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# Evaluation of Dose Dependent Changes in Flow Rate, Ph, Total Protein and Alpha Amylase Content of Whole Saliva in Patients Undergoing Head and Neck Radiation.

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***Abstract:***
***Objective:****The aim of the study was to estimateand evaluate the dose dependent changes in the salivary flow rate, pH, level of total protein, and αamylase during the course of radiotherapy in oropharyngeal cancer patients.*
***Patients and method****: The study group comprised of 50 patients undergoing radiotherapy for oropharyngeal carcinoma in a tertiary care centre in Chennai. Cobalt 60 equipment was used for radiation treatment. A weeklydose of 10 Gy for about 6 to 7 weeks with total dose of 60 to 66 Gy was administered. The whole saliva was collected from these patients in eppendrof sample tubes by spitting method after stimulation by a piece of paraffin wax. The salivary samples were collected prior to and during radiation treatment of 10 Gy, 40 Gy and 60 Gy.. The salivary flow rate, pHwas measured and the level of total protein and alpha amylase were estimated by using chemical diagnostic test kits.*
***Result****s:The study revealed that there was 42.5% reduction in the salivary flow rate after the dose of 10 Gy, 65% reduction after the dose of 40 Gy and about 77.5% reduction after the dose of 60 Gy. there was reduction in the pH of saliva for about 0.46 units after the dose of 10 Gy, for about 0.82 units after 40 Gy and for about 1.14 unit after 60 Gy. It was found that there was reduction in the level of salivary amylase for about 36.1% after the dose of 10 Gy (after 1 week), for about 78.5% after the dose of 40 Gy (after 4 weeks) and for about 90.9% after the dose of 60 Gy.). The present study revealed that there was increase in the level of total protein for about 18.9% after 10Gy, 6.16% increase after 40Gy and 83% increase in the level after 60 Gy(6 weeks).*
***Key Words:*** *radiotherapy, salivary flow rate, pH, salivary amylase, protein*

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## I. **Introduction**:

Radiotherapy is indicated for majority of head and neck cancer patients. Depending on the extent and stage of the malignancy it can be either used alone or in combination with chemotherapy or surgery. Radiation therapy for malignant tumor in oral and oropharyngeal region usually involves the salivary glands in the irradiated volume and hence has a damaging effect on the salivary glands located within the radiation beam. The extent of damage to the serous and mucous acini in the salivary glands depends upon on their sensitivity to radiation injury.[(1,2,3)]

Treatment of oral carcinoma conventionally involves the administration of a dose of 60Gy to 70Gy and this can lead to a rapid decrease in flow during the first week of radiation. By 5 weeks of radiation, the flow virtually ceases and rarely recovers completely. Both resting and stimulated salivary flow are inhibited.[(4,5,6)]

The small volume of viscous saliva that is secreted in these patients usually has a pH value 1 unit below normal (i.e. an average of 5.5 in irradiated patients compared to 6.5 in unexposed individuals). This pH is low enough to initiate decalcification of normal enamel.[(7).] There are numerous antimicrobial systems in saliva, which perform modulatory and protective roles mucin, electrolytes and proline – rich polypeptides govern microbial attachment to oral tissues. Secretory IgA binds to bacteria and fungi leading to aggregation. Aggregated IgA binds to polymorphs and can also activate the alternate complement pathway. Histidine – rich polypeptides are thought to play a major role in the prevention of oral candidiasis.[(8)]

**1.OBJECTIVES:**

The main objectives of this study were to estimate the pH and flow rate, total protein and alpha amylase level whole stimulated saliva in a group of subjects undergoing head and neck radiation and to evaluate the dose dependent changes in the concentration of total protein, amylase, pH and flow rate of whole stimulated saliva before and after $1^{st}$, $4^{th}$ and $6^{th}$ week of radiotherapy

## II. Methodology

2-1:The study group comprised of 50 subjects undergoing treatment in the Department of Radiation Oncology, in a tertiary care hospital Chennai.The individuals who were planned to undergo a minimum of 6 weeks of radiation treatment for carcinoma in the head and neck region were selected. Of the fifty individuals, the group consisted of 34 males and 16 females. The minimum age of female was 35 years and maximum age was 70 years and the mean age was 53.6 years. The minimum age of males was 28 years and the maximum age was 69 years and the mean age was 55.5 years.The location of tumours are as follows ,Oropharynx (thirteen), nasopharynx (four), tongue (fifteen), maxilla (three), mandible (two), and buccal mucosa (thirteen).The individuals in the study group did not have any systemic diseases like hypertension, diabetes, cardiac problems and renal failure. No other medications known to affect salivary flow existed among these patients.Cobalt-60 equipment was used for radiation treatment. The weekly dosage was 10 Gy. The total dosage given varied from 60 to 66 Gy and administered 5 days in a week over a period of 6 to 7 weeks.

