

Microflora analysis in the postchemotherapy patients of oral cancer

ABSTRACT

Background: To assess changes in oral microflora in dental plaque from cancer patients within 7 days of the first course of chemotherapy and the relationship of the changes with mucositis.

Materials and Methods: Thirty cancer patients, divided into a test group undergoing chemotherapy and a control group not undergoing chemotherapy, were enrolled in this pilot study. Oral microflora was cultured from three samples of dental plaque at t0 (before chemotherapy), t1 (1 day after chemotherapy), and t2 (7 days after chemotherapy). Single and crossed descriptive analyses were used to establish prevalence, and the Chi-square test was used to establish the statistical significance of the differences observed in distributions (significance level: $P < 0.05$).

Results: In most patients (55%), oral microflora consisted mainly of Gram-positive cocci, while the remaining 45% of the bacterial flora also had periodontal-pathogenic species. No *Porphyromonas gingivalis* appeared in the test group. *Actinobacillus* was the least frequently found bacterium among periodontal pathogens in the test group, while *Fusobacterium nucleatum* was the most frequently found. No significant differences were found in quantitative bacterial changes between t0, t1, and t2 in either the test or control groups, or between the two groups. According to World Health Organization scores, oral mucositis developed in 10 patients (66.6%) in the test group.

Conclusions: The results of this pilot study indicate that there were no changes in microflora in dental plaque in cancer patients within 7 days of the first course of chemotherapy. No correlations between oral mucositis and specific microorganisms were assessed.

Keywords: Cancer patients, chemotherapy, dental plaque, oral microflora

INTRODUCTION

The human oral cavity is inhabited by upward of 500 species of bacteria,^[1] most of which are harmless commensal organisms. Others, however, are pathogenic and are involved in the development of dental caries, periodontal diseases, and acute or chronic infections. Cytotoxic chemotherapy compromises the oral defense mechanisms, either by direct mucosal damage or by neutropenia, potentially causing an overall shift in oral microflora. In patients undergoing chemotherapy, there is an increase in the number and proportion of some bacteria associated with periodontal diseases (*Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum*),^[2-4] along with a diminished effectiveness of immune defense against infection.^[5] Mucositis is an oral complication that affects 30%–40% of patients receiving chemotherapy and radiotherapy and 80% of those undergoing hematopoietic

stem cell transplantation. It is a multifactorial disease defined as epithelial thinning associated with intense erythema, ulceration, pain, bleeding, and increased risk of infection.^[6] The cytotoxic effects of anticancer drugs against high turnover tissues such as the oral epithelium and the local effects of radiation on the oral mucosa are responsible for this

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event, which compromises quality of life and may interfere with management of the disease. Mucositis typically appears between 7 and 14 days after the initiation of chemotherapy and is usually preceded by a subjective complaint of soreness or a burning sensation. Drugs most likely to cause mucositis include doxorubicin, bleomycin, fluorouracil, and methotrexate.^[7] It is thought that the incidence and severity of cancer chemotherapy-associated mucositis is caused in part by changes in the oral bacterial microflora. Oral microorganisms are believed to be involved in the ulceration phase, where they probably intensify the inflammatory process and aggravate or promote the formation of ulcers.^[8] However, until now, it has been unclear whether there is an association between periodontal pathogens and mucositis.^[2] Given the significant impact of oral mucositis on quality of life, it is essential to try to prevent it by all means possible. Currently, there are several treatments, but none of them has been validated definitively.^[9] The aim of this pilot study was to assess changes in oral microflora in dental plaque in cancer patients within 7 days from the first course of chemotherapy and their relationship with mucositis.

MATERIALS AND METHODS

Following protocols are followed in the experiment.

Procedure

We enrolled 29 patients (18 men and 11 women, aged 32–59 years) with solid malignancy that had no previous adjuvant radiotherapy or recent antimicrobial or antiviral treatment. The primary, Stage II, squamocellular cancer was located in the lungs (8 men, 3 women), colon–rectum (6 men and 1 woman), prostate (4 men), and breast (7 women). The study was conducted at the Department of Microbiology, King Georges Medical University, Lucknow, UP, India. The study was approved by the Institutional Ethical Committee of King Georges Medical University, Lucknow, UP, India. All patients gave their written informed consent. The patients were divided into groups, the test group consisted of patients undergoing a first course of chemotherapy with docetaxel or 5-fluorouracil and oxaliplatin; the control group consisted of patients not undergoing chemotherapy because of the stage of their disease and because they did not have adequate numbers of platelets and leukocytes.

