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Salivary IgA as a Surrogate Biomarker for Microbial Infections in Postoperative Patients Receiving Chemo-Radio-Therapy for Head and Neck Cancer

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Abstract

Objective Radiotherapy (RT) and chemotherapy (CT) are important treatment options in patients with head and neck cancers. A common complication of this is microbial colonization or infection of mucosal surfaces. These infections may commonly be due to bacteria or yeasts. Salivary proteins with their buffering activity and immunoglobulin, especially immunoglobulin A (IgA), protect oral tissue, mucosal surfaces, and teeth from various microorganisms. This study characterizes the common microorganisms encountered and evaluates the role of salivary IgA in predicting microbial infections in this group of patients with mucositis.

Methods A total of 150 adult head and neck cancer patients on CTRT were evaluated at baseline and at the end of 3 and 6 weeks, respectively. Oral swabs collected from buccal mucosa were processed in the microbiology laboratory for the presence of microorganisms. Saliva was processed for IgA level estimation on Siemens Dimension Automated biochemistry analyzer.

Results *Pseudomonas aeruginosa* and *Klebsiella pneumonia*e were the most common organisms found in our patients, followed by *Escherichia coli* and group A beta-hemolytic *Streptococci*. A significant increase ($p = 0.0203$) in the incidence of bacterial infection was observed in post-CTRT patients (61%) compared to pre-CTRT patients (49.33%). There was significant increase in levels of salivary IgA ($p = 0.003$) in patients with bacterial and fungal infection ($n = 135/267$) when compared to those in samples showing no growth ($n = 66/183$).

Keywords
- mucositis
- IgA levels
- fungal infection
- bacterial infection
- biomarker

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Conclusion A significant increase in the incidence of bacterial infection in post-CTRT patients was observed in this study. This study also indicated that postoperative head and neck cancer patients with oral mucositis that developed an infection were associated with high salivary IgA levels, and it may serve as a surrogate biomarker of infection in these patients.

Introduction

Head and neck (H&N) cancers account for 4 to 5% of all cancers. It is more common in men than in women (4:1) and in those above 40 years of age.[1] One of the treatment options in cancers is radiotherapy (RT), on its own or in combination with other treatments like chemotherapy (CT). However, both radiation and CT affect not only malignant cells but damage the surrounding disease-free buccal and peri-buccal tissue. Oral mucositis is one of the commonest side effects of these procedures and may lead to microbial colonization or infection of damaged mucosal surfaces usually by gram-negative bacteria and yeast. Antimicrobial interventions may help in arresting this cascade of events that culminates in the development of ulcerative oral mucositis and thus prevent or reduce the attendant clinical manifestations. The presence of mucositis was also identified as an independent risk factor in the development of bacteremia due to organisms such as viridans streptococci among neutropenic patients, whether treated for cancer or receiving a hematopoietic stem cell transplant.[2]

Under certain physiological and pathological conditions, the yeast may change status from that of commensal to a pathogen, particularly in patients with malignancies who may be immunosuppressed.[3] Hence, it is important to detect these infections as early as possible and initiate timely interventions in the form of antimicrobial treatment.

Although culture and susceptibility methods are generally employed for detecting these infections, the role of salivary immunoglobulin A (IgA) as a biomarker for infections in this setting can be considered. Saliva consists of various chemical components such as inorganic compounds, organic compounds, proteins, and hormones. Salivary proteins with their buffering activity and immunoglobulin, especially IgA, protect oral tissue, mucosal surfaces, and teeth from various microorganisms. Immunoglobulins have antimicrobial activity and protect against various viruses, bacteria, and fungi. Compromised salivary function secondary to destruction of glandular tissue by radiation is thought to be a major factor leading to *Candida* infection.

In view of the above, the aim of this study was to study the incidence of microbial infection/colonization in H&N cancer patients on CTRT and to identify the common organisms present. We also wanted to study the role of salivary IgA in predicting infections and to know if it can be used as a potential surrogate biomarker for microbial infections in diagnostic laboratories.

Materials and Methods

A total of 150 adults (133 men and 17 women) with H&N cancer on RT and CT both were included in the study. The study was approved by the Institutional Ethics Committee (IEC Project no. 131 ACTREC)

Inclusion Criteria

Postoperative patients with H&N cancers starting on CTRT (> 50 Gray) and above the age of 18 years were included in this study.

Exclusion Criteria

Human immunodeficiency virus (HIV) reactive patients and patients who had received prior neoadjuvant CT were excluded.

Sample Collection

Informed consents were taken from all patients included in the study. All these patients were evaluated at baseline on day 0 before starting the chemo-/radiotherapy (pre-CTRT) and after starting the CTRT (post-CTRT) at the end of week 3 and 6, respectively. Oral swabs were collected from the buccal mucosa and processed as per microbiology standard laboratory protocol for the presence of microorganisms. A total of 450 swabs were collected from 150 patients.