**2-2:Collection of saliva:**

The subjects were instructed to refrain from eating, drinking, and smoking one hour preceding the saliva collection. The collections were made on non-fasting subjects between 8:00 to 10:00 a.m. and 2:00 to 4:00 p.m.The patient was first instructed to chew a piece of paraffin wax of $1cm^3$. After 30 seconds the patient was asked to expectorate (spit) into the spittoon to discard their first generated saliva. Then, they were allowed to continue chewing the piece of wax for a further 5 minutes, expectorating every 60 sec into a preweighed graduated Eppendorf sample tube for 5 minutes.The paraffin stimulated whole saliva was collected of every patient as mentioned above once 2 o 3 days before the first irradiation and then during the treatment after the cumulative dosage of 10 Gy, 40 Gy and 60 Gy. For patients who had very sparse saliva secretion, the collection was extended to 15 minutes. Then the saliva samples were stored at $-20^oC$ until analysed.

**2-3: Estimation of salivary flow rate and pH**

The samples were centrifuged at 4500 rpm for 15 minutes to remove debris. The volume of saliva was measured by weighing the sample tubes in the electronic weighing machine. Salivary flow rate was expressed in – ml/min.To assess the pH of saliva ,the stimulated saliva was collected from patients as mentioned earlier. The pH indicator strip was placed into the sample of saliva for 10 seconds. Then the colour of the strip was checked and it should be compared with the testing chart provided along with the kit. The range of pH from 5.0 to 8.0 can be checked using this saliva pH indicator.The salivary protein was estimated by Biuret method [(9).]The salivary amylasewas estimated byPNPG blocked method (Modified Saccharogenic method of Wellenfels et al) [(10,11)].

## III. Results

Our study was directed to analyze the flowrate, pH, amylase and total protein in the saliva of 50 patients with oral and oropharyngeal cancers before and consecutively after 1 week, 4 weeks and 6 weeks of radiotherapy. The study subjects were given a total dose of radiation of about 60 to 66 Gy, with a fractionation dose of 200 mGy per day in the range of 6 to 7 weeks.

3-1:SALIVARY FLOW RATE**:**Out of 50 study subjects before radiation, the minimum salivary flow rate was 0.32 ml/min and the maximum salivary flow rate was 0.7 ml/min. The mean value of salivary flow rate for 50 study subjects before radiation was 0.80 ± 0.32 ml/min (Table -1). the salivary flow rate was assessed after 1 week of radiation for the same 50 patients. The mean value of salivary flow rate after I week was 0.46 ± 0.25 ml/min (Table -1). There was a significant reduction noted between the preradiation flow rate and flow rate measured after I week of radiation (p= 0.000). The salivary flow rate was measured after 4 weeks of radiation, for the same 50 study subjects. It was observed that there was reduction between the preradiation flow rate and the value measured after 4 weeks of radiation which was statistically significant (P = 0.000) (Table – 1). After 6 weeks of radiation, the salivary flow rate was assessed for the same 50 study subjects. The mean value of salivary flow rate was 0.18 ± 0.14 ml/min A significant reduction mean flow rate was observed betweenpreradiation flow rate and the value assessed after 6 weeks of radiation, which was statistically significant (p=0.000) (Table 1)

3-2: SALIVARY pH**:**In the 50 study subjects, the minimum value of salivary pH was 5.40 and the maximum value of salivary pH was 1.32 before radiation. The preradiation mean value of salivary pH for the was 6.99 ± 0.45 (Table -2). Out of 50 study subjects, the salivary pH was assessed after I week of radiation. The minimum salivary pH of 5.80 and a maximum salivary pH of 7.40 was observed. The mean value of salivary pH after I week of radiation was 6.53 ± 0.38. There was a significant reductionin the pH after I week of radiation. (P = 0.000) (Table – 2). with a mean value of 6.53±0.38**.** The mean value of salivary pH after 4 weeks of radiation was observed. A significant reduction was noted between the pH of saliva before radiation and after 4 weeks of radiation (P = 0.000) (Table – 2). It was found that the difference between the pH of the saliva before radiation the pH of the saliva before radiation and after 6 weeks was statistically significant (P= 0.000) (Table 2).