Microbial analysis

Oral mucositis was scored according to World Health Organization (WHO) criteria^[10] at eight nonkeratinized anatomical sites (labial and buccal mucosa, lateral and ventral tongue, floor of mouth, and soft palate) by one trained dentist (V.C.). Oral micro flora was cultured from plaque specimens. All patients were sampled at time

zero (t0) (immediately before chemotherapy) and on t1 (1 day after infusion) and t2 (7 days after infusion). Controls were sampled on equivalent dates. Sampling was done at the same time of day, approximately 2 h after breakfast. For each individual, the supragingival plaque of the right lower premolars was collected with a sterile swab. All specimens were processed within the following 4 h. Following serial dilution, 100 µl of each dilution was plated on Schaedler Selective Blood Agar plates supplemented with 5% bovine blood (Biolife Italiana, Milan, Italy) and incubated in 80% nitrogen/10% hydrogen/10% CO₂ at 35°C to monitor *P. gingivalis*, *F. nucleatum*, *Actinobacillus* spp. and *Peptostreptococcus micros*. An additional 100 µl was plated on Columbia agar containing 5% bovine blood (Biolife Italiana) in 5% CO₂ to monitor *Gemella* spp., *Streptococcus* spp., *Leuconostoc* spp., and *Granulicatella* spp. Microorganisms were identified by standard procedures^[11] as well as the production of a set of metabolic enzymes (as tested with Rapid ID 32A and Rapid ID32 Strep).^[12,13] With regard to bacterial counts, the results were expressed in MCF, equivalent to 1.5×10^8 cells/ml.

RESULTS

Oral mucositis, according to WHO scores, involving nonkeratinized sites developed in 10 patients (66.6%) in the test group: eight with Grade 1 and two with Grade 2. No ulcerations on the keratinized mucosa were scored. No mucositis developed in the control group. Table 1 shows 16 patients (55%) who developed plaque that consisted predominantly of saprophytic Gram-positive cocci (*Streptococcus* spp., *Leuconostoc* spp., *Granulicatella* spp., and *Gemella* spp.). The other 13 patients (45%) developed periodontal pathogens (*F. nucleatum*, *P. gingivalis*, *Actinobacillus* spp., and *P. micros*). *Actinobacillus* spp. was the least frequently found periodontal pathogen in the test group (6.6%), while *F. nucleatum* was the most frequently found (20%). No significant differences were found in bacterial changes between t0, t1, and t2 in the test group [Figure 1]. In the control group, the bacterial count remained unchanged during the observation period [Figure 2]. At t0, t1, and t2, differences in qualitative and quantitative variations between the two groups were not significant [Figure 3].

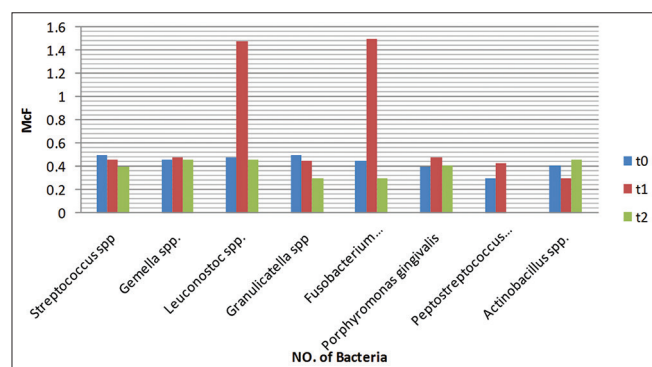
DISCUSSION

Supragingival plaque is influenced by saliva and gingival fluid and allows the growth of aerobic and anaerobic organisms,^[14] ultimately leading to complex microflora dominated by Gram-positive bacteria, particularly streptococci. This flora can be representative of the oral flora during

