Research Article
Effect of Head and Neck Radiotherapy on Saliva Biochemical Indicators
Donia Sadri, Alireza Abdollahi, Zahra Tehrani, Saeede Ghanbari

Abstract
Purpose: Saliva plays a crucial role in preserving and maintaining oral health. Hyposalivation due to head and neck radiation therapy can induce changes in the biochemical properties of the saliva. The aim of this study was to Analyze and compare saliva properties before and after radiation therapy in patients with head and neck cancers. Materials & Methods: In this experimental study eighteen patients with head and neck cancers were evaluated about the flow rate of saliva, buffering capacity, amylase, total protein, IgM, IgA, IgG and albumin concentration of saliva prior to and following radiotherapy using schirmer test, pH meter, Biuret test, turbidimetry and spectrophotometry respectively. The oral symptoms were evaluated according to the patient’s complaints. Statistical analysis of the results was performed with paired t test. Results: There were statistically significant changes in flow rate (28.27±7.8 Vs 10.94±12.2), salivary buffering capacity (6.47±0.68 Vs 5.06±0.52), amylase (2536.5±707.9 Vs 1053.23±343.5), and IgM concentration (0.87±1.8 Vs 0.04 ±0.07) before and after radiation therapy. (p<0.05). There were no significant changes in total protein, albumin IgA and IgG concentration after radiotherapy. Clinically problems like Xerostomia, loss of taste, difficulty in swallowing, eating and speaking were also found associated. Conclusion: Results of the present study shows that head and neck radiation therapy leads to the reduction of salivary flow rate, buffering capacity, amylase and IgM concentrations.

Key words: Saliva; Radiation Therapy; Amylase; Buffering Capacity; Immunoglobulin; Flow Rate.

Introduction
Head and neck carcinoma (HNC) is the sixth most common cancer worldwide, accounting for 2.8% of all malignancies. Although the use of chemotherapy is increasing, radiation therapy and surgery are still two main modalities of head and neck cancer treatment.\(^1\) Radiotherapy (RT) causes both acute and long term side effects on normal tissues. Acute side effects are mucositis, dysphagia, skin erythema, and desquamation.\(^2\) Long term complications are the result of damage to vessels, salivary glands, mucosa, connective tissue, and bone. Type and severity of these changes directly depend on the dosimetry, total dose received, amount of radiation divisions (fraction size), and duration of treatment. Some late sequelae such as osteonecrosis, fibrosis, trismus, loss of taste sensation, dental decay and destruction of tissues in the middle or inner ear may occur.\(^3\)

Xerostomia is one of the most prominent complications of radiation therapy in patients with head and neck cancer since, high-dose radiation cause damage to salivary glands bilaterally.\(^4\) Dysfunction of salivary glands changes the volume, consistency and pH of secreted saliva. Patients suffer from oral disorders or pain with difficulty in speaking, chewing and swallowing. Changes in the amount of saliva and its biochemicals (amylase, proteins, Immunoglobulins and buffering capacity) increase the risk of dental caries and oral fungal infection which lead to decreased nutritional intake and weight loss in patients undergoing treatment. Complications of radiation therapy affect the quality of life and health issues of HNC patient’s negatively.\(^5\)

Radiation-induced xerostomia rapidly occurs during treatment: in the first week there is a reduction in salivary flow to 50-60% and in the seven weeks salivary flow diminishes to 20 percent.\(^6\) Saliva producing cells evidently do not vanish, but lose their function during the first days after radiation therapy. Koning et al proposed two mechanisms for radiation-induced salivary dysfunction: First, impairment of selective membrane and receptor-mediated signaling pathways of water excretion lead to cell dysfunction but, immediate cell death and lysis do not occur. Second, classical cell killing of progenitor cells and stem cells interrupt the proper cell renewal, and cause damage to the cellular...
environment thus, secretary cells function improperly for a short time.\textsuperscript{7} Salivary function continues to decline for several months after radiotherapy.\textsuperscript{8}

Depending on the dose received and the amount of radiation exposure of salivary glands, a recovery may happen after 12 to 18 months. But, generally xerostomia as an irreversible problem remains throughout the lifetime.\textsuperscript{9,10} Considering the effect of ionizing radiation on salivary gland tissue structures, the purpose of this study was to analyze some salivary biochemical properties after radiation therapy.

