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REVIEW PAPER

Radiation Induced Xerostomia: Current Concepts and Future Trends

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ABSTRACT

Radiation induced xerostomia is a persistent clinical presentation that affects the quality of life in head and neck cancer patients even with the best of the intensity modulated radiotherapy protocols. Comprehensive review of the anatomic, histologic, developmental and neuronal entities of salivary glands from a regenerative perspective, ensuing radiation is taken. It also evaluates the systemic and glandular radiation responses that form the early and late clinical changes. From these, the article submits probable strategies; based on the current knowledge and future challenges involved, in reversing radiation induced xerostomia. Further, it elaborates on the status of radioprotectors and mitigators including the recently reported biologic and chemical derivatives and proposes the rationale of using combination radioprotector therapy in radiation injuries. A brief of palliative regimes, alternate treatment modes and technologic advancements in radiotherapy are also explained. Salivary gland histologic components, which need to be protected in the emerging radiotherapy protocols and can be targeted in different salivary gland regeneration therapies is highlighted. The paper contributes to an improved understanding in radiation induced xerostomia and deliberates on novel mechanisms towards enhancing quality of life in head and neck cancer radiotherapy patients.

Keywords: Radiation induced xerostomia; Quality of life; Radioprotectors, Radiation countermeasures

1. INTRODUCTION

The world wide annual incidence of head and neck cancers is around 5.5 lakhs with 3 lakhs death and a male predisposition[1]. They mostly constitute squamous cell carcinomas affecting oral cavity, pharynx and larynx, where treatment is in isolation or in combinations of surgery, chemotherapy and intensity modulated radiotherapy (IMRT). In IMRT, focused radiation is given to a tumor/lesion, at 2 Gy/day in a 5 day week schedule for 35-50 days amounting to 50-70 Gy. Fractionation is meant to ensure balance between cancer control and normal tissue damage prevention. However, 40 per cent of the IMRT patients still experience different types of oral side effects, either 'acute' or 'chronic', because salivary glands inadvertently fall in the line of ionising radiation[2]. Acute changes include xerostomia, mucositis, taste disturbances, periodontal pain, esophagitis, dysphagia and infections. Chronic complications would result in xerostomia, trismus, fibrosis, dental caries, osteoradionecrosis and malnutrition[3]. Among all these, xerostomia or hyposalivation is an incapacitating oral effect seen in both acute and chronic stages of radiotherapy. It is imperative to note that xerostomia is also seen in Sjogren's syndrome, Bell's palsy, cystic fibrosis, granulomatous/graft-versus-host/thyroid/liver diseases, uncontrolled diabetes, amyloidosis and in human immunodeficiency virus infections as a sequel of the respective disease processes. However, 'radiation induced xerostomia' is a separate entity in the landscape of radiation biosciences. It causes subjective feelings such as dryness in the oral cavity/frequent desire to take drinks/change of diet or objective changes as assessed by saliva volume evaluation-Schirmer's test or by salivary scintigraphy/dynamic magnetic resonance imaging. The ill-effects of radiation induced xerostomia is graded by the LENT-SOMA scale (LENT-Late Effects Normal Tissues, with grades 1-4 and SOMA-Subjective, Objective, Management, and Analytic descriptors of toxicity) given by the European Organisation for Research and Treatment of Cancer (EORTC) and the Radiation Therapy Oncology Group (RTOG). Overall, radiation induced xerostomia markedly affects the quality of life (QoL) of head and neck cancer patients, to the extent of even disrupting the radiotherapy protocol[4].

This review takes stock of the current understanding of radiation induced xerostomia from three different perspectives: biologic, pharmacologic and technologic. Biologic advancements would have to be viewed in 2 view points; one aiming at effective future biomimetic means for glandular regeneration consequent to radiation by identifying the right histologic components to be protected in the current and

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emerging head and neck cancer radiotherapy protocols. Two, it would seek further insight both into the radiation insult on cells and its response/repair mechanisms and pathways. This improved biologic pedestal would aid in the pharmacological synthesis of new 'radioprotectors' and 'mitigators' either 'biological' or 'chemical' with a greater therapeutic efficacy. Technological innovations may see refinements in radiotherapy procedures and a shift from the use of 'photons' towards 'protons' in radiotherapy, which may guarantee better ionising beam conformation and normal tissue protection.

