asked to rinse the mouth with distilled water thoroughly to remove any food debris and then after 10 minutes, requested to salivate into a sterile plastic. Salivary flow rate (ml/min) was measured by the following formula:[26]

$$= \frac{\text{Weight of container with Saliva (g) - Weight of container without saliva (g)}}{\text{Duration of saliva collection}}$$

Once saliva was collected in the plastic container, the saliva was immediately transported to the biochemistry laboratory in an ice box. The collected saliva was centrifuged at 3000 rpm for 10 minutes and the supernatants were stored in cold refrigerator (-20ºC).

Table 4. Incidence of development of different grades of dysfunction based on the severity and radiation dose to the parotids

Week	CTCAE grading for Salivary dysfunction	Salivary dysfunction grade		Chi square/ Fishers exact test	Radiation dose to the parotid gland		Chi square/ Fishers exact test
		Medium N = 28	Severe N = 32	*P* value	Less than 25 Gy	Above 25 Gy	*P* value
Week 0	0	28 (100)	32 (100)	-	24 (100)	36(100)	-
Week 1	0	28 (100)	32 (100)	-	24 (100)	36 (100)	-
Week 2	0	28 (100)	19 (59.4)	0.0001	18 (75)	29 (80.56)	0.61
	1	0 (0)	13 (40.6)		6 (25)	7 (19.44)	
Week 3	0	28 (100)	8 (25)	0.0001	17 (70.8)	19 (52.78)	0.255
	1	0 (0)	22 (68.8)		7 (29.2)	15 (41.67)	
	2	0 (0)	2 (6.3)		0 (0)	2 (5.56)	
Week 4	0	23(82.1)	0(0)	0.0001	11 (45.8)	12 (33.33)	0.16
	1	5 (17.9)	18 (56.3)		11 (45.8)	12 (33.33)	
	2	0 (0)	13 (40.6)		2 (8.3)	11 (30.56)	
	3	0 (0)	1 (3.1)		0 (0)	1 (2.78)	
Week 5	0	18 (64.3)	0 (0)	0.0001	7 (29.2)	1 1(30.56)	0.35
	1	9 (32.1)	4 (12.5)		8 (33.3)	5 (13.89)	
	2	1 (3.6)	23 (71.9)		8 (33.3)	16 (44.44)	
	3	0 (0)	5 (15.6)		1 (4.2)	3 (8.33)	
Week 6	0	5 (17.9)	0 (0)	0.0001	3 (12.5)	2 (5.56)	0.155
	1	16 (57.1)	0 (0)		6 (25)	10 (27.78)	
	2	7 (25)	19 (59.4)		13 (54.2)	13 (36.11)	
	3	0 (0)	13 (40.6)		2 (8.3)	11 (30.56)	
Week 7	1	6 (21.4)	0 (0)	0.0001	3 (12.5)	3 (8.33)	0.04
	2	22 (78.6)	0 (0)		13 (54.2)	9 (25)	
	3	0 (0)	32 (100)		8 (33.3)	24 (66.67)	

CTCAE: Common terminology criteria for adverse events; Salivary amylase activity in units/Liter (one U/L is the activity of enzyme which converts one micromole of the substrate to product in one minute under standard assay conditions)

Estimation of Amylase in the saliva

The stored saliva was removed from cold refrigerator, thawed, and analyzed using appropriate blanks, controls, and standards using the UV-, visible spectrophotometer (Shimadzu, Japan). We assayed the amylase activity in saliva with the kinetic spectrophotometric method as previously described by Balcom and co-workers.[27] The reagent kit was obtained from Crest Diagnostics. The assay was based on hydrolysis of a 2 –chloro–4 nitro phenol salt to chloro nitrophenol (CNP). Quality control procedures were included to ensure accuracy and precision of amylase values.

Clinical evaluation for salivary dysfunction

Salivary dysfunction grading

The changes in the salivary dysfunction were evaluated in accordance to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 (CTCAE, 2006).[28] Briefly, the CTCAE 2006 is categorised as follows: grade 1 = slightly thickened saliva with slightly altered taste (metallic); grade 2 = thick, ropy, and sticky saliva along with markedly altered taste, alteration in diet indicated, and secretion-induced symptoms not interfering with activities of daily living; grade 3 = acute salivary gland necrosis, severe secretion-induced symptoms interfering with activities of daily living, and grade 4 = disabling.