For salivary sample collection, patients were asked to refrain from eating or drinking anything for at least half an hour prior to collection of saliva. A piece of paraffin film was given to chew as it stimulated the flow of saliva.[4] Saliva was collected in sterile containers. At least 2 mL of sample was collected. Sample was immediately stored at 2 to 4°C and transported to laboratory within 6 hours.

Sample Processing

Samples were processed as per laboratory protocol and cultured on Saboraud's dextrose agar (SDA—plain) and with antibiotic chloramphenicol (SDA with antibiotics), blood agar, and MacConkey agar plates to look for yeasts and pathogenic bacteria. Blood and MacConkey agar plates were incubated and observed for 48 hours and SDA plates for 2 weeks. Any growth on SDA plates was processed further to confirm presence of yeast as per microbiology laboratory protocol.

Salivary samples were processed for IgA level estimation by immunoturbidimetry method on Siemens Dimension fully automated biochemistry analyzer. A reference range of 12.43 to 33.53 mg/dL (124.3–335.3 mg/L) was used for the evaluation of salivary IgA.[5]

Incidence of bacterial and fungal infections was compared in pre-CTRT and post-CTRT groups. Results of IgA in salivary samples were also compared between samples showing no growth and those with microbial infection as well as between in pre-and post-CTRT samples calculating the *p*-value. All *p*-values were two-sided and SPSS v21 was used for statistical analysis.

Results

Baseline characteristics of the patients are as described in ►**Table 1**. *Pseudomonas aeruginosa* and *Klebsiella pneumonia*e were the commonest organisms found in our patients, followed by *Escherichia coli* and group A beta-hemolytic *Streptococci*. A significant increase ($p = 0.0203$) in the incidence of bacterial infection was observed in post-CTRT patients (61%) compared to pre-CTRT patients (49.33%; ►**Table 2**). Among the yeasts, *Candia albicans* and *Candida tropicalis* were most commonly isolated in our setting. However, there was no significant difference ($p = 0.217$) between the incidence of *Candida* infection in pre-CTRT (23.33%) and post-CTRT (29.33%) patients. (►**Table 3**) In salivary samples, there was a significant increase observed in levels of salivary IgA ($p = 0.003$) in patients with bacterial and fungal infections ($n = 135/267$), when compared to those in samples showing no growth ($n = 66/183$; ►**Table 4**) There was no statistically significant difference between pre- and post-CTRT groups for levels of IgA in salivary samples in the presence of infection.

Discussion

RT and CT are important options in treatment of H&N cancers. However, these procedures can lead to increase in the chances of bacterial and yeast infections in these patients. The incidence of *Candida* infections was observed to be 17 to 19% in some studies.[6] In our study, the incidence of *Candida* infection was around 29% in postoperative post-CTRT patients. A study by Raj et al in 42 H&N cancer patients showed a 57.14% incidence of *Candida* infection.[7] The common species found in this study was *Candida tropicalis* (28.57%) and *Candida albicans* (14.28%) followed by *Candida parapsilosis* (14.28%). In our study, the most common *Candida* species isolated was *Candida albicans* at (18.66%) followed by *Candida tropicalis* (5.66%) and *Candida glabrata* at (2%).

Table 1 Baseline characteristics of the patients

Cancer	No of Cases
Buccal mucosa	67
Tongue	50
Alveolus	24
Gingivobuccal sulcus	5
Lip	3
Retro molar cancer	1

Note: $n = 150$ (male: 133; female: 17). Age (years; median: 44.5).

Table 2 Incidence of bacterial infection and types of organism (CTRT)

Organism	Pre-CTRT ($n = 150$)	Post-CTRT ($n = 300$)
1. *Klebsiella pneumoniae*	14	57
2. *Pseudomonas aeruginosa*	23	48
3. *Escherichia coli*	5	23
4. *Group A beta hemolytic Streptococci*	19	21
5. *Enterobacter* species	1	1
6. *Shewanella* species	3	10
7. *Staphylococcus aureus*	4	8
8. *Acinetobacter* species	3	3
9. *Proteus mirabilis*	1	2
10. *Methicillin-resistant Staphylococcus aureus*	1	2
11. *Enterobacter cloacae*	0	1
12. *Enterobacter aerogenes*	0	3
13. *Providencia* species	0	2
14. *Serratia marcescens*	0	2
Total	74 (49.33%)	183 (61%)
p-Value		0.0257

Abbreviation: CTRT, chemotherapy-radiotherapy.