2. ANATOMIC, HISTOLOGIC, DEVELOPMENTAL AND NEURONAL PERSPECTIVES IN SALIVARY GLANDS

Paired parotid glands are anteroinferior to ears and superficial to mandibular angle and ramus, while paired submandibular and sublingual glands are inferior to mandible in the mouth floor. Minor salivary glands, which are numerous, are widely distributed just below oral mucosa in the lips, anterior mouth floor, cheeks, soft/posterior hard palate, tonsillar pillars and in the anteroventral/posterodorsal tongue. Rodents don't have minor salivary glands in the lips, hard palate and ventral tongue. Salivary glands have 2 types of cells: 80 per cent acinar and 20 per cent ductal. Acinar cells are either serous or mucous, which produce proteins, water and electrolytes. Parotids are predominated with serous acini, sublingual with mucous acini and submandibular more of serous but less of mucous[5]. Accordingly, parotid saliva is watery, sublingual viscous and submandibular moderately viscous. Around 1.5 l of saliva is produced per day; submandibular glands: 70 per cent, parotids: 20 per cent and sublingual: 5 per cent. Rest is from the numerous minor salivary glands. Saliva mainly consists of water, electrolytes, proteins, and carbohydrates. It has important functions: phonation, chewing, taste perception, swallowing, and re-hydration/lubrication/protection of oral mucosa by bactericidal thiocyanates/proteolytic enzymes/ antibodies. Saliva also aids in starch digestion, maintains pH by buffering action and prevents tooth enamel dissolution and do a cleansing of oral cavity by washing away of accumulated food debris[6].

Secretory end pieces from acini successively increase in diameter to form the duct system: intercalated, striated and excretory. Acini consist of pyramidal cells with electron dense secretory granules, whereas intercalated ducts are made of cuboidal cells with less dense granules. Secretory granules are subject to circadian rhythm, which undergoes exocytosis during feeding activities with secretion of α-amylase. In rodents, intercalated ducts join to form 'granular convoluted tubules', which produce several bioactive proteins[5]. Striated and excretory ducts are made of columnar cells with large mitochondria and basolateral invaginations, which give increased surface area for ion exchange. Larger excretory ducts are made of stratified/pseudo stratified columnar epithelial cells. Acinar cell contribute to the fluid and protein part of saliva whereas gland ducts express inorganic content. Myoepithelium covers the acini and intercalated ducts but not the striated and excretory ducts. The long processes of myoepithelial cells circumvent the acini where it is spirally arranged in the long axis of the intercalated ducts. Myofilaments in these processes contain intermediate filaments made of cytokeratin, which rhythmically contract to expel saliva. Progenitor cells are found between the intercalated and striated ducts, whereas stem cells occur in between excretory and striated ducts[7]. Though, both can self renew and differentiate, stem cells are more primitive and radio resistent compared to progenitor cells. During embryogenesis, salivary glands develop from 'epithelial-mesenchymal' interaction. Thereafter, different cell lineages develop from the stem and progenitor cells. Acinar, myoepithelial and duct differentiation are postnatal events. Intercalated ducts have the ability to differentiate to acinar and duct cells, while acinar cells cannot differentiate to other cell types[4]. Other than these histologic constituents, aquaporins (AQP) are important water channel proteins that regulate salivary flow and secretion. They are of different types; AQP1 (endothelial cells), AQP3 and AQP5 (basolateral and apical cell membrane respectively of acinar cells) and AQP8 (basolateral membrane of myoepithelial cells)[8]. Right perception of the histologic elements and key molecules involved in saliva secretion are essential for deducing suitable radiation reversal means[9].

Neuron-epithelial interaction and autonomous nervous system (ANS) (parasympathetic/sympathetic) involvement are crucial towards gland development and in saliva secretion[10]. Therefore, it is presumed that re-visiting the different types of cell interactions during salivary glandular histogenesis may open up novel 'biomimetic' approaches for its regeneration. Nerves and vessels enter into the salivary glands through the main excretory duct. Arterial supply goes upstream against the salivary flow, which branches to smaller ducts and then to the acini. Venous return follows the salivary down flow, where ion exchange occurs. Acetylcholine is the main neurotransmitter for the sympathetic/parasympathetic neurons whereas noradrenalin is the chief neurotransmitter for sympathetic neurons. Vasoactive intestinal peptide (VIP), substance P and calcitonin gene regulated peptide (CGRP) also mediate parasympathetic signalling while neuropeptide Y (NPY) regulate sympathetic signalling. Cholinergic and muscarinic parasympathetic stimulation articulate the watery component of saliva while α and β adrenergic sympathetic stimulation elicits the organic matter through secretory granular exocytosis[11,12].

Anatomically, preganglionic parasympathetic fibers from the inferior salivatory nucleus in medulla of brain stem synapse to the otic ganglion in the infra temporal fossa. Postganglionic fibers then pass through auriculotemporal nerve of fifth cranial cranial nerve for the secretion of serous saliva from parotids. For submandibular/sublingual glands, preganglionic parasympathetic fibers from the superior salivatory nucleus in the pons region of the brainstem pass through nervus intermedius to join facial nerve. In the mastoid area, these fibers pass through chorda tympani nerve and in the infra temporal nerves they join the lingual nerve, to synapse at the submandibular ganglion for finally innervating submandibular/ sublingual glands. Sympathetic salivary centers for all the glands are in the upper thoracic segments of the spinal cord. Preganglionic fibers from thoracic ganglion synapse with the superior cervical ganglion and postganglionic sympathetic

