DOI: https://doi.org/10.47391/JPMA.1115

Salivary and imaging-based biomarkers of radiation therapy-induced xerostomia

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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 needs 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.

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 tissue.

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]

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.[9] 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.[9] 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.[10]

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.[11] 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.[12] Acute and late observer-related xerostomia is significantly reduced in IMRT compared to 3D-CRT.[11] 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.[13] Dosimetric sparing of parotid glands improved subjective xerostomia[13] and reduction in radiation dose to 24-26 Gy aids recovery of salivary flow.[14] 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.[15] In these patients, xerostomia did not resolve significantly.[16] 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.[14] Incidence of post-IMRT xerostomia at 12-month is 38% and at 24-months is 29% compared to post-conventional RT xerostomia of 74% at 12-month and 83% at 24-month.[14] 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.[17] 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.[18] In parotid saliva, total protein secretion rate was decreased and lactoferrin and beta-2-microglobulin concentrations were increased 3-6

months after IMRT.[19] Epidermal growth factor (EGF), secreted by parotid and submandibular salivary glands, was expressed in saliva.[20] 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.[21] Secretory immunoglobulian A (SIgA) was higher in saliva of post-RT xerostomic patients than in healthy controls but the difference was not significant.[22] 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.[23] 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.[24] Table 2 provides a brief overview of these biomarkers.

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.[24] 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] 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]

c. Fractional standard uptake value

[^{18}F]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 [^{18}F]FDG by parotid gland and fractional standard uptake value (SUV) have proved to be quantifiable imaging-based biomarkers of parotid gland function.[29] Fractional SUV positively correlates with salivary flow rate measurements and observer-reported xerostomia assessment.[23]

d. Net metabolic clearance of 11C-methionine

Salivary gland hypofunction could also be measured by dynamic ^{11}C-methionine PET-CT, which exhibits a sigmoidal response of net metabolic clearance of ^{11}C-methionine with increasing radiation dose and could be used as an imaging-based biomarker of salivary flow rate measurement and salivary gland function.[30, 31]

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.[32] Increase in ADC at two weeks post-RT is associated with increased degree of xerostomia at six months following RT.[33]

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[34] 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.[35] 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.[35] 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_{-3dB}) 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.

Disclaimer: None to declare

Conflict of interest: None to declare

Funding disclosure: None to declare

References

1. Aronson JK, Ferner RE. Biomarkers-a general review. Curr Protoc Pharmacol. 2017;76:9.23.1-9.17.
2. Francavilla VC, Vitale F, Ciaccio M, Bongiovanni T, Marotta C, Caldarella R, et al. Use of saliva in alternative to serum sampling to monitor biomarkers modifications in professional soccer players. Front Physiol. 2018;9:1828.
3. Keshavarzi M, Darijani M, Momeni F, Moradi P, Ebrahimnejad H, Masoudifar A, et al. Molecular imaging and oral cancer diagnosis and therapy. J Cell Biochem. 2017;118(10):3055-60.
4. Jiang N, Zhao Y, Jansson H, Chen X, Mårtensson J. Experiences of xerostomia after radiotherapy in patients with head and neck cancer: a qualitative study. J Clin Nurs. 2018;27(1-2):e100-e8.
5. Villa A, Connell CL, Abati S. Diagnosis and management of xerostomia and hyposalivation. Ther Clin Risk Manag. 2015;11:45.

6. Acauan MD, Figueiredo MAZ, Cherubini K, Gomes APN, Salum FG. Radiotherapy-induced salivary dysfunction: Structural changes, pathogenetic mechanisms and therapies. Arch Oral Biol. 2015;60(12):1802-10.
7. García-Chías B, Figuero E, Castelo-Fernández B, Cebrián-Carretero J, Cerero-Lapiedra R. Prevalence of oral side effects of chemotherapy and its relationship with periodontal risk: a cross sectional study. Support Care Cancer. 2019:1-12.
8. Ying NJ, Thomson W. Dry mouth - an overview. Singapore Dent J. 2015;36:12-7.
9. Marur S, Forastiere AA. Head and neck squamous cell carcinoma: update on epidemiology, diagnosis, and treatment. Mayo Clin Proc. 2016;91(3):386-96.
10. Iyer NG, Tan DS, Tan VK, Wang W, Hwang J, Tan NC, et al. Randomized trial comparing surgery and adjuvant radiotherapy versus concurrent chemoradiotherapy in patients with advanced, nonmetastatic squamous cell carcinoma of the head and neck: 10-year update and subset analysis. Cancer. 2015;121(10):1599-607.
11. Gupta T, Agarwal J, Jain S, Phurailatpam R, Kannan S, Ghosh-Laskar S, et al. Three-dimensional conformal radiotherapy (3D-CRT) versus intensity modulated radiation therapy (IMRT) in squamous cell carcinoma of the head and neck: a randomized controlled trial. Radiother Oncol. 2012;104(3):343-8.
12. Chao KC, Deasy JO, Markman J, Haynie J, Perez CA, Purdy JA, et al. A prospective study of salivary function sparing in patients with head-and-neck cancers receiving intensity-modulated or three-dimensional radiation therapy: initial results. Int J Radiat Oncol Biol Phys. 2001;49(4):907-16.
13. Amosson CM, Teh BS, Van TJ, Uy N, Huang E, Mai WY, et al. Dosimetric predictors of xerostomia for head-and-neck cancer patients

treated with the smart (simultaneous modulated accelerated radiation therapy) boost technique. Int J Radiat Oncol Biol Phys. 2003;56(1):136-44.