This may be attributed to the higher sample size in our study and different sites of sample collection, oral swabs in our study, and throat swabs in the study by Raj et al.[7] However, an increase in fungal infection was observed in both these studies post-RT intervention. In another study by Panghal et al, *Candida albicans* was the commonest fungi observed.[8] The incidence of bacterial infection observed in the above-mentioned study was around 27.27% which was much lower than that observed in our study (61%). The organisms

Table 3 Incidence of fungal infection and types of organism (CTRT)

Organism	Pre-CTRT ($n = 150$)	Post-CTRT ($n = 300$)
1. *Candida albicans*	22	56
2. *Candida tropicalis*	8	17
3. *Candida krusei*	0	3
4. *Candida glabrata*	0	6
5. *Candida kefyr*	1	1
6. *Candida parapsilosis*	3	2
7. *Candida rugosa*	1	1
8. *Candida albicans*	0	2
Total	35 (23.33%)	88 (29.33%)
p-Value		0.2224

Abbreviation: CTRT, chemotherapy-radiotherapy.

Table 4 Comparison of IgA levels in samples with and without infection

	(Pre-CTRT)		(Post-CTRT)	
	No. of samples	IgA > 33.53 mg/dL (> 335.3 mg/L)	No. of samples	IgA > 33.53 mg/dL (> 335.3 mg/L)
Total no. of samples	150	76 (50.7%)	300	130 (43.3%)
All samples with infection (fungal and bacterial)	81	39 (48.1%)	186	96 (51.6%)
Samples showing no growth	69	32 (46.4%)	114	34 (29.8%)
All samples with fungal infection	35	14 (40%)	70	40 (57.1%)
All samples with bacterial infection	65	29 (44.6%)	164	80 (48.8%)
		No of samples	IgA > 33.53 mg/dL (> 335.3 mg/L)	
Total no. of samples showing no growth		183	66	
Total no. of samples with infection (fungal + bacterial)		267	135	
p-Value			0.003	

Abbreviations: CTRT, chemotherapy-radiotherapy; IgA, immunoglobulin A.

isolated in both studies included *E. coil*, *P. aeruginosa*, and *K. pneumoniae*. The most common organism isolated in oral cavity infections in the above study was *K. pneumoniae* which concurs with the findings of our study.

Saliva has the advantage over other body fluids used in diagnosis as collection is easier, the process of collection is noninvasive and it is readily available. Saliva plays a major role in the protection of oral cavity mucosa with its antibacterial, antifungal, and antiviral properties.[9] Saliva has been used in diagnosis of infectious disease such as HIV and HBV as well as in forensic analysis, hormonal assays, drug assay, etc.[10] One such important aspect of saliva is immune response to various agents. The immune response of saliva to infections is manifested by presence of antibodies in saliva. It can be measured by the presence of IgA in saliva that is derived from plasmacytes in salivary glands. In our study, we noticed significant rise in IgA levels in samples with infections when compared to those samples that showed no growth on microbiological analysis. This can be explained by the fact that salivary IgA antibodies have long been known to have existed in neonates for various mucosal bacteria such as *E. coli*, *Streptococci*, *Enterococcus faecalis* and to achieve adult levels by the age of 2 years.[11–14] Pedersen et al have already shown in their study that increased levels of IgA have been observed in cystic fibrosis patients against *Pseudomonas* aeruginosa.[12] The local IgA production in saliva was observed to be increased more in comparison to the serum levels in these patients. Another study by Maurer et al has also demonstrated the antiviral properties of salivary IgA and its immune effector function against Influenza A virus.[15] It was observed that the IgA probably helps keep the microbes away from mucous membranes and reduce the clinical symptoms. Our study showed a significant increase in levels of salivary IgA levels ($p = 0.003$) in patients with bacterial and fungal infections in comparison to those in samples showing no growth.

From these findings, it can be proposed that salivary IgA evaluation may be a useful tool in prediction of infections in cases of mucositis. A further prospective study with a larger sample size will help establish the usefulness and effectiveness of this noninvasive test in a routine diagnostic laboratory setup.

Conclusion

A significant increase in the incidence of bacterial infection in post-CTRT patients was observed in this study. The commonest bacteria isolated were *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *E. coli*, and group A beta hemolytic *Streptococci.* The commonest fungi observed were *Candida albicans* and *Candida tropicalis*. Our findings also indicated that postoperative H&N cancer patients with oral mucositis that developed infection were associated with high salivary IgA levels, and it may serve as a surrogate biomarker of infection in these patients.

Note

Part of this study was presented at the 70th AACC annual scientific meeting and clinical lab expo organized in Chicago in 2018 as a poster.

Authors' Contributions

P.D.C., V.G.B., and A.J. researched literature and conceived the study. P.D.C., V.G.B., A.J., T.G., V.M., V.N., R.D., and K.P. were involved in protocol development, gaining ethical approval, and patient recruitment. P.D.C. and V.G.B. did data analysis. P.D.C. wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Ethical Approval

This study was approved by Institutional Ethics Committee (ref no: project no 134, TMC-ACTREC IEC).

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Conflict of Interest
None declared.

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