fibers reach salivary glands through the external carotid artery plexus. Superior cervical ganglionectomy experiments have shown the control of sympathetic fibers in salivary secretion[10]. Parasympathetic and sympathetic neurons are derived from the neural crest cells. The neural-epithelial communication for salivary gland development is evident from the knockout mice experiments, which proved the importance of glial cell line derived neurotrophic factor (GDNF) in the development of submandibular glands[11]. Similarly, sympathectomy experiments have shown reduction in parotid gland weight and in its protein synthesis[10]. This is because; sympathetic nerves innervate the gland at birth along with blood vessels and assist in acinar and ductal cell differentiation and maturation. Other contributions of gland development come from inputs arising out of dietary stimulations, cues from circulating hormones from thyroid/adrenal/pancreas/gonads and signals from extracellular matrix/cell membrane messengers/micro RNAs[12]. An obvious implication of all these would be the role of neurons in salivary gland progenitor cell differentiation either directly or indirectly. Future research towards salivary gland regeneration will therefore have to take into account the above mentioned equilibrium between sympathetic and parasympathetic contributions.

3. LOCAL AND SYSTEMIC RADIATION RESPONSES

Salivary gland cells are post mitotic and well differentiated. They have low proliferation rate compared to other tissues in the oral cavity like lips, gums and tongue. However, salivary glands are highly radiosensitive and show drastic fall in saliva production within 24 h following exposure. This is quite contrasting and intriguing, reasons of which are now presumed to be a combination of acinar cell dysfunctions and cell death. The current information of radiation response of salivary glands has come from total body irradiation (TBI) animal experiments done using radiation from Cobalt-60 or Cesium-137 on rats: 5-40 Gy, mouse: 1-15 Gy, rhesus monkey: 2.5-15 Gy and mini pigs: 15-20 Gy, though none of them closely simulate to humans[3]. Post radiation, gland size and acinar cell number decreases; cytoplasm shows mononuclear inflammatory infiltrate, but looses secretory granules and eosinophilic staining, nucleus enlarges, becomes hyperchromatic and ducts dilate and accumulate cell debris. Nerves lose vesicles but shows increase in neuropeptides. Proximal portions are more affected, allowing re-growth from distal side. In vascular compartment, endothelium is affected. Stroma undergoes adiposis and fibrosis, which hinder transport of nutrients and oxygen to cells[13].

Radiation affects multiple systems, but death within first 30days of exposure is due to 'gastrointestinal' and 'hematopoietic' effects and are known as 'acute radiation syndrome' (ARS). Gastrointestinal manifestations results from fluid and electrolyte impairments, bacterial sepsis and depletion of intestinal stem cells (ISCs). These are usually seen within 10-12 days of exposure to 8-20 Gy of radiation. Hematopoietic effects appear after exposure to 3-8 Gy with thrombocytopenia/ neutropenia due to exhaustion of hematopoietic progenitor cells (HPCs). Skin, kidneys and lungs are then affected.

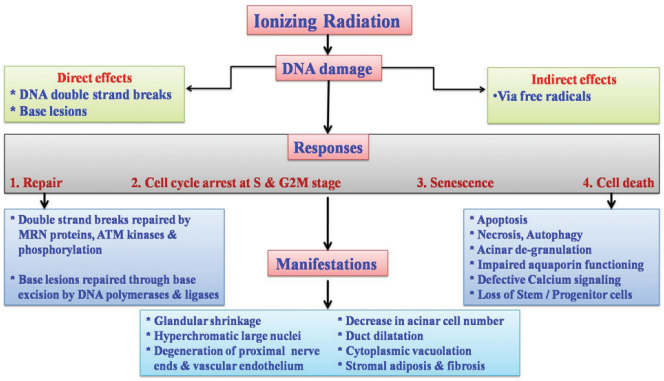

Figure 1. Systemic and local (salivary gland) events following ionising radiation exposure