**Materials and Methods**

This study was approved by the Ethics committee with the Helsinki Declaration of 1975, as revised in 2000. All patients received detailed information about the study and signed an informed consent form. This quasi-experimental study was done on eighteen patients with head and neck cancer referring to Cancer Institute and Radiotherapy Department from Feb 2009-June 2010. All patients met the following inclusion criteria: Having head and neck cancer, at least one of major salivary glands should be within the radiation fields, no history of previous radiotherapy.

Considering the related articles and pilot study, 18 patients were evaluated before and after radiotherapy. The participants were asked to not to eat, drink, and smoke one hour prior to saliva collection, for preventing circadian changes sampling was done from 9 to 11o clock in the morning at the separate room and suitable condition.\textsuperscript{9} In order to collecting whole saliva samples, the patients were asked to spit at least 2 ml of their saliva into measuring containers. Modified Schirmer test was used to calculate saliva flow rate on a mm/5 min basis and pH was measured by pH meter.\textsuperscript{12}

Samples were collected before RT and once again in the last session of radiotherapy and centrifuged at 2500 RPM at 4°C for 5 minutes and stored frozen at -70°C for future analysis: Biuret test was used to determine total protein concentration. In this technique, the copper atoms of Biuret solution will react with peptide bonds, producing a color change. A deep violet color indicates the presence of proteins. A photometric color test was used to determine the amount of Albumin. Albumin with Bromocrosol green in an acidic pH will form a green complex.\textsuperscript{13,14}

The amounts of IgA, IgG, and IgM (using kit bio system) were assessed by Turbidimetric method.\textsuperscript{15,16} All of the above factors were measured by auto analyzer system BT3000" Biotechnica ™ Italy”.

Saliva samples were diluted with Normal saline (saliva: saline ratio was 1:10) to measure the amount of amylase using manual spectrophotometry and the values were recorded in IU/L. After the last session of radiotherapy (before taking saliva samples), oral signs and symptoms were recorded and expressed as percentage. Statistical analysis using pair t-test was done to compare the amount of salivary flow rate, PH, total protein, albumin, amylase, IgA, IgG, and IgM before and after radiation therapy.

**Results**

A total of 18 patients were assessed for this study (mean age 57.05 ± 13.3 years); 11 patients had Squamous cell Carcinoma (SCC) and 7 subjects were diagnosed with other types of cancer of the oral cavity. Five patients (27.8%) had the history of smoking, and 14 patients (77.8%) had history of previously surgery. Average dose of radiation received by patient was 5822.2 ± 942 CGy and the average number of RT sessions was (30.05 ± 3.7) minimum 20 and maximum 35 sessions. Clinical evaluation determined that all patients were suffering from loss of sense of taste and dry mouth especially at night. Three patients (16.7%), reported difficulty in swallowing and fifteen patients (83.3%) had difficulty in speaking.

According to this study, the flow rate, PH, the amount of amylase (Graph 1), and IgM (Graph 2) were significantly reduced in saliva samples but, there were no significant changes in the amount of IgG, IgA, total protein, and albumin after the last session of radiotherapy (Table 1).

**Discussion**

The present study shows a decrease in salivary flow rate after radiation therapy which leads to xerostomia and other problems in patients. Recent studies confirm these findings.\textsuperscript{11,12,18,19} This is because the excretory and acinar cells of the salivary glands are highly differentiated with a slow mitotic rate and turnover, but they behave like acute responding tissues to radiation and are very radio-sensitive. Therefore, an irreversible degenerative process in salivary glands leads to fibrosis of parenchyma
[nerves, vessels] and selective membrane damage, confounding the receptor mediated signaling pathways of water excretion.\(^5\)

In the present study IgA and IgG levels demonstrated a tendency to decrease but only IgM levels had a statistically significant reduction (P<0.05)(Graph 2). IgA in saliva is produced by plasma cells in the connective tissue around the intra-lobular ducts of the major and minor salivary glands and its secretion is regulated by interaction between T and B lymphocytes.\(^22\) It seems that high dose radiation has an influence on these cells and IgA concentration.\(^23\)

Salivary IgM are primarily derived from serum via GCF, It can be suggested that an atrophic oral mucosa and neuropathy caused by RT prevent transudation of IgM from serum into saliva.\(^24\)

In this study, we observe no significant increase in IgG level after radiation therapy. Other similar studies show that there is no difference between salivary IgG concentration before and after RT or a slight increase in that because of mucosal and sulcal transudation of serum IgG into saliva.\(^23\) Evaluation of IgM and IgG requires more investigations in future due to lack of information in this area. Some research show an increase in IgA concentration after radiation therapy.\(^19,25\) The lack of agreement in these findings may be due to different sampling time after radiotherapy and methods of immunoglobulin measurement.