3-3: SALIVARY AMYLASE:Estimation of salivary amylase before radiation in 50 study subjects showed a minimum value of 168.90 U/L and a maximum value of 427.80 U/L. The mean value of salivary amylase of 267.09 was observed (Table – 3). Estimation of salivary amylase after 1 week of radiotherapy in the same 50 study subjects showed a minimum value of 74.30 U/L and a maximum value of 379.10 U/L. The mean of salivary amylase after 1 week of radiation was 170.62 U/L. There was significant reduction in between the mean value before radiation and the value recorded after 1 week of radiation, (P = 0.000) (Table – 3). The level of salivary amylase was estimated after 4 weeks of radiotherapy in the same 50 individuals of the study group. A minimum a value of 7.02 U/L and a maximum value of salivary amylase of 264.63 U/L was observed. It revealed a mean value of 57.41 U/L of salivary amylase after 4 weeks of radiation. There was a significant reduction between preradiation value and value estimated after 4 weeks of radiation (P = 0.000) (Table- 3 &).In the same 50 study subjects, the level of salivary amylase was measured after 6 weeks of radiation. A minimum value of 107.13 was observed. The mean value of salivary amylase after 6 weeks of radiation was 24.04 U/L. Between the preradiation value and the value estimated after 6 week of radiation which was statistically significant (P = 0.000) (Table-3).

3-4:SALIVARY PROTEIN**:**In the present study, the total protein in the whole stimulated saliva was measured prior to radiation. The minimum value of salivary protein of 0.81 g/dl and a maximum value of 1.94 gl/dl were observed. The mean value of salivary protein prior to radiation was 1.37 g/dl (Table- 4). After 1 week of radiation, the level of total protein in the whole saliva was estimated and it showed a minimum value of 0.95 g/dl and a maximum value of 2.52 g/dl was observed after 1 week of radiotherapy. There was a significant increase in the level of total protein in the saliva between the preradiation value and the value after 1 week of radiation (P = 0.000) (Table- 4 ).Estimation of total protein after 4 week of radiation in the same 50 study subjects showed a minimum value of salivary protein of 0.82 g/dl and a maximum value of salivary protein of 2.01 g/dl. The level of total protein had significantly increased b/w the preradiation and after 4 weeks (P = 0.000), but showed significant decrease between the levels after 1 week and after 4 week (P = 0.000) (Table- 4). The level of total protein in the stimulated whole saliva was measured in the same 50 study subjects after 6 weeks of radiation. A minimum value of 1.12 g/dl and a maximum value of 2.98 g/dl were observed. The mean value of salivary protein of 2.05 g/dl was observed after 6 weeks of radiation. A statistically significant elevation was noted in the level of total protein between the preradiation level and level after 6 weeks radiation (Table-4).

**Table No:1**

| Parameter | Mean | SD | Median | Minimum | Maximum |
|---|---|---|---|---|---|
| Flow rate – BR | 0.80 | 0.32 | 0.84 | 0.32 | 1.32 |
| Flow rate – 1 | 0.46 | 0.25 | 0.42 | 0.11 | 0.96 |
| Flow rate – 4 | 0.28 | 0.18 | 0.23 | 0.05 | 0.74 |
| Flow rate – 6 | 0.18 | 0.14 | 0.12 | 0.04 | 0.56 |

**Table No: 2**

| Parameter | Mean | SD | Median | Minimum | Maximum |
|---|---|---|---|---|---|
| pH-BR | 6.99 | 0.45 | 6.90 | 5.40 | 7.80 |
| pH -1 | 6.55 | 0.38 | 6.40 | 5.80 | 7.40 |
| pH -4 | 6.17 | 0.38 | 6.20 | 5.40 | 7.00 |
| pH -6 | 5.85 | 0.40 | 5.80 | 5.20 | 6.80 |

**Table No: 3**

| Parameter | Mean | SD | Median | Minimum | Maximum |
|---|---|---|---|---|---|
| Amylase-BR | 267.09 | 62.07 | 270.50 | 168.90 | 427.80 |
| Amylase-1 | 170.62 | 69.57 | 164.50 | 74.30 | 379.10 |
| Amylase-4 | 57.41 | 45.68 | 45.86 | 7.08 | 264.63 |
| Amylase-6 | 24.04 | 23.22 | 14.41 | 4.02 | 107.13 |

**Table No: 4**

| Parameter | Mean | SD | Median | Minimum | Maximum |
|---|---|---|---|---|---|
| Total protein-BR | 1.37 | 0.24 | 1.38 | 0.81 | 1.94 |
| Total protein-1 | 1.69 | 0.30 | 1.69 | 0.95 | 2.52 |
| Total protein-4 | 1.46 | 0.25 | 1.50 | 0.82 | 2.01 |
| Total protein-6 | 2.05 | 0.44 | 1.97 | 1.12 | 2.98 |