Carcinogenesis and teratogenesis are late effects[14]. Ionising radiation induces DNA damage through 'direct' and 'indirect' actions. Directly, it breaks the DNA double strand, while indirectly it acts through several free radicals. Both can cause cell mutation and subsequently cell death. Radiolysis of water within the cells is a major event in radiation injury. It produces reactive species like hydroxyl ions (OH), which damages DNA, proteins and lipids. DNA damages can be of 2 types: double-strand breaks (DSBs) and base lesions. Correction of double strand breakages is mediated through MRN protein complex (Mre11, Rad50 and Nbs1). MRN complex binds to the DNA breakage points over which ataxia telangiectasia mutated (ATM), kinases also attach. It then undergoes phosphorylation targeting several key molecules that elicit any one of these: DNA repair/DNA survival with residual damage/arrest of cell cycle or senescence/apoptosis or cell death. ATM signaling also synchronises cell cycle check points like S and G2M, which would give adequate time for cells to repair and not to let pass the damages to daughter cells. Further, ATM also activates cell survival pathways like NF-kB signalling[15].Base lesions are rectified by base excision repair pathways that use DNA polymerases and ligases. Other mechanisms attributed for acinar cell death though with less clarity are (i) de-granulation of secretory granules, (ii) impairment in aquaporin water channels, (iii) calcium signaling mechanisms, and (iv) loss of stem/progenitor cells[13]. All these would cause early and late effects of head and neck cancer radiotherapy. Early changes (phase 1: 0-10 days, phase 2: 10-60 days) are marked by rapid decrease in the salivary flow and late changes (phase 3: 60-120 days and phase 4: 120-240 days) by senescence of remaining acinar cells or death of salivary gland stem and progenitor cells. Novel radiation reversal approaches in salivary glands need to take all these molecular events into consideration. Figure 1 illustrates the systemic and salivary glandular events consequent to radiation.

4. BIOLOGIC AND SURGICAL ADVANCEMENTS IN SALIVARY GLAND RADIATION COUNTERMEASURES

Salivary acinar cell death through apoptotic pathways have been well studied, but role of necrosis and autophagy is yet to be deciphered. Acinar apoptosis involve activation of tumor suppressor gene p53; regulated by Akt, a serine/

threonine specific protein kinase. Activation of Akt is by the binding of growth factors to kinase subunits like p85 and p110. Akt in turn acts on Mouse Double Minute 2 homolog (MDM2) gene leading to apoptosis of acinar cells. Mouse models have shown increased apoptosis within 8 h - 24 h of radiotherapy that accounts for the glandular shrinkage and reduction/ compositional changes in saliva[3]. Reversing radiation induced apoptosis is therefore now an intensely researched area. Kinase inhibitors (Roscovitine) have been found to suppress apoptosis and temporarily inhibit cell cycle progression (G2/M cell cycle) to allow for DNA repair[16].

Ligating the main excretory duct is reported to cause proliferation of ductal cells, key to which would be the number of surviving stem/progenitor cells[17]. Ascl3+ progenitor cells were identified in developing rat submandibular glands. They had the capacity to develop into acinar and ductal cells[18]. Other proteins studied for salivary gland progenitor cells are: intermediate filament proteins like Keratins (K5, K14 and K19) in the cytoplasm, cell surface proteins like aquaporin3, c-kit, CD24, CD 49, CD133 and transcription factors like Oct3/4, Nanog and Sox2/10 in the nucleus. A prudent approach would be to identify the right progenitor population with multiple markers, which would have requisites for regeneration[9]. Insulin growth factor 1, keratinocyte growth factor, fibroblast growth factors have promoted survival and proliferation of progenitor cells[13]. Isolation of stem cells from salivary glands prior to radiotherapy and re-populating them in the 'ductal area' after radiotherapy is another approach[17]. In vitro salivary gland culture has also been attempted. The salispheres expressed salivary gland stem cell markers: CD24, CD29, CD34, CD44, CD49, CD90, and CD117 though maintaining pluripotency of acinar cells in culture is still a challenge[19]. Other upcoming methods would involve isolation of mesenchymal stem cells from bone marrow/adipose/epithelial tissues or from induced pluripotent stem (iPS) cells and inducing in vitro differentiation into salivary gland cells. They would be then injected into the damaged gland sites[20]. It would employ biodegradable scaffolds as templates in oral mucosa from which salivary glands could be tissue engineered. In a similar method, efforts to differentiate ductal cells into artificial salivary glands with acinar and ductal cells have also been attempted. To stimulate stem cell population, activation of Wnt/b catenin pathway has also been attempted[21,22].

Gene therapy is an emerging option in treating radiation induced xerostomia[23]. Transfer of required genes is done through viral/non viral vectors, though viral vectors are currently explored. However, they carry risk of insertion mutations, undue immune responses and the threat of virus itself becoming replication competent. One such approach focuses on expressing the water channel protein Aquaporin-1 through an adenoviral vector. Unlike other aquaporins, AQP-1 has no specificity to cell sides: apical/lateral, but can improve water permeability of cell membrane from all sides. Introduction of gene AQP1 has increased the saliva secretion in rat and pig salivary glands after radiation[8]. Heat shock proteins 25 and 70 injected via adenoviral vector administered prior to radiation have given only incomplete protection[9]. Potential toxicities with gene insertions are another issue. On the other hand, transfer of DNA through non viral genes is challenging and is still in developing stages. Transfer of siRNAs through nanoparticles to block pro-apoptotic genes is another method. Needless to mention, success of gene therapy is directly related to the amount of intact salivary glandular parenchyma[24].