Secretory IgA protects the mucosa against microbial infections. Secretory IgA is produced by plasma cells and receptors pass it through the epithelial cells into lumens.\(^22\) It seems that radiation causes damage to epithelial cells and impairment of the transudation of secretory immunoglobulin into saliva.

The results of this study as well as Cassio de Barros pontes’s study show an increase in the amount of total protein and albumin after RT but not statistically significant.\(^12\) But in the other study this increase was significant.\(^19,11\) This is probably due to the difference in sampling time after radiation therapy and number of studied cases. In mentioned studies, the sampling time was 2-6 months after completion of radiation therapy. However, sampling time of this study was immediately after the last session of radiotherapy. Radiation therapy causes acute complications and mucositis and impairment of transudation of serum albumin in saliva.\(^24\)

Reduced salivary flow causes an increase in the number of acidogenic microorganisms in saliva and dental plaque. The lower pH enhances the risk of candidiasis infection and dental caries.\(^19\) The present study shows a significant decrease in salivary amylase levels following radiotherapy (P<0.05) (Graph 1). The results are similar to other studies.\(^11,12,16,19\)

Salivary amylase enzyme is considered as a reliable enzyme to determine the serous cell functionality. Acini in the parotid glands are almost exclusively of the serous type and makes parotid glands more sensitive to radiotherapy than sublingual and submandibular glands.\(^20\) Considering the results of this study, reduced amylase levels may be caused by parotid gland serous cells dysfunction. But, Takei T et al., suggest that the reduction in overall salivary amylase levels may be result of both salivary dysfunction and reduction of food intake during RT treatment.\(^21\)
Table 1: Salivary Flow rate and biomarkers changes in 18 irradiate patients

<table>
<thead>
<tr>
<th>Flow rate (mm/5min)</th>
<th>PH</th>
<th>IgA (mg/dl)</th>
<th>IgM (mg/dl)</th>
<th>IgG (mg/dl)</th>
<th>Total protein (mg/dl)</th>
<th>Amylase IU/L</th>
<th>Alb (mg/dl)</th>
<th>irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.27±7.8</td>
<td>6.47±0.68</td>
<td>22.11±11.69</td>
<td>0.87±1.18</td>
<td>0.5±1.15</td>
<td>0.63±0.64</td>
<td>2536.5±707.9</td>
<td>0.05±0.06</td>
<td>Pre-Treatment</td>
</tr>
<tr>
<td>10.94±12.24</td>
<td>5.06±0.52</td>
<td>16.25±6.37</td>
<td>0.04±0.07</td>
<td>0.94±1.1</td>
<td>0.93±0.89</td>
<td>1053.22±343.5</td>
<td>0.09±0.72</td>
<td>Post-Treatment</td>
</tr>
</tbody>
</table>

P<0.05  P<0.05  P=0.09  P<0.05  P=0.28  P<0.27  P<0.05  P=0.12  P VALUE

The present study demonstrated that decreased saliva flow causes dry mouth and sense of taste loss in all of the patients. 83.3% of patients suffer from dysphagia and 16.7% of them reported speech problems after radiation therapy. Similar studies support these findings. Xerostomia is caused by dysfunction of salivary gland Acinar and secretory cells. Reduced saliva flow, changes in salivary biochemical indicators, and lingual papillary atrophy affect the sense of taste. Difficulties in swallowing and speech are due to pharyngeal muscle inflammation and dry mouth respectively.

Conclusion

The results of this study show that head and neck radiotherapy reduces salivary flow rate, PH, amylase and IgM (as biochemical indicators of saliva). Qualitative and quantitative changes in saliva cause many problems in irradiated patients.

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