Patient care

All the patients were provided with the standard oral, dental, medical, and supportive care. The patients were provided with povidone-iodine solution diluted 1:100 (Betadine 1 ml and 100 ml water) as recommended by Madan and co-workers.[29] Dental cleaning was recommended thrice daily (early morning, after lunch and before retiring for the day) using a soft tooth brush. The patients with spontaneous gum bleeds were provided with cleaning solutions. The patients were asked to eat 30 minutes after mouth wash at least. As all the patients were in the hospital during the treatment period, it was easy to monitor their adherence to diet, medications, practice of oral hygiene, and mouthwash.

Statistical analysis

The values were expressed as mean with standard deviation. The demographic and tumor details were expressed as frequency and percentage. The biochemical data was stratified as those who received less than 25 and above 25 Gy to the parotids and minimum or severe grade

of salivary dysfunction by the end of the treatment. We evaluated the significance of the difference of the values between the groups via Analysis of Variance (ANOVA) and Bonferroni multiple comparison. Statistical significance for the difference in the amylase was carried out using the paired "t-test". The correlation between the mean dose to the parotids and the severity of salivary dysfunction (moderate versus severe; grades (1 versus 2 versus 3) were analyzed through Karl Pearson's Correlation Analysis. Statistical analyses were performed with SPSS software (SPSS Inc., Chicago, IL) and a value of $P < 0.05$ was considered significant.

Results

The sociodemographic and clinical characteristics of the participants are summarized in table 1. The mean age of the patients was 53.88 ± 10.89, with the majority in the age group of 51 to 60 (45%) and 76.67% of the patients were men (Table 1). Majority of the patients had a smoking (cigarette or beedi) and alcohol habit (Table 1). The patients affected with cancer in the oropharynx region were highest in the study population 13.33% (8/60) (Table 2). With regard to tumor pathology, most patients had T3 (40%) and N2 (50%) (Table 2). Majority of the patients received chemo-radiation (93.33%), 69.13 ± 1.66 radiation dose and in 34.46 ± 0.89 fraction size (Table 2). Data on the radiation dosimetry, such as GTC, CTV, and dose to parotids, are represented in table 3. The correlation between the mean dose to the parotids and the severity (R=0.4718; P = 0.0001; Figure 1) and grades in accordance to CTCAE version 3.0 (R = 0.4425; P = 0.0004; Figure 2) was significant.

On exposure to radiation, the salivary flow rate decreased from 0.29 ± 0.02 ml/min on day 0 to 0.28 ± 0.05 on day 1 and to 0.20 ± 0.04 on day 22 (Table 3). The change in salivary flow rate between days 0 to 22 was statistically significant (P = 0.0001; Table 3). In comparison with those with mild salivary gland dysfunction, the salivary flow rate on day 22 was significantly lower in the patients with severe salivary gland dysfunction (P = 0.045). Salivary amylase activity was significantly changed from 147.69 ± 11.15 U/L on day 1, 158.28 ± 11.74 IU/L on day 2 to 109.07 ± 23.21 U/L on day 22 following radiation therapy. The change in salivary amylase between days 1 / 2 and day 22 was of statistical significance (Table 2). The salivary amylase on day 22 was significantly lower in the patients with severe salivary gland dysfunction compared with those with mild salivary gland dysfunction (P = 0.014). The increase in salivary amylase on day 1 post-RT was significant (P = 0.05; Table 2). The change in the incidence of salivary dysfunction was considered on a weekly basis from both the CTCAE grading grade and dose to the parotid gland and was significant at week 7 (Table 4).

Discussion

The results of the current study indicates that on exposure to radiation, there is a decrease in the salivary flow rate (0.29 ± 0.02 to 0.20 ± 0.04; P = 0.0001) and in the activity of salivary amylase (147.69 ± 11.15 to 109.07 ± 23.21 U/L; P = 0.0005). The salivary flow rate and amylase were less in the patients with severe salivary gland dysfunction (P = 0.014) and in those who had a cumulative dose of radiation more than 25 Gy to the parotid glands (P = 0.04).

Clinically, curative radiation to HNC region causes salivary dysfunction, leading to hypofunctioning of the glands and xerostomia and affects the oral health, functioning, general health, and quality of life of the affected individual.[2] Our results were in agreement with those of earlier studies reporting a decrease in salivary flow rate from a baseline value (Tables 3 and 4) one to six months post-radiation therapy by different investigators.[2, 6]