Growth factors can be potential radiation reversal agents, by their role in cell signaling and DNA repair as with protein kinase Akt. Prior treatment with Insulin like growth factor (IGF1) was found to reduce Akt dependent apoptosis. Similarly, keratinocyte growth factor (KGF) and fibroblast growth factor (bFGF) could stimulate epithelial cell proliferation in oral/ esophageal mucosa and inhibit apoptosis[25]. Here, growth factors were given to animals prior to radiation or by gene transfer through adenovectors[26]. Nevertheless, expression of the transferred gene outside the purview of targeted cell is a potential problem. Role of fibroblast growth factor signaling in the progenitor cell differentiation was understood from the mutation studies done on fibroblast growth factor 10 (FGF10) and its receptor FGFR2b, which causes aplasia of lacrimal and salivary glands (ALSG: OMIM 602115) and lacrimo-auriculo-dento-digital syndrome (LADD: OMIM 149730)[7].

Considering the importance of submandibular salivary glands in resting salivary secretion, benefits of surgically relocating the gland has also been explored. Here, salivary gland and ganglion are transplanted to the submental space away from the direct field of radiation, though the procedure is not free from side effects[9].

5. PHARMACOLOGICAL AND TECHNOLOGICAL ADVANCEMENTS IN SALIVARY GLAND RADIATION COUNTERMEASURES

Chemical/biological agents used against tissue damages from radiation are (i) radioprotectors: given prior to radiation and (ii) mitigators: given after radiation exposure but before appear of symptoms. They have applications both in cancer radiotherapy and in nuclear warfare/accidental radiation exposures. They are expected to satisfy certain criteria: selective protection to normal tissues, easy deliverability (self administered) and capability to reverse the adverse effects with minimal toxicity. Radioprotectors might be of critical use in protecting salivary gland functions during head and neck cancer radiotherapy. However, when used for radiotherapy purposes, these carry the risk of tumor protection also.

Though several agents have been aggressively pursued for long time, few have been able to see clinical realisation. Amifostine (WR-2721) is a prototype for radioprotectors. It was the first drug developed against radiation effects. Its mechanism involves de-phosphorylation of the drug by alkaline phosphatase, which would liberate an active metabolite (WR-1065); a free thiol group that can kill free radicals that attack DNA, proteins and lipids. Selective deposition of WR-1065 in the oral mucosa and salivary glands has been demonstrated. A phase III clinical trial in 1999 proved the radioprotective effects of amifostine on salivary glands and was approved by FDA as a drug for radiation induced xerostomia. However, later it was found to cause allergy, vomiting and hypotension. There

were also concerns over its tumor protection, but afterwards it was proved wrong since tumor cells showed low levels of alkaline phosphate activity. Yet, as of now, amifostine is not used against radiation induced xerostomia. Nitroxidetempol and soy-isoflavones are other free radical scavengers evaluated in this line[27].

Reversing 'radiation induced accelerated senescence' in acinar cells is also an emerging option: inhibition of 'mechanistic target of rapamycin (mTOR)' was found to annul radiation induced mucositis in mice. Certain antioxidants and few other biologic derivates have also been cited for radioprotective action. When administered as a transgene in a replication deficient adenovirus, superoxide dismutase, an antioxidant showed radiation protection by metabolising reactive oxygen species in oral, esophagus and lung tissues. Gamma-Tocotrienol, an isomer of vitamin E has also shown similar actions. Indole derivatives (3, 3'- Diindolylmethane) can activate ATM signalling and initiate responses against DNA damage. Flagellin protein derivatives (CBLB502/Entolimod) from salmonella bacteria have shown binding to toll-like receptor 5 (TLR5) in cells and activate NFkB signalling, rendering radioprotection in mice. Chlorobenzylsulfone derived kinase inhibitors (ON01210 or Ex-RAD) act by repealing ATM/p53 signalling. Intestinal cell mitogens (R-spondin1; 29 kDa, 263 amino acid protein) have been reported to stimulate ISC and undo gastrointestinal radiation features through Wnt/β-catenin pathway. Certain antibiotics (tetracycline) and anti-hypertensives (captopril) have also been reported

