Episode 68 – Effect of Radiation Therapy on Oral Innate Immune Response & Oral Microbiome

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Introduction

- The oral cavity hosts over 700 different bacteria species that colonize various surfaces and prime (activate) oral neutrophils.[1] [1]
- Neutrophils[2] are typically recruited to the oral cavity from blood circulation.
- Dr. Glogauer and colleagues have demonstrated oral neutrophils[3] activated by oral inflammation can prime systemic innate immune responses.[4] [2]
- Circulating neutrophils recruited to the oral cavity become oral neutrophils that perform immune surveillance functions and symbiotically interact with commensal microbiota to maintain homeostasis.
- Oral neutrophils support normal periodontal homeostasis by forming a barrier between dental biofilm and oral epithelium. In severe chronic periodontal disease, an increased influx of oral neutrophils, with a hyperactive phenotype, swarm to the oral cavity leading to an overactive inflammatory response.
- Neutrophils form a major component of innate immune response.[5] Interaction between oral microbiota and neutrophils is a key determinant of oral health. [3]

Research objective

- Objective of this prospective cohort study was to describe effects of intensity-modulated radiotherapy (IMRT)[6] during head and neck tumour[7] treatment on the quantity and activation of oral neutrophils and the potential role the oral microbiome[8] plays in these changes.
- If radiation therapy (radiotherapy) causes changes in oral neutrophils and the oral microbiome, further research into how these changes can influence development and/or progression of radiation-induced oral complications is merited. Oral

[1] Refer to Episode 32 for discussion on oral microbiome metabolites that promote health or contribute to disease.
[2] Refer to Episodes 44 & 45 for additional information on neutrophils.
[3] Oral neutrophils are also known as oral polymorphonuclear leukocytes (oPMNs).
[4] Refer to Episode 6 for discussion on oral inflammation priming systemic innate immune response.
[5] Innate immunity, also known as natural immunity, is the level of immunity an individual is born with. Refer to Episode 45 for more information on innate immune response.
[6] IMRT is a type of conformal radiotherapy, which shapes the radiation beams to closely fit the shape of the tumour. IMRT allows for higher, more effective doses of radiation while reducing exposure to healthy tissue to limit treatment side effects and tissue damage. [6] [7]
[7] The most common type of head and neck cancer is squamous cell carcinoma.
[8] Refer to Episode 63 for discussion on the oral microbiome.

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complications of head and neck radiation therapy include osteoradionecrosis, oral mucositis, and xerostomia. [3]

Participant recruitment

Participant recruitment involved the following:

- Ethics approval was obtained.
- Participants with head and neck tumours presented to the dental oncology clinic at Princess Margaret Cancer Centre for dental assessment and treatment prior to radiotherapy commencement.
- Participants were over 18 years, not pregnant, or with immunocompromised conditions (e.g., HIV) or treatments, other than chemotherapy.
- Before oral sample collection, participants were seen by a dentist at the dental oncology clinic for a complete oral examination and radiographs. [3]

Participant characteristics

- Total of 68 participants were recruited and completed the pre-radiotherapy visit.
- Most participants were ≥65 years of age (48%).
- Majority of participants were male (69%).
- Most participants were diagnosed with pharynx/pharyngeal cancer (60%) followed by oral cancer (18%), and larynx cancer (10%).[9]
- Most frequent cancer treatment was chemoradiation (38%), followed by radiation (31%), and surgery with adjuvant radiation[10] (24%).
- Oral samples were collected at pre-radiotherapy for 68 participants. Three participants succumbed to their illness and did not complete any further visits after the pre-radiotherapy visit. [3]

Oral sample collection

- Oral rinse samples and biofilm samples were collected from participants during four different time points (pre-radiotherapy, mid-radiotherapy, one-month post-radiotherapy, six-months post-radiotherapy).
- To collect oral rinse samples, participants rinsed with 5 mL of isotonic sodium chloride solution for 30 seconds and then expectorated into a sterile 50 mL tube. This procedure was repeated six times (for a total of 30 mL) with 1-to-2.5-minute intervals between each rinse sample.
- Biofilm samples were collected from the tongue dorsum, and supragingival and subgingival plaque using three separate sterile endodontic paper points.
- Oral neutrophil counts and activation states were analyzed using flow cytometry,[11] and the oral microbiome was analyzed using 16S rRNA gene sequencing.[12] [3]