Table 1: Mean numbers of bacteria in study population

Patient	Sex	Bacteria	Chemotherapy	N (MCF = 1.5×10^9 cells/ml)		
				t0	t1	t2
1	Male	<i>Granulicatella</i> spp.	Yes	0.5	0.5	0.5
2	Male	<i>F. nucleatum</i>	Yes	0.3	0.4	0.45
3	Male	<i>Gemella</i> spp.	Yes	0.5	0.45	0.45
4	Male	<i>P. micros</i>	No	0.3	0.3	0.3
5	Female	<i>Actinobacillus</i> spp.	No	0.3	0.45	0.3
6	Male	<i>Streptococcus</i> spp.	Yes	0.5	0.5	0.5
7	Female	<i>Granulicatella</i> spp.	Yes	0.45	0.5	0.5
8	Female	<i>Gemella</i> spp.	No	0.5	0.5	0.45
9	Female	<i>Leuconostoc</i> spp.	Yes	0.5	0.45	0.5
10	Male	<i>F. nucleatum</i>	No	1.5	1.5	1.5
11	Male	<i>Streptococcus</i> spp.	Yes	0.5	0.5	0.5
12	Male	<i>P. gingivalis</i>	No	0.3	0.45	0.45
13	Female	<i>Actinobacillus</i> spp.	No	0.3	0.3	0.3
14	Male	<i>Streptococcus</i> spp.	Yes	0.5	0.5	0.5
15	Female	<i>P. micros</i>	Yes	0.4	0.45	0.45
16	Male	<i>Leuconostoc</i> spp.	No	1.5	1.45	1.45
17	Female	<i>F. nucleatum</i>	Yes	0.3	0.3	0.3
18	Female	<i>Streptococcus</i> spp.	No	0.5	0.45	0.45
19	Female	<i>Streptococcus</i> spp.	No	0.4	0.4	0.4
20	Male	<i>P. gingivalis</i>	No	0.5	0.45	0.5
21	Male	<i>Gemella</i> spp.	No	0.5	0.5	0.5
22	Male	<i>Streptococcus</i> spp.	Yes	0.45	0.45	0.45
23	Female	<i>Granulicatella</i> spp.	No	0.3	0.3	0.3
24	Male	<i>Gemella</i> spp.	Yes	0.45	0.45	0.45
25	Male	<i>Leuconostoc</i> spp.	Yes	0.5	0.45	0.55
26	Male	<i>F. nucleatum</i>	Yes	0.3	0.4	0.4
27	Female	<i>Streptococcus</i> spp.	No	0.5	0.5	0.5
28	Male	<i>P. gingivalis</i>	No	0.4	0.45	0.4
29	Male	<i>Actinobacillus</i> spp.	Yes	0.4	0.5	0.5

F. nucleatum: *Fusobacterium nucleatum*, *P. micros*: *Peptostreptococcus micros*, *P. gingivalis*: *Porphyromonas gingivalis*

**Figure 1: Mean number of bacteria in the samples of test group**

chemotherapy,^[15] as found in our present study. The microflora undergo modifications during the day, particularly due to eating, and for this reason, all sampling was done 2 h after breakfast. The standardization of sampling allowed us to minimize variations related to this parameter. The microorganisms monitored in this study were saprophytic species of the oral cavity (*Streptococcus* spp., *Leuconostoc* spp., *Granulicatella* spp., and *Gemella* spp.) and species associated

with periodontal pathology (*P. gingivalis*, *Actinobacillus* spp., *Peptostreptococcus* spp., and *F. nucleatum*). These periodontal pathogens are known for their association with periodontal diseases in immunosuppressed individuals.^[15-17] In our pilot study, *F. nucleatum* was the most frequently found periodontal pathogen in dental plaque of patients undergoing chemotherapy. However, none of our patients showed any sign of serious periodontal pathology and periodontal probing, was not part of the standard of care during our study. Therefore, it was not possible to make such conclusions from this study. The dental plaque flora is constantly influenced by external sources, such as nosocomial infections, gastroesophageal reflux, and systemic and oral treatments. Topical, oral, and parenteral antimicrobials before and during cancer chemotherapy should alter the quantitative and qualitative oral microflora profile.^[18] For this reason, the use of antimicrobial agents was an exclusion criterion for our study. Children differ from adults in their oral microflora, and in their response to chemotherapeutic regimens. Most of the oral bacterial changes noted in pediatric studies involved

