Salivary and imaging-based biomarkers of radiation therapy-induced xerostomia
Saira Atif1, Norsila Abdul Wahab2, Sarah Ghafoor3, Muhammad Qasim Saeed4, Azlina Ahmad5

Abstract
Biomarkers are anatomical characteristics or naturally occurring measurable molecules indicating physiological or pathological state of an individual. These biomarkers have the potential to detect or predict diseases at an early stage, which is particularly beneficial in timely management of common complications of radiation therapy done in head and neck cancer treatment regime. Xerostomia is one of the most common oral complaints of radiation therapy. Saliva has an abundance of protein biomarkers; however, those related to post-radiation therapy xerostomia need to be explored further. Textural and imaging-based biomarkers are helpful in predicting xerostomia in such patients. This narrative review provides an account of salivary protein and imaging-based biomarkers of radiation therapy-induced xerostomia in head and neck cancer patients.

Keywords: chemotherapy, mouth dryness, head and neck cancer, radiotherapy, salivary glands.

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Introduction
Biomarkers are biological indicators of a condition or state, which can be measured and analysed objectively.1 These biomarkers are molecular, physiological, anatomical, or biochemical in nature. Biological fluid such as saliva and serum have potential molecular and biochemical biomarkers, which could be used for detection, prognosis, and monitoring of head and neck cancers (HNC). Furthermore, saliva could be used to monitor complications associated with the treatment of HNC. Early detection of these complications is important for starting appropriate treatment at an early stage, which could improve prognosis of the disease.

Whole saliva is a complex oral fluid secreted by major and minor salivary glands and has been studied extensively over the past few decades as a potential diagnostic tool for various diseases. Saliva could be easily and non-invasively obtained from the patients and is a suitable alternative to serum.2 Imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI) scans are useful tools in HNC diagnosis and treatment planning, and provide useful textural and anatomical information about cancer and its surrounding healthy tissues.3 Imaging-based biomarkers are these anatomical and textural features, derived from in vivo images, which could differentiate between normal and pathological tissues.

Salivary protein biomarkers could be helpful for understanding pathophysiology of salivary gland dysfunction, for screening and risk assessment of developing xerostomia after radiation therapy (RT), for monitoring of a treatment outcome in xerostomic patients, and for selecting appropriate therapy in HNC patients. Xerostomia is a common complication in post-RT patients affecting 60-90% of individuals.4 It is defined as a subjective sensation of dryness of mouth, which might or might not be associated with hyposalivation.5 Xerostomia due to hyposalivation is suggestive of salivary gland dysfunction. This reduced salivary flow in post-RT patients is attributed to functional and histological changes in salivary glands, which is characterized by parenchymal loss, acinar atrophy, interstitial fibrosis, and proliferated and dilated ducts in the glands.6 Chemotherapy is also concurrently done in most of cancer patients besides RT and/or surgery, in which xerostomia is the highest (73.4%) self-reported side effect followed by distortion of sense of taste (61.8%), and dry lips (54.2%).7 Xerostomia is assessed by both objective and subjective methods. When patient reports oral dryness verbally or through standardized questionnaires, it is called as self- or patient-reported xerostomia. Observer-reported xerostomia is when physicians/dentists examining the oral cavity of patients report oral dryness.

Prolonged xerostomia leads to multiple problems such as dental caries, periodontal disease, candida infections, oral ulcers, mucositis, halitosis, tongue fissuring, tongue depapillation, burning mouth syndrome, dental prostheses instability, difficulty in chewing, difficulty in swallowing, and altered taste, which negatively impacts oral health-related quality of life. These oral complications might also restrict the type and amount of food taken, which might lead to malnutrition and subsequent weight loss.8

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HNC comprises of different malignancies; the most common is squamous cell carcinoma arising from epithelial lining of nasal cavity, paranasal sinuses, oral cavity, larynx, and pharynx. Patients with HNC undergo different combination treatment modalities based on the type and stage of cancer and expected treatment outcome. RT might be advised alone without surgery and/or chemotherapy. In RT, high-energy X-ray radiation is used to destroy cancer cells whereas normal cells are spared as much as possible. The prescribed target dose of radiation ranges from 50-70 Gy (2 Gy per fraction). RT is given 5-7 days a week, with either one or more than one session a day, and lasts for 3-7 weeks.

Three-dimensional conformal RT (3D-CRT) is the most common type of RT used in head and neck region. The radiation beams are cautiously arranged matching the shape of tumour, reducing radiation exposure of the surrounding healthy tissues. Intensity-modulated RT (IMRT) is an advanced form of conformal technique, where radiation is more accurately delivered to the tumour, based on its location and severity, sparing surrounding normal tissues such as major salivary glands. Most of the patients with HNC treated with IMRT experienced some degree of xerostomia. Acute and late observer-related xerostomia is significantly reduced in IMRT compared to 3D-CRT. The subjective assessment of xerostomia significantly correlates with the dosimetric parameters of IMRT such as mean and maximum doses, and volume and percent above tolerance of parotid glands. Dosimetric sparing of parotid glands improved subjective xerostomia and reduction in radiation dose to 24-26 Gy aids recovery of salivary flow.