BR- Before Radiation ;1 – After 1 week; 4 – After 4 weeks ; 6 – After 6 weeks

## IV. Discussion

The normal individuals will have an unstimulated salivary flow rate of 0.1-0.5ml/ min and a stimulated flow rate of 1.1-3.0 ml/min.Ionizing radiation causes glandular tissue damage, which may result in a rapid, irreversible loss of salivary fluid secretion. The glandular architecture is replaced by ductal remnants and loose fibrous connective tissue which is moderately infiltrated with lymphocytes and plasma cells. This progressive glandular, atrophy, fibrosis and reduced salivary output begins slowly after initial exposure and intensifies thereafter.

Reduced salivary flow and simultaneous qualitative changes in the protein and electrolyte content of whole saliva have been considered the main cause for the frequently detected rapid development of post radiation caries and the quantitative and qualitative shifts in oral microflora. Cross sectional and longitudinal studies of the influence of radiation induced xerostomia on the human oral microbiota reveal that the reduction in salivation is parallel by a shift to a highly cariogenic microflora[12,13,14].Dreizen S in 1990, reported that greater than 50 percent decrease in salivary flow has occurred after one week (10 Gy over five days), with a greater than 75% decline in salivary flow after six weeks treatment (60 Gy total dose) [15] .The results from our study revealed that there was 42.5% reduction in the salivary flow rate after the dose of 10 Gy, 65% reduction after the dose of 40 Gy and about 77.5% reduction after the dose of 60 Gy.This indicates that salivary glands in the field of radiation gets affected as the radiation dose increases from first week to sixth week of radiotherapy.

The normal pH of saliva ranges from6.2 to7.6.T. Vuotila et al in 2002[16] studied the whole saliva samples of 39 head and neck cancer patients having radiation therapy, which were collected before, during and after radiation therapy. They found that the salivary flow rate, buffer capacity and pH decreased, and the levels of lactobacilli increased significantly during the first half of the radiation therapy.

Peter Moller et al in 2004[17], who conducted study on 54 patients with advanced squamous cell carcinoma who were treated with radiation alone or in combination with surgery or chemotherapy or both. The pH of whole stimulated saliva (WSS) and whole saliva resting saliva (WRS) was measured electromechanically by using an Orion pH meter 230 A and a Ross electrode model 81 – 35From our resultswe noticed that there was a significant reduction in the pH of saliva for about 0.46 units after the dose of 10 Gy, 0.82 units after 40 Gyand 1.14 unit after 60 Gy.

Tomasik A et al in 1994[18] measured the serum and salivary alpha-amylase for controls and patients with laryngeal carcinoma, before and after localized irradiation including salivary glands. They observed significant increase in serum amylase after irradiation. Alpha-amylase activity in saliva was decreased after irradiation but differences were not statistically significant due to the significant decrease of protein in saliva of irradiated group.From our study it was observed that as the course of radiotherapy advanced there was reduction in the level of salivary amylase for about 36.1% after the dose of 10 Gy (after 1 week), for about 78.5% after the dose of 40 Gy (after 4 weeks) and for about 90.9% after the dose of 60 Gy (after 6 weeks).

Tuula A. Makkonen et al in 1986[19] studied eleven patients treated for malignant conditions of the head and neck and analyzed the radiation induced changes in the flow rate and protein composition of stimulated whole saliva of those patients. They observed unusually high salivary concentrations of albumin, lactoferrin, lysozyme, salivary peroxidase, myeloperoxidase, and total protein during the therapy, but most values slowly returned to pretreatment levels after cessation of radiation.In our study it was revealed that there was an initial increase in the level of total protein for about 18.9% after 10 Gy , just 6.16% increase after 40Gy and 83% increase in the level after 60 Gy (6 weeks) of radiation .Thisdose dependent increase in total protein level may be correlated with the reduction in mean salivary flow .

## V. Conclusion

From this study we had observed that there were a dose dependent qualitative and quantitative changesin pH,flow rate,alpha amylase and total protein content of saliva. At the end of radiation treatment, the patients suffer with severe xerostomia,candidiasis,difficulty in swallowing. More research is necessary in the field of radiotherapy in sparing the salivaryglands which liesin field of radiation while treating head and neck cancer patients in order to restore the function of saliva and to maintain an optimum oral health.

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