[9] Refer to Episode 53 for signs and symptoms of oropharyngeal cancer.
[10] Adjuvant therapy is often used after primary treatments, such as surgery, to lessen risk of the cancer returning.
[11] Flow cytometry is a procedure used to detect and measure physical and chemical characteristics of a population of cells. Flow cytometry studies are used to identify and quantify immune cells.
[12] 16S rRNA gene sequencing is commonly used for identification, classification, and quantitation of microbes within complex biological mixtures (e.g., within human gut microbiome and oral microbiome). [8]

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Effect of radiotherapy on absolute oral neutrophil counts

The research data showed:

- Average (mean) absolute oral neutrophil[13] counts increased during mid-radiotherapy compared to numbers before radiotherapy. However, the increase was not statistically significant.
- There was a statistically significant decrease in mean absolute oral neutrophil counts from mid-radiotherapy to six months post-radiotherapy.
- Mean absolute oral neutrophil counts significantly decreased from pre-radiotherapy to 6-months post-radiotherapy and did not return to the same counts seen pre-radiotherapy.
- Results indicate radiotherapy played a vital role in reducing oral neutrophil numbers recruited to the oral cavity. [3]

Effect of radiotherapy on absolute oral neutrophil marker counts and activation states

- Oral neutrophil activation is characterized by expression levels of certain cell surface CD[14] markers. A panel of seven oral neutrophil CD and H3Cit[15] were used to evaluate radiotherapy's effect on oral neutrophil priming (activation) state.
- Statistically significant drops in activation states of CD11b, CD16, CD18, CD64, and H3Cit markers from pre-radiotherapy to post-radiotherapy were observed. [3]

Effect of radiotherapy on oral microbiota

The following results were observed:

- Statistically significant shift in beta (*β*) diversity[16] was seen between pre-radiotherapy and mid-radiotherapy samples, and pre-radiotherapy and 1-month post-radiotherapy samples. *β*-diversity was not altered significantly between samples collected from mid-radiotherapy compared to 1-month post-radiotherapy. These data suggest radiotherapy may directly impact the overall oral microbiome.
- Actual abundance profiling showed overall bacteria numbers were significantly reduced after radiotherapy. The bacterial load continued to decline one-month post-radiotherapy.
- Proportional analysis of each bacterial phylum showed *Firmicutes* (containing the most abundant Gram-positive bacteria) increased by 5-6% within the overall oral microbiome of samples collected after radiotherapy. There was a slight increase in *Spirochaetes* after radiotherapy while *Proteobacteria* were reduced.
- Markable increase in numbers of *Streptococcus* occurred at mid-radiotherapy.

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[13] Absolute oral neutrophil count is a measure of the number of neutrophils, which is an estimate of the body's ability to fight infections.

[14] CD is an abbreviation for "cluster of differentiation." CD molecules are cell surface markers which are useful for the identification and characterization of leukocytes and different subpopulations of leukocytes. Neutrophils (white blood cells) are a type of leukocyte. CD markers are especially useful for identification of leukocyte population using flow cytometry. [9]

[15] Citrullinated histone H3 or H3Cit is a marker of neutrophil extracellular traps (NETs) and thus neutrophil activation. Neutrophils release NETs (a meshwork of chromatin fibres) to immobilize and kill invading pathogens to help prevent them from spreading. [4] [5]

[16] *β*-diversity measures the change in diversity of species from one environment to another. [10]

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- The lactobacilli group (which usually corresponds to dental caries) increased after radiotherapy.
- Genera affected by treatment and reduced after radiotherapy included *Veillonella* (mid-radiotherapy only), *Haemophilus* (1-month post-radiotherapy only), *Neisseria*, *Actinomyces* (mid-radiotherapy only), *Leptotrichia*, and *Capnocytophaga*. [3]