Table 1. Strategies in radiation induced xerostomia management

Approach	Target/ Means	Current status	Challenges and future trends
Histologic	(i) Progenitor cells	Progenitor cell markers (i) Ascl3+ cells (ii) Cytoplasm filament proteins; keratins (K5, K14, K19) (iii) Cell surface proteins; AQP3, c-kit, CD 24, CD49, CD133 (iv) Transcription factors; Oct3/4, Nanog, Sox2/10	(i) Identifying and isolating right progenitor cells with necessary markers for glandular regeneration (ii) Growth factor (FGF) induced differentiation of progenitor cells
	(ii) Stem cells	(i) Isolation of stem cells from salivary glands prior to radiotherapy and ductal re-population (ii) In-vitro salivary gland culturing (iii) MSCs and iPS differentiation in suitable scaffolds for tissue engineering of artificial salivary glands	Maintaining pluripotency of salivary stem cells is yet to be surmounted
Biologic	(i) Apoptosis	(i) Apoptosis blocking through kinase inhibitors (ii) Down regulation of pro-apoptotic genes through siRNAs in nanoparticles (iii) Growth factor (IGF1, KGF, FGF) mediated apoptosis inhibition	Intensely researched
	(ii) Gene therapy	Gene transfer (AQPs and HSPs) through adenoviral vectors	Risks of mutations, altered immune responses and virus transformation
	(iii) Reversal of radiation induced accelerated senescence	Inhibition of mTOR	Potential area of research
	(iv) Water channel proteins	Regulation of aquaporins (AQP1, AQP3, AQP5 and AQP8)	Intensely researched
Biomimetics	Neural (sympathetic-parasympathetic) -epithelial interactions	Improved understanding	New salivary gland regeneration protocols would have to take cues from neural-epithelial interaction
Surgical	(i) Ligation of duct	Ductal proliferation	Based on pending stem/progenitor cells
	(ii) Gland repositioning	Submandibular gland to sub mental space	Refinements awaited
Pharmacologic	(i) Radioprotectors	None available as of now	Emerging theme: combination therapy of radioprotectors; chemical and biologic
	(ii) Radiation mitigators	Experimental stages- Biologic derivatives, antioxidants, certain vitamins and antibiotics	Risk of tumour protection with radioprotectors needs addressing
	(iii) Palliative	Saliva substitutes / lubricants - artificial saliva	(i) Not free of side effects
		Saliva stimulants - cholinergic parasympathomimetic agents	(ii) Limited efficacy in improving QoL
Alternate therapies	(i) Hyperbaric oxygen	Evolving	Warrant evidences
	(ii) Acupuncture	Evolving	Warrant evidences
Technologic	Proton therapy	Evolving	To save crucial glandular components

radioprotective, though with less ambiguity in its mechanism[15]. Considering the complex nature of radiation damages in terms of the multiple systems it affects and the numerous response mechanisms it initiates, future applications may warrant use of novel formulations of chemical and biologic radioprotectors in combinations for effective radiation reversal in salivary glands. A change from single to multiple radioprotector regimes may be a promising and discreet approach for successful radiation protection in salivary glands.

Agents used for symptomatic/palliative treatment of xerostomia are saliva substitutes/lubricants or saliva stimulants. Former includes certain mouthwashes and gels containing artificial saliva. Latter are mostly cholinergic parasympathomimetic agents, which increase salivary secretion by stimulation of acinar muscarinic receptors. Pilocarpine is a prototype. Its most accepted dose is 5 mg three times daily. However, this also causes side effects like headache, increased sweating, lacrimation, urination, vasodilatation, nausea, dizziness and dyspepsia. Further, it needs to be administered with caution in patients with cardiovascular and pulmonary diseases. Similar other formulations are cholinergic agonists with greater affinity for muscarinic M3 receptors like cevimeline and bethanechol which show little cardiac and pulmonary effects. Palliative care using these medications has the objective of improving QoL of patients but overall efficacy of all these are limited[28] .

Alternative modes of therapies reported for radiation induced xerostomia include hyperbaric oxygen (HBO), acupuncture, and electro stimulation. But long term results from these still warrant evidences[29]. New improvements in radiotherapy would aim for better conformed radiation beam to the best fit of the lesion with reduced volume of normal tissue exposure. Improved sparing of salivary glands has been attempted with ‘protons’ instead of ‘photons’. Protons permit better dose distribution and reduced spot intensity, compared to photon radiotherapy[30]. This has been proved to save the excretory ductal area, which houses the stem cell compartment and therefore probably could be a better case for glandular regeneration after radiotherapy. Table 1 summarises the strategies in radiation induced xerostomia.

6. CONCLUSIONS

Radiation induced xerostomia is a debilitating oral condition seen in head and neck cancer patients undergoing radiotherapy. The review has sought to bring out the crucial histologic elements which could be given preferential sheltering during radiotherapy and cell-cell interactions that can be simulated for salivary gland regeneration post radiotherapy. Further, in the backdrop of systemic responses, the effect of radiation on the microscopic and macroscopic aspects of salivary glands was assessed. DNA damage and ensuing consequences: acinar cell cycle-changes, cell-senescence, cell-death; through apoptosis, de-granulation, aquaporins and signalling and probable resolution of it through appropriate interventions in the anti-apoptotic pathways, re-activation of stem/progenitor cells, tissue engineering and gene therapy with particular impetus to growth factors were also evaluated. Mechanism of action of currently available radioprotectors based on their ability to act on key molecules and pathways were also examined. Accordingly; need for a paradigm shift from the traditional use of single drugs to multiple ones, chemical and biologic radioprotectors, for effective radiation countermeasures have been suggested. It also took an account of the palliative care, alternate therapies and upcoming innovations in radiotherapy which can address radiation induced xerostomiafor improving the quality of life of head and neck cancer patients.