14. Nutting CM, Morden JP, Harrington KJ, Urbano TG, Bhide SA, Clark C, et al. Parotid-sparing intensity modulated versus conventional radiotherapy in head and neck cancer (PARSPORT): a phase 3 multicentre randomised controlled trial. Lancet Oncol. 2011;12(2):127-36.
15. Lee AW, Ng WT, Chan LL, Hung WM, Chan CC, Sze HC, et al. Evolution of treatment for nasopharyngeal cancer–success and setback in the intensity-modulated radiotherapy era. Radiother Oncol. 2014;110(3):377-84.
16. Pow EH, Kwong DL, McMillan AS, Wong MC, Sham JS, Leung LH, et al. Xerostomia and quality of life after intensity-modulated radiotherapy vs. conventional radiotherapy for early-stage nasopharyngeal carcinoma: initial report on a randomized controlled clinical trial. Int J Radiat Oncol Biol Phys. 2006;66(4):981-91.
17. Kawamoto T, Nihei K, Nakajima Y, Kito S, Sasai K, Karasawa K. Comparison of xerostomia incidence after three-dimensional conformal radiation therapy and contralateral superficial lobe parotid-sparing intensity-modulated radiotherapy for oropharyngeal and hypopharyngeal cancer. Auris Nasus Larynx. 2018;45(5):1073-9.
18. Russo N, Bellile E, Murdoch-Kinch CA, Liu M, Eisbruch A, Wolf GT, et al. Cytokines in saliva increase in head and neck cancer patients after treatment. Oral Surg Oral Med Oral Pathol Oral Radiol 2016;122(4):483-90.e1.
19. Richards TM, Hurley T, Grove L, Harrington KJ, Carpenter GH, Proctor GB, et al. The effect of parotid gland-sparing intensity-modulated

radiotherapy on salivary composition, flow rate and xerostomia measures. Oral Dis. 2017;23(7):990-1000.

20. Ino M, Ushiro K, Ino C, Yamashita T, Kumazawa T. Kinetics of epidermal growth factor in saliva. Acta Otolaryngol. 1993;113(sup500):126-30.

21. Epstein JB, Gorsky M, Guglietta A, Le N, Sonis ST. The correlation between epidermal growth factor levels in saliva and the severity of oral mucositis during oropharyngeal radiation therapy. Cancer 2000;89(11):2258-65.

22. Ohyama K, Moriyama M, Hayashida JN, Tanaka A, Maehara T, Ieda S, et al. Saliva as a potential tool for diagnosis of dry mouth including Sjögren's syndrome. Oral Dis. 2015;21(2):224-31.

23. Cannon B, Schwartz DL, Dong L. Metabolic imaging biomarkers of postradiotherapy xerostomia. Int J Radiat Oncol Biol Phys. 2012;83(5):1609-16.

24. van Dijk LV, Brouwer CL, van der Schaaf A, Burgerhof JG, Beukinga RJ, Langendijk JA, et al. CT image biomarkers to improve patient-specific prediction of radiation-induced xerostomia and sticky saliva. Radiother Oncol. 2016;122(2):185-91.

25. van Dijk LV, Brouwer CL, van der Laan HP, Burgerhof JGM, Langendijk JA, Steenbakkers R, et al. Geometric image biomarker changes of the parotid gland are associated with late xerostomia. Int J Radiat Oncol Biol Phys. 2017;99(5):1101-10.

26. Belli ML, Broggi S, Scalco E, Cattaneo GM, Dell'Oca I, Logghe G, et al., editors. Analysis of serial CT images for studying the RT effects in head-neck cancer patients. 2015 37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC); 2015: IEEE.