Summary of findings

- The data suggest radiation may, directly and indirectly, influence oral neutrophil counts and activation states with a shift in oral polymicrobial communities.
- Oral samples collected during and after radiotherapy showed a significant reduction in abundance of Gram-negative bacteria and an increase in the genera *Streptococcus*, *Lactobacillus*, *Treponema*, and *Prevotella*. This change shifted the oral microbiome's *β*-diversity, positively correlated with reduced oral neutrophils counts and suppressed CD marker activation.
- Changes in the oral microbiome and oral neutrophils did not return to baseline pre-radiotherapy levels at 6-months post-radiotherapy. [3]

Take home messages

- Oral health professionals should routinely conduct oral cancer screenings, including visual inspection and tissue palpation, for all clients and refer any suspicious finding to the appropriate medical or dental provider.
- Radiotherapy can cause considerable changes in the oral microbiome by reducing the relative abundance of commensal Gram-negative bacteria and increasing the commensal Gram-positive bacteria. While this may be seen as a desired outcome, disturbing normal flora may result in disease-inducing microbial communities, including aciduric microbes.
- Changes in the oral innate immune response and oral microbiome due to radiotherapy provide opportunity to determine how these changes may affect and contribute to radiotherapy-induced oral complications (e.g., osteoradionecrosis, oral mucositis, xerostomia), which significantly impact quality of life.
- More frequent preventive periodontal and follow-up appointments may be required, and administration of oral probiotics[17] may be recommended to counteract the radiotherapy changes in the oral microbiome to improve oral health outcomes of individuals post-radiotherapy.
- Targeted oral health treatments and preventive measures need to be included into the standard of care for individuals undergoing radiotherapy to help maintain good oral health and function.

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[17] Refer to Episode 63 for discussion on probiotics.

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Client resources

Oral Cancer Screening, ODHA Factsheet
https://odha.on.ca/wp-content/uploads/2016/08/Oral-Cancer-Screening.14.1-copyright.pdf

Oral Cancer Self-Examination, ODHA Factsheet
https://odha.on.ca/wp-content/uploads/2016/08/Oral-Cancer-Self-Exam14.1-copyright.pdf

7-Step DIY Oral Cancer Screening, CDHO Factsheet
https://www.cdho.org/docs/default-source/pdfs/oral-health-rpt/article-7-step-oral-cancer-screening.pdf

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Seven-Step “Look and Feel” Oral Cancer Screening, CDHO Factsheet
https://www.cdho.org/docs/default-source/pdfs/isc/oralcancer_pullout.pdf

CDHO Seven-Step Oral Cancer Self-Exam (2 mins), CDHO instructional video
https://www.youtube.com/watch?v=6SMMq4traNc

CDHO Seven-Step Oral Cancer Self-Exam (5 mins), CDHO instructional video
https://www.youtube.com/watch?v=FJBQ3tserp0

Additional Resources

The effect of intensity-modulated radiotherapy to the head and neck region on the oral innate immune response and oral microbiome: A prospective cohort study of head and neck tumour patients, Mojdami, Z; Barbour, A; Oveisi, M; Sun, C; Fine, N; Saha, S; Marks, C; Elebyary, O; Watson, E; Tenenbaum, H; Azarpazhooh, A; Glogauer, M. *International Journal of Molecular Sciences*, Volume 23, Issue 17, Article 9594, August 24, 2022, p 1-29
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Expression, role, and regulation of neutrophil Fcγ receptors, Wang, Y; *Frontiers in Immunology*, Volume 10, Article 1958, August 27, 2019, p 1-13
https://www.frontiersin.org/articles/10.3389/fimmu.2019.01958/full

Oral neutrophils characterized: Chemotactic, phagocytic, and neutrophil extracellular trap (NET) formation properties, Moonen, C; Hirschfeld, J; Cheng, L; et al. *Frontiers in Immunology*, Volume 10, Article 635, March 29, 2019, p 1-15
https://www.frontiersin.org/articles/10.3389/fimmu.2019.00635/full

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