REFERENCES

1. Jemal, A.; Bray, F.; Center, M.M.; Ferlay, J.; Ward, E. & Forman, D. Global cancer statistics. *CA Cancer J. Clin*, 2011, **61**(2), 69-69. doi: 10.3322/caac.20107
2. Cannon, D.M. & Lee, N.Y. Recurrence in region of spared parotid gland after definitive intensity modulated radiotherapy for head and neck cancer. *Int. J. Radiat. Oncol. Biol. Phys.*, 2008, **70**(3), 660-665. doi: 10.1016/j.ijrobp.2007.09.018
3. Grundmann, O.; Mitchell, G.C. & Limesand, K.H. Sensitivity of salivary glands to radiation: from animal models to therapies. *J. Dent. Res*., 2009, **88**(10), 894-903. doi: 10.1177/0022034509343143
4. Deasy, J.O.; Moiseenko, V.; Marks, L.; Chao, K.S.C.; Nam, J. & Eisbruch, A. Radiotherapy dose-volume effects on salivary gland function. *Int. J. Radiation Oncology Biol. Phys*., 2010, **76** (3 Suppl), S58-63. doi: 10.1016/j.ijrobp.2009.06.090
5. Patel, V.N.; Rebustini, I.T. & Hoffman, M.P. Salivary gland branching morphogenesis. *Differentiation*, 2006, **74**(7), 349-364. doi: 10.1111/j.1432-0436.2006.00088.x
6. De Almeida Pdel, V.; Grégio, A.M.; Machado, M.A.; de Lima, A.A. & Azevedo, L.R. Saliva composition and functions: a comprehensive review. *J. Contemp Dent Pract*, 2008, **9**(3), 72-80.
7. Redman, R.S. On approaches to the functional restoration of salivary glands damaged by radiation therapy for head and neck cancer, with a review of related aspects of salivary gland morphology and development. *Biotech Histochem*, 2008, **83**(3), 103-130. doi: 10.1080/10520290802374683
8. Lombaert, I.M.; Knox, S.M. & Hoffman, M.P. Salivary gland progenitor cell biology provides a rationale for therapeutic salivary gland regeneration. *Oral Dis*, 2011, **17**(5), 445-449. doi: 10.1111/j.1601-0825.2010.01783.x
9. Baum, B.J.; Zheng, C.; Cotrim, A.P.; Goldsmith, C.M.; Atkinson, J.C.; Brahim, J.S.; Chiorini, J.A.; Voutetakis, A.; Leakan, R.A.; Waes, C.V.; Mitchell, J.B.; Delporte, C.; Wang, S.; Kaminsky, S.M. & Illei, G.G. Transfer of the AQP1 cDNA for the correction of radiation-induced salivary hypofunction. *Biochim Biophys Acta*, 2006,**1758** (8), 1071-1077. doi: 10.1016/j.bbamem.2005.11.006
10. Proctor, G.B.; Carpenter, G.H. Regulation of salivary

gland function by autonomic nerves. *Auton Neurosci*, 2007,**133**(1), 3-18.
doi: 10.1016/j.autneu.2006.10.006