27. Belli ML, Scalco E, Sanguineti G, Fiorino C, Broggi S, Dinapoli N, et al. Early changes of parotid density and volume predict modifications at the end of therapy and intensity of acute xerostomia. Strahlenther Onkol. 2014;190(11):1001-7.
28. Sanguineti G, Ricchetti F, Wu B, McNutt T, Fiorino C. Parotid gland shrinkage during IMRT predicts the time to xerostomia resolution. Radiat Oncol. 2015;10:19-.
29. van Dijk LV, Noordzij W, Brouwer CL, Boellaard R, Burgerhof JG, Langendijk JA, et al. 18F-FDG PET image biomarkers improve prediction of late radiation-induced xerostomia. Radiother Oncol. 2018;126(1):89-95.
30. Buus S, Grau C, Munk OL, Bender D, Jensen K, Keiding S. 11C-methionine PET, a novel method for measuring regional salivary gland function after radiotherapy of head and neck cancer. Radiother Oncol. 2004;73(3):289-96.
31. Buus S, Grau C, Munk OL, Rodell A, Jensen K, Mouridsen K, et al. Individual radiation response of parotid glands investigated by dynamic 11C-methionine PET. Radiother Oncol. 2006;78(3):262-9.
32. Zhou S, Qian J, Xu L, Tian Y, Fan Q, Shen J, et al. The quantitative evaluation of early radiation-induced changes in the salivary glands using MRI. Zhonghua Yi Xue Za Zhi. 2017;97(7):492-5.
33. Zhang Q, Wei Y-M, Qi Y-G, Li B-S. Early changes in apparent diffusion coefficient for salivary glands during radiotherapy for nasopharyngeal carcinoma associated with xerostomia. Korean J Radiol. 2018;19(2):328-33.
34. Yang X, Tridandapani S, Beitler JJ, David SY, Yoshida EJ, Curran WJ, et al. Ultrasound GLCM texture analysis of radiation-induced parotid-gland injury in head-and-neck cancer radiotherapy: An in vivo study of late toxicity. Med Phys. 2012;39(9):5732-9.

35. Yang X, Tridandapani S, Beitler JJ, Yu DS, Yoshida EJ, Curran WJ, et al. Ultrasound histogram assessment of parotid gland injury following head-and-neck radiotherapy: a feasibility study. Ultrasound Med Biol. 2012;38(9):1514-21.

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

Saliva	Type of secretion	Post-IMRT	Protein biomarkers	Reference
Stimulated	Whole saliva	12-months	GROα, TNFα, VEGF, IL-1α, IL-1β, IL-6, IL-8	Russo *et al*., 2016[18]
Stimulated and unstimulated	Parotid saliva	3-6 months >12-months	Lactoferrin, Beta-2-microglobulin Lactoferrin	Richards *et al*., 2017[19]
Stimulated and unstimulated	Whole saliva	Not specified	Secretory immunoglobulin A*	Ohyama et al., 2015[22]

IMRT: Intensity-modulated radiation therapy, GROα: Growth-regulated oncogene α, TNFα: Tumour necrosis factor α, VEGF: Vascular endothelial growth factor, IL: Interleukin.
*Not significantly raised.

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

Imaging modality	Scan/ analysis type	Imaging-based biomarker of xerostomia	Reference	Primary tumour site

Imaging modality	Scan/ analysis type	Imaging-based biomarker of xerostomia	Reference	Primary tumour site
CT	-	Short-run-emphasis	van Dijk *et al*., 2016[24]	A, B, G, J
		Volume changes	van Dijk *et al*., 2017[25],	A, B, G, J
			Belli *et al*., 2015[26],	A, C, D
			Sanguineti *et al*., 2015[28],	A, C, D
			Belli *et al*., 2014[27]	A, B
		Density changes	Belli *et al*., 2015[26],	A, C, D
			Belli *et al*., 2014[27]	A, B
FDG-PET-CT	-	Fractional standard uptake value	Cannon *et al*., 2012[23],	A, B
			van Dijk *et al*., 2018[29]	A, B, G
^{11}C-methionine PET-CT	-	Net metabolic clearance of ^{11}C-methionine	Buss *et al*., 2004[30],	C, D, E, J
			Buss *et al*., 2006[31]	A, B, D, G, J
MRI	T1-T2 weighted scans	Volume changes	Zhou *et al*., 2017[32]	Nasopharyngeal carcinoma, primary site not specified
		Relative signal intensity		
		Apparent diffusion coefficient	Zhou *et al*., 2017[32], Zhang *et al*., 2018[33]	
Ultrasound	GLCM textural analysis	Angular second moment	Yang *et al*., 2012[34]	laryngeal and oropharyngeal malignancies, primary site not specified
		Inverse differential moment		
		Contrast		
		Variance		
		Correlation		
		Entropy		
		Cluster shade		
		Cluster prominence		

Imaging modality	Scan/ analysis type	Imaging-based biomarker of xerostomia	Reference	Primary tumour site
Ultrasound	Echo-intensity histogram	Area of low intensity Area of high intensity Low-intensity width High-intensity width Peak intensity value of the histogram -3dB intensity width of the histogram	Yang *et al*., 2012[35]	laryngeal and oropharyngeal malignancies, primary site not specified

CT: Computed tomography, FDG-PET: [^{18}F]fluorodeoxyglucose-labelled positron emission tomography, MRI: Magnetic resonance imaging, GLCM: Gray level co-occurrence matrix, A: Pharynx, B: Larynx, C: Tongue, D: Tonsil, E: Soft palate, F: Sinus, G: Oral cavity, H: Nasal cavity I: Orbit, J: Primary tumour site unknown