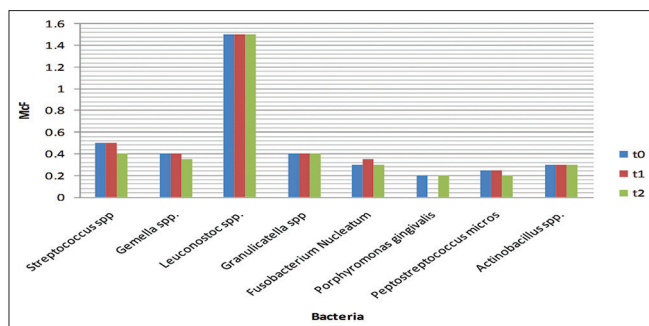


Figure 2: Mean numbers of bacteria in the samples in the control groups

Gram-positive streptococci and staphylococci, whereas in studies of adults, most changes involved Gram-negative organisms such as *Enterobacteriaceae* and *Pseudomonas* spp.^[15] There is no consensus regarding qualitative and quantitative changes in oral microflora during cancer chemotherapy, or a clear pattern or association between mucositis and changes in oral microflora.^[2] Previous studies have differed in many important aspects, including patient populations and presence of a control group, chemotherapeutic regimens, and use of antimicrobials during chemotherapy, sample sites and number of samples collected, collection times and methods, microorganisms cultured, and the scoring method for mucositis. Thus, it is difficult to compare our results to those of other studies. Our results showed that although there was a reduction in the number of oral bacteria in 5% of patients in the test group, in the remaining 95%, there was no significant change in the number of bacteria analyzed from t0 to t2. Similarly, the test group showed no change in bacterial microflora between beginning chemotherapy and at the end of treatment. The cross-sectional analysis showed no significant differences between the test and control groups. In slightly more than half of the patients (57%), the oral microflora consisted mainly of Gram-positive cocci (saprophytic species of the oral cavity), while the remaining 43% of the patients had bacterial flora that also had periodontal pathogenic species. The only difference between the two groups was the incidence of mucositis, which was present only in the test group. These results suggest that bacterial pathogenicity is due to less changes in the intrinsic microhabitat of the oral cavity and more to a decrease in the efficiency of the immune response.^[19] However, in this study, the relationship between leukocyte counts and quantitative oral microflora changes was not determined. The combination of mucositis and granulocytopenia increases the risk of systemic infection resulting from invasion of oral microflora into the bloodstream. However, although it is postulated that some oral bacteria may exacerbate mucositis, it cannot be determined from the results that the presence of local or systemic bacterial infection correlates with the onset and severity of mucositis.^[20] *P. gingivalis* was consistently

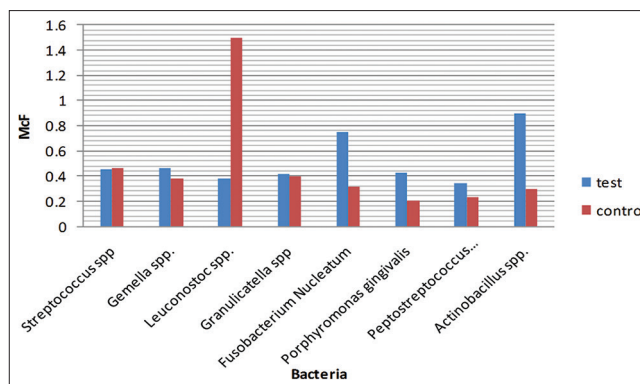


Figure 3: Cross-sectional analysis of mean bacterial counts between the test and control groups

associated with oral ulcerations in a study of hematopoietic stem cell transplant patients and had a positive predictive value.^[8] *P. gingivalis* possesses several virulence factors such as fimbriae that enable the bacterium to attach and invade epithelial cells,^[21] and a lipopolysaccharide capsule that is highly antigenic and can induce the production of pro-inflammatory cytokines.^[22] These virulence factors might prolong or intensify oral ulcerations and could explain the role of *P. gingivalis* in mucositis. Nevertheless, in our study, no patient undergoing chemotherapy had *P. gingivalis* in the plaque samples.