11. Knox, S.M.;Lombaert, I.M.; Reed, X.; Vitale-Cross, L.; Gutkind, J.S. & Hoffman, M.P. Parasympathetic innervation maintains epithelial progenitor cells during salivary organogenesis. *Science*, 2010, **329**(5999), 1645-1647.
doi: 10.1126/science.1192046
12. Coppes, R.P.; Zeilstra, L.J.W.; Kampinga, H.H.; Konings, A.W.T. Early to late sparing of radiation damage to the parotid gland by adrenergic and muscarinic receptor agonists. *Br. J. Cancer*, 2001, **85**(7), 1055-1063.
doi: 10.1054/bjoc.2001.2038
13. Vissink, A.; van Luijk, P.; Langendijk, J.A. & Coppes, R.P. Current ideas to reduce or salvage radiation damage to salivary glands. *Oral Dis*, 2015, **21**(1), e1-e10.
doi: 10.1111/odi.12222
14. Lavin, M.F. & Kozlov, S. ATM activation and DNA damage response. *Cell Cycle*, 2007, **6**, 931-942.
doi: 10.4161/cc.6.8.4180
15. Rosen, E.M.; Day, R. & Singh, V.K. New approaches to radiation protection. *Front Oncol*, 2014, **4**, 381.
16. Arany, S.; Xu, Q.; Hernady, E.; Benoit, D.S.; Dewhurst, S. & Ovitt, C.E. Pro-apoptotic gene knockdown mediated by nanocomplexedsiRNA reduces radiation damage in primary salivary gland cultures. *J. Cell Biochem*, 2012,**113**(6), 1955-1965.
doi: 10.1002/jcb.24064
17. Pringle, S.; van Os, R. & Coppes, R.P. Concise review: Adult salivary gland stem cells and a potential therapy for xerostomia. *Stem Cells*, 2013, **31**(4), 613-619.
doi: 10.1002/stem.1327
18. Bullard, T.; Koek, L.; Roztocil, E.; Kingsley, P.D.; & Mirels, L. Ovitt, C.E. Ascl3 expression marks a progenitorpopulation of both acinar and ductal cells in mouse salivary glands. *Dev. Biol.*, 2008, **320**(1), 72-78.
doi: 10.1016/j.ydbio.2008.04.018
19. Nanduri, L.S.Y.; Lombaert, I.M.; van der Zwaag, M.; Faber, H.; Brunsting, J.F.; van Os, R.P. & Coppes, R.P. Salisphere derived c-Kit (+) cell transplantation restores tissue homeostasis in irradiated salivary gland. *Radiother Oncol*, 2013,**108**(3), 458-463.
doi: 10.1016/j.radonc.2013.05.020
20. Tran, S.D.; Wang, J.; Bandyopadhyay, B.C.; Redman, R.S.; Dutra, A.; Pak, E.; Swaim, W.D.; Gerstenhaber, J.A.; Bryant, J.M.; Zheng, C.; Goldsmith, C.M.; Kok, M.R.; Wellner, R.B. & Baum, B.J. Primary culture of polarized human salivary epithelial cells for use in developing an artificial salivary gland. *Tissue Eng.*, 2005, **11**(1-2), 172-181.
doi: 10.1089/ten.2005.11.172
21. Aframian, D.J. & Palmon, A. Current status of the development of an artificial salivary gland. *Tissue Eng. Part B Rev.*, 2008, **14**(2), 187-198.
doi:10.1089/ten.teb.2008.0044
22. Hai, B.; Yang, Z.; Millar, S.E.; Choi, Y.S.; Taketo, M.M.; Nagy, A. & Liu, F. Wnt/β- Catenin signaling regulates postnatal development and regeneration of the salivary gland. *Stem Cells Dev.,* 2010, **19**(11), 1793-1801.
doi.org/10.1089/scd.2009.0499
23. Vitolo, J.M. & Baum, B.J. The use of gene transfer for the protection and repair of salivary glands. *Oral Dis.*, 2002, **8**(4), 183-191.
doi: 10.1034/j.1601-0825.2002.02865.x
24. Arany, S.; Benoit, D.S.; Dewhurst, S. & Ovitt, C.E. Nanoparticle mediated gene silencing confers radioprotection to salivary glands in vivo. *Mol. Ther.*, 2013, **21**(6), 1182-1194.
doi:10.1038/mt.2013.42
25. Limesand, K.H.; Avila, J.L.; Victory, K.; Chang, H.H.; Shin, Y.J.; Grundmann, O. & Klein, R.R.Insulin-like growth factor-1 preserves salivary gland function after fractionated radiation. *Int. J. Radiat. Oncol. Biol. Phys.*, 2010, **78** (2), 579-586.
doi:10.1016/j.ijrobp.2010.03.035
26. Palaniyandi, S.; Odaka, Y. & Green, W. Adenoviral delivery of Tousled kinase for the protection of salivary glands against ionizing radiation damage. *Gene Ther.*, 2011, **18**, 275-282.
doi:10.1038/gt.2010.142
27. Cotrim, A.P.; Hyodo, F.; Matsumoto, K.; Sowers, A.L.; Cook, J.A.; Baum, B.J.; Krishna, K.C. & Mitchell, J.B. Differential radiation protection of salivary glands versus tumor by Tempol with accompanying assessment of Tempol by magnetic resonance imaging. *Clin. Cancer Res.*, 2007, **13**(16), 4928-4933.
doi:10.1158/1078-0432.CCR-07-0662
28. Goldstein, N.E.; Genden, E. & Morrison, R.S. Palliative care for patients with head and neck cancer: I would like a quick return to a normal lifestyle. *JAMA*, 2008, **299**(15), 1818-1825.
doi:10.1001/jama.299.15.1818
29. Simcock, R.; Fallowfield, L.; Monson, K.; Solis-Trapala, I.; Parlour, L.; Langridge, C. & Jenkins, V. ARIX: a randomized trial of acupuncture v oral care sessions in patients with chronic xerostomia following treatment of head and neck cancer. *Ann Oncol.*, 2013, **24**(3), 776-783.
doi:10.1093/annonc/mds515
30. van de Water, T.A.; Lomax, A.J.; Bijl, H.P.; Schilstra, C.; Hug, E.B. & Langendijk, J.A. Using a reduced spot size for intensity modulated proton therapy potentially improves salivary gland sparing in oropharyngeal cancer. *Int. J. Radiat. Oncol. Biol. Phys.*, 2012, **82**(2), e313-319.
doi:10.1016/j.ijrobp.2011.05.005

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