Two-dimensional conventional RT causes a more severe and sustained functional damage to salivary glands as the RT dose is greater than tolerance dose of major salivary glands. In these patients, xerostomia did not resolve significantly. Clinically, significant recovery of saliva secretion and improvement in xerostomia and quality of life scores were reported at 12- and 24-months post-IMRT compared to conventional RT. Incidence of post-IMRT xerostomia at 12-month is 38% and at 24-month is 29% compared to post-conventional RT xerostomia of 74% at 12-month and 83% at 24-month. With the availability of contralateral superficial lobe parotid-sparing-IMRT, the incidence of post-RT xerostomia has further reduced to 26% and 18% at 12- and 24-months, respectively. However, a significant number of patients still suffer from this condition.

Saliva has an abundance of protein biomarkers, which could reflect physiological state of the patient. Growth-regulated oncogene-α (GROα), tumour necrosis factor-α (TNFα), vascular endothelial growth factor (VEGF), and cytokines such as interleukin (IL)-1α, IL-1β, IL-6, and IL-8 were reported to be increased in stimulated saliva samples 12 months after initial diagnosis and post-IMRT. In parotid saliva, total protein secretion rate was decreased and lactoferrin and beta-2-microglobulin concentrations were increased 3-6 months after IMRT. Epidermal growth factor (EGF), secreted by parotid and submandibular salivary glands, was expressed in saliva. In the oral mucosa, EGF plays an important role in wound healing and maintaining epithelial barrier. Oral mucositis is a common complication due to xerostomia in post-RT patients. High EGF levels before and during RT were associated with less mucosal damage measured by Oral Mucositis Assessment Scale. Secretory immunoglobulin A (SIgA) was higher in saliva of post-RT xerostomastic patients than in healthy controls but the difference was not significant. It is unfortunate that there is limited literature available on salivary protein biomarkers of xerostomia in post-RT patients. Brief overview of salivary protein biomarkers has been provided in Table 1.

Salivary flow rate is affected by gender, age, and baseline measurements across individuals. Temporal variations are also common within the same person. Imaging-based biomarkers provide an objective assessment of salivary gland function, which are quantifiable and reproducible. Current treatment planning of HNC advocates pre-RT CT scan to calculate the required radiation dose distribution and to attain three-dimensional information of the target and surrounding anatomical structures at risk. These pre-CT scans are useful because they are reproducible, provide a record of geometrical and textural changes in salivary glands, and significantly contribute to prediction of xerostomia before and after RT. Table 2 provides a brief overview of these biomarkers.

### Table 1: Potential salivary protein biomarkers of radiation-induced xerostomia in HNC patients.

<table>
<thead>
<tr>
<th>Saliva Type of secretion</th>
<th>Post-IMRT</th>
<th>Protein biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulated</td>
<td>Whole saliva</td>
<td>12 months</td>
</tr>
<tr>
<td>Stimulated and unstimulated</td>
<td>Parotid saliva</td>
<td>3-6 months</td>
</tr>
<tr>
<td>Stimulated and unstimulated</td>
<td>Whole saliva</td>
<td>&gt;12 months</td>
</tr>
<tr>
<td>Unstimulated</td>
<td>Not specified</td>
<td></td>
</tr>
</tbody>
</table>


### 1. Biomarkers of xerostomia in CT scan

#### a. Short-run-emphasis

Short-run-emphasis is a CT imaging-based biomarker, which is predictive of developing xerostomia at 12 months post-RT. It measures the non-functional fatty tissue that has replaced functional parenchyma, and patients with higher
fatty tissue to parenchymal tissue ratio have greater risk of developing xerostomia. Parotid gland surface reduction and acute xerostomia scores are significantly associated with development of late xerostomia (6 to 12 months after completing RT).25

**b. Density and volume changes in parotid gland**
According to megavoltage and kilovoltage CT images, a density reduction of parotid gland is seen at the end of RT which also significantly correlates to volume shrinkage. Kinetic analysis of parotid gland variation during RT showed a stronger correlation between early (first week of RT) and late density variation rate compared to volume.26,27

**c. Fractional standard uptake value**
[18F] fluorodeoxyglucose-labelled positron emission tomography-CT (FDG-PET-CT) is widely used in HNC management for staging and response assessment, which also gives useful information about surrounding tissues. Uptake of [18F]FDG by parotid gland and fractional

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**Table 2:** Imaging-based biomarkers of radiation-induced xerostomia in HNC patients.