CONCLUSIONS

Within the limitations of the small sample size of our pilot study, it can be concluded that no changes occur in microflora in dental plaque in cancer patients within 7 days from the first course of chemotherapy. No correlations between oral mucositis and specific microorganisms were assessed. More patients are required to increase the reliability of the results, and more detailed studies are necessary to understand the relationship between chemotherapy, alterations in the nature and magnitude of the oral microflora, and the presence of mucositis. Better characterization of changes in oral microflora would be obtained using molecular biological techniques. This would help our understanding of the potential role of oral microflora in the development and exacerbation of oral mucositis. Data from such work could be directed toward developing and testing selective antimicrobial therapies for the prevention and management of mucositis during cancer chemotherapy.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Paster BJ, Boches SK, Galvin JL, Ericson RE, Lau CN, Levanos VA, *et al.* Bacterial diversity in human subgingival plaque. *J Bacteriol* 2001;183:3770-83.
2. Napeñas JJ, Brennan MT, Bahrani-Mougeot FK, Fox PC, Lockhart PB. Relationship between mucositis and changes in oral microflora during cancer chemotherapy. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007;103:48-59.
3. He J, Huang W, Pan Z, Cui H, Qi G, Zhou X, *et al.* Quantitative analysis of microbiota in saliva, supragingival, and subgingival plaque of Chinese adults with chronic periodontitis. *Clin Oral Investig* 2012;16:1579-88.
4. Slots J, Rams TE. New views on periodontal microbiota in special patient categories. *J Clin Periodontol* 1991;18:411-20.
5. Paju S, Pussinen PJ, Suominen-Taipale L, Hyvönen M, Knuutila M, Könönen E. Detection of multiple pathogenic species in saliva is associated with periodontal infection in adults. *J Clin Microbiol* 2009;47:235-8.
6. Feller L, Essop R, Wood NH, Khammissa RA, Chikte UM, Meyerov R, *et al.* Chemotherapy – And radiotherapy-induced oral mucositis: Pathobiology, epidemiology and management. *SADJ* 2010;65:372-4.
7. Raber-Durlacher JE, Weijl NI, Abu Saris M, de Koning B, Zwinderman AH, Osanto S. Oral mucositis in patients treated with chemotherapy for solid tumors: A retrospective analysis of 150 cases. *Support Care Cancer* 2000;8:366-71.
8. Laheij AM, de Soet JJ, von dem Borne PA, Kuijper EJ, Kraneveld EA, van Loveren C, *et al.* Oral bacteria and yeasts in relationship to oral ulcerations in hematopoietic stem cell transplant recipients. *Support Care Cancer* 2012;20:3231-40.
9. Clarkson JE, Worthington HV, Furness S, McCabe M, Khalid T, Meyer S. Interventions for treating oral mucositis for patients with cancer receiving treatment. *Cochrane Database Syst Rev* 2010;(8):CD001973.
10. Freitas AC, Campos L, Brandão TB, Cristófaró M, Eduardo Fde P, Luiz AC, *et al.* Chemotherapy-induced oral mucositis: Effect of LED and laser phototherapy treatment protocols. *Photomed Laser Surg* 2014;32:81-7.
11. Murray PR, Traynor P, Hopson D. Critical assessment of blood culture techniques: Analysis of recovery of obligate and facultative anaerobes, strict aerobic bacteria, and fungi in aerobic and anaerobic blood culture bottles. *J Clin Microbiol* 1992;30:1462-8.
12. Pattyn SR, Ieven M, Buffet L. Comparative evaluation of the rapid ID 32A kit system, miniaturized standard procedure and a rapid fermentation procedure for the identification of anaerobic bacteria. *Acta Clin Belg* 1993;48:81-5.
13. Freney J, Bland S, Etienne J, Desmonceaux M, Boeufgras JM, Fleurette J. Description and evaluation of the semiautomated 4-hour rapid ID 32 strep method for identification of streptococci and members of related genera. *J Clin Microbiol* 1992;30:2657-61.
14. Fujinaka H, Takeshita T, Sato H, Yamamoto T, Nakamura J, Hase T, *et al.* Relationship of periodontal clinical parameters with bacterial composition in human dental plaque. *Arch Microbiol* 2013;195:371-83.
15. Sixou JL, De Medeiros-Batista O, Gandemer V, Bonneure-Mallet M. The effect of chemotherapy on the supragingival plaque of pediatric cancer patients. *Oral Oncol* 1998;34:476-83.
16. Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, *et al.* *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* 2013;14:207-15.
17. Katz J, Onate MD, Pauley KM, Bhattacharyya I, Cha S. Presence of *Porphyromonas gingivalis* in gingival squamous cell carcinoma. *Int J Oral Sci* 2011;3:209-15.
18. Lanzós I, Herrera D, Santos S, O'Connor A, Peña C, Lanzós E, *et al.* Microbiological effects of an antiseptic mouthrinse in irradiated cancer patients. *Med Oral Patol Oral Cir Bucal* 2011;16:e1036-42.
19. Parisi E, Glick M. Immune suppression and considerations for dental care. *Dent Clin North Am* 2003;47:709-31, vii.
20. Vanhoecke B, De Ryck T, Stringer A, Van de Wiele T, Keefe D. Microbiota and their role in the pathogenesis of oral mucositis. *Oral Dis* 2015;21:17-30.
21. Amano A. Bacterial adhesins to host components in periodontitis. *Periodontol* 2000 2010;52:12-37.
22. Vernal R, León R, Silva A, van Winkelhoff AJ, Garcia-Sanz JA, Sanz M. Differential cytokine expression by human dendritic cells in response to different *Porphyromonas gingivalis* capsular serotypes. *J Clin Periodontol* 2009;36:823-9.