In the current work, a marginal increase in the salivary amylase on day 1 post-RT and a significant decrease in salivary amylase (1.4-fold) on 22 days post-RT were observed and are represented in table 3. A significant change from the baseline value was evident on day 22 post-RT and the decrease was 1.4-fold. Decreased α-amylase activity was observed after six weeks of radiation treatment of oral cancer in a previous study,[6] which is attributed to the reduction in the

number of acinar cells, incomplete tissue regeneration, and late stromal effects, such as delayed vascular damage due to radiation. Vedam and co-workers reported a significant decrease in the levels of salivary amylase from baseline to three weeks of RT, followed by an increase in quantity at six weeks of RT.[16] From a histopathological view point, the decrease in the activity of salivary amylase activity post irradiation at three weeks has been attributed to the decrease in quantitative number of the acinar cells, compromised tissue regeneration, and changes in the stromal vascular changes. Concomitantly, the increase in the levels after six weeks is shown to be due to hyperamylasemia and significant reduction in the saliva flow rate due to chronic inflammation and parotitis.[16] Other researchers have reported decreased salivary amylase post-RT even with stimulated saliva samples.[30]

There have been studies with serum levels of salivary isoenzyme of amylase which observed an increase in the enzyme levels post-RT. Leslie and co-workers (1992) estimated the serum amylases prior to and at 24-h intervals following the start of RT in HNC patients.[31] The treatment volume in this study included all the major salivary glands and the results suggested a significant increase in the serum amylase, with peak values being observed at 24–48 hours post irradiation.[31] Moreover, reports do suggest that the peak rise in serum amylase was more in people undergoing hyper fractionated accelerated schedule in comparison with conventional fractionation, clearly indicating that the fractionation size has a role.[31] Reports also suggest that transient hyperamylasemia occurs after exposure to a dose of 1–2.75 Gy, with an optimal increase being observed at 9–36 hours post radiation.[32] Additionally, exposure to fractionated doses in the range of 1.8 to 4 Gy per day is also shown to increase serum amylase levels.[33]

From a clinical perspective, the increase in serum salivary amylase on exposure to salivary glands irradiation is important. Histopathological studies have revealed this to be due to the damage to serous cells and alternations in their cell membrane permeability, which then consequentially results in the release of intracellular amylase into the saliva. Additionally, exposure to ionizing radiation causes loss in the architecture of acinar cells, infiltration of inflammatory cells, and concomitant damage and vacuolation of the serous cells.[31] From a radiobiological view point, the parotid glands producing salivary amylase are highly radiosensitive and exposure to radiation severely affects the tissues and serous secretion.[16] Our study group had observed previously that the amylase levels in saliva and serum (1 day post-RT) significantly increased after exposure of HNC patients to 2 Gy of radiation, thereby suggesting that they did not have any utility as a predictive biomarker.[17] Studies have demonstrated that amylase is a sensitive biomarker for stress-related changes in the body, which reflects activity of the sympathetic nervous system and in chronic stress.[34] Stress induced by the disease and the radiation therapy among the HNC patients could be a factor for the increase in salivary amylase, observed on day 1 post-RT in the present study.

Herein, we observed a significant correlation between salivary amylase and salivary flow rate both in patients with mild salivary gland dysfunction and severe dysfunction on days 1 and 22 after radiation therapy. Previously, Arhakis and co-workers[35] observed a positive correlation between salivary amylase and age along with a negative correlation between salivary amylase and the interaction of flow rate and age in healthy young adults. In the absence of a stressful stimulus, the flow rate, age, and the interaction of these two factors affects the secretion of salivary amylase in healthy young adults.[35] There is a lack of convincing data on the correlation of salivary amylase with the salivary flow rate in HNC patients undergoing RT and the present research provides such evidence of a negative correlation of salivary amylase with flow rate. The contradicting observations of increased or decreased salivary amylase with RT in various studies could be due to the type of salivary sample (stimulated or unstimulated whole saliva), dose, and duration of radiation in RT, volume of tissue affected by the radiation, and confounding factors, such as stress and age.

The greatest drawback of the study is that we considered only two time points post irradiation. Studies should be planned to understand the most effective saliva sampling time point during the course of the curative treatment especially after the first fraction of 2 Gy and before the next fraction of 2 Gy considering radiation to all the salivary glands and considering the DVH of the parotid gland. The outcome of the planned extended study will be of immense help in asserting which time point of saliva collection and amylase assay will be important in predicting the salivary dysfunction.

Conclusion

The results of the study indicated for the first time that quantification of salivary amylase levels on day 22 post irradiation could be an important marker to predict salivary dysfunction. The limitation of this study was that we considered only two time point post-irradiation and studies should ascertain the most effective time point post irradiation as this will enable researchers determine the optimal evaluation time point for the assay to be performed to develop salivary amylase as a predictive assay for salivary dysfunction.

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Conflict of Interest

None declared.

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