<table>
<thead>
<tr>
<th>Imaging modality</th>
<th>Scan/analysis type</th>
<th>Imaging-based biomarker of xerostomia</th>
<th>Reference</th>
<th>Primary tumour site</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>Short-run-emphasis</td>
<td>van Dijk et al., 2016&lt;sup&gt;24&lt;/sup&gt;</td>
<td>A, B, G, J</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Volume changes</td>
<td>van Dijk et al., 2017&lt;sup&gt;25&lt;/sup&gt;</td>
<td>A, B, G, J</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Density changes</td>
<td>Belli et al., 2015&lt;sup&gt;26&lt;/sup&gt;</td>
<td>A, C, D</td>
<td></td>
</tr>
<tr>
<td>FDG-PET-CT</td>
<td>Fractional standard uptake value</td>
<td>Cannon et al., 2012&lt;sup&gt;23&lt;/sup&gt;</td>
<td>A, B</td>
<td></td>
</tr>
<tr>
<td>PET-CT</td>
<td>Net metabolic clearance of &lt;sup&gt;11&lt;/sup&gt;C-methionine</td>
<td>Belli et al., 2014&lt;sup&gt;27&lt;/sup&gt;</td>
<td>A, B, G, J</td>
<td></td>
</tr>
<tr>
<td>MRI T1-T2 weighted scans</td>
<td>Volume changes</td>
<td>Zhou et al., 2017&lt;sup&gt;32&lt;/sup&gt;</td>
<td>Nasopharyngeal carcinoma, primary site not specified</td>
<td></td>
</tr>
<tr>
<td>Ultrasound GLCM textural analysis</td>
<td>Angular second moment</td>
<td>Yang et al., 2012&lt;sup&gt;24&lt;/sup&gt;</td>
<td>Laryngeal and oropharyngeal malignancies, primary site not specified</td>
<td></td>
</tr>
<tr>
<td>Ultrasound echo-intensity histogram</td>
<td>Area of low intensity</td>
<td>Yang et al., 2012&lt;sup&gt;25&lt;/sup&gt;</td>
<td>Laryngeal and oropharyngeal malignancies, primary site not specified</td>
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The stratified analysis of patients based on median values of both mid-treatment shrinkage and mean weighted parotid dose showed that patients with higher mean dose and small shrinkage at mid-treatment have a higher risk to experience persistent xerostomia compared to other patients.26,28

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standard uptake value (SUV) have proved to be quantifiable imaging-based biomarkers of parotid gland function. Fractional SUV positively correlates with salivary flow rate measurements and observer-reported xerostomia assessment.

**d. Net metabolic clearance of 11C-methionine**

Salivary gland hypofunction could also be measured by dynamic 11C-methionine PET-CT, which exhibits a sigmoidal response of net metabolic clearance of 11C-methionine with increasing radiation dose and could be used as an imaging-based biomarker of salivary flow rate measurement and salivary gland function.

**2. Biomarkers of xerostomia in MRI scan**

MRI scans are commonly used around the world and provide detailed images of tissues and organs in the body. Early changes in salivary glands could be quantitatively assessed using T1- and T2-weighted MRI scans before and after IMRT.

**a. Volumetric changes, relative signal intensity, and apparent diffusion coefficient**

Volumetric changes, T1-weighted relative signal intensity (RSI), T2-weighted RSI, and apparent diffusion coefficient (ADC) are effective imaging-based biomarkers for objective assessment of salivary gland function. Increase in ADC at two weeks post-RT is associated with increased degree of xerostomia at six months following RT.

**3. Biomarkers of xerostomia in ultrasound imaging**

**a. Gray level co-occurrence matrix (GLCM) textural analysis**

GLCM textural analysis of ultrasound images of salivary gland provides a quantitative evaluation of radiation-induced injury. Sonographic features such as angular second moment, inverse differential moment, contrast, variance, correlation, entropy, cluster shade, and cluster prominence indicate histological changes in salivary glands and could be utilized as biomarkers of post-radiation salivary gland damage and xerostomia.

**b. Echo-intensity histogram**

Echo-intensity histogram method in ultrasound imaging quantitatively assesses parotid glands utilizing echogenicity, heterogeneity, and homogeneity sonographic features. Post-RT parotid gland has brighter lines and spots on B-mode images compared to normal parotid gland, in addition, area of high-intensity ($A_{high}$) and high-intensity width ($W_{high}$) echo-intensity histogram features showed the highest significance. Histogram parameters including area of low-intensity ($A_{low}$), $A_{high}$, low-intensity width ($W_{low}$) and $W_{high}$ could accurately diagnose acute toxicity of parotid glands resulting in xerostomia compared to healthy parotid glands. $A_{high}$, $W_{high}$, peak intensity value of the histogram ($I_{peak}$), and -3dB intensity width of the histogram ($W_{3-DB}$) features could differentiate late toxicity of parotid glands from healthy controls. $I_{peak}$, $A_{high}$, and $W_{high}$ have excellent diagnostic accuracy in classifying acute and late toxicity.

**Conclusion**

Salivary and imaging-based biomarkers could be promising tools for xerostomia prediction and diagnosis in post-radiation therapy cancer patients. Imaging-based biomarkers such as volume and density changes in salivary glands are promising markers for prediction of early and late xerostomia.

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**References**


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