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A HOSPITAL BASED OBSERVATIONAL ASSESSMENT OF THE MICROFLORA IN THE POST-CHEMOTHERAPY PATIENTS OF ORAL CANCER

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Abstract

Aim: The aim of this study to evaluate the microflora in the post-chemotherapy patients of oral cancer.

Methods: The present study was conducted in the Department of Dentistry Ananta Institute of Medical Sciences and Research Centre, Tehsil Nathdwara, Distt. Rajsamand, Rajasthan, India, from July 2020 to June 2021. We enrolled 24 patients (15 men and 9 women, aged 20–55years) with solid malignancy that had no previous adjuvant radiotherapy or recent antimicrobial or antiviral treatment. Sampling was done at the same time of day, approximately 2 h after breakfast. Microorganisms were identified by standard procedures as well as the production of a set of metabolic enzymes (as tested with Rapid ID 32A and Rapid ID32 Strep). With regard to bacterial counts, the results were expressed in MCF, equivalent to 1.5 × 108 cells/ml.

Results: Oral mucositis, according to WHO scores, involving nonkeratinized sites developed in 8 patients (33.33%) in the test group: 7 with Grade 1 and 1 with Grade 2. No ulcerations on the keratinized mucosa were scored. No mucositis developed in the control group. 12 patients (50%) who developed plaque that consisted predominantly of saprophytic Gram-positive cocci (Streptococcus spp., Leuconostoc spp., Granulicatella spp., and Gemella spp.). The other 12 patients (50%) 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 (8.33%), while F. nucleatum was the most frequently

found (16.67%). No significant differences were found in bacterial changes between t0, t1, and t2 in the test group. In the control group, the bacterial count remained unchanged during the observation period. At t0, t1, and t2, differences in qualitative and quantitative variations between the two groups were not significant.

Conclusion: 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.

Keywords: microflora, chemotherapy, oral mucositis

Introduction

About 30 trillion bacterial cells are living in or on every human. That is around one bacterium for each cell in the human body.[1] These microorganisms are on the whole known as the microbiome. Since the completion of the Human Microbiome Project[2], we have witnessed an increased interest in the role that the human microbiome plays in human health, many studies have linked changes in microbial communities to systemic conditions such as allergies, diabetes, inflammatory bowel disease, and atherosclerosis.[3-7] Among the systemic conditions influenced by the microbiome, cancer has not been an exception. We have learned that chronic infections contribute to carcinogenesis, with approximately 13% of the global cancer burden being directly attributable to infectious agents.[8] Many viruses promote cancer through well-described genetic mechanisms. Around 10–15% of human cancers worldwide are caused by seven human viruses, which include Epstein-Bar Virus (EBV), Hepatitis B Virus (HBV), Human T-lymphotropic virus-I (HTLV-I), Human papillomaviruses (HPV), Hepatitis C virus (HCV), Kaposi's sarcoma herpesvirus (KSHV) and Merkel cell polyomavirus (MCV).[9] However, the first evidence that bacteria were directly involved in cancer development did not come until the 1980s with the work of Marshall and Warren.[10] When they presented their results, entrenched was the belief that lifestyle caused ulcers that it was difficult for them to convince the scientific world of Helicobacter pylori's role in gastric cancer. To provide even more conclusive evidence, in 1985, Marshall deliberately infected himself with the bacterium and established his stomach illness. Since then, it has been firmly proven by many researchers worldwide that H. pylori cause more than 90% of duodenal ulcers and up to 80% of gastric ulcers, and has been classified as a class I carcinogen by the World Health Organization due to its ability to promote stomach cancer after chronic infection.[11-13] Disease-promoting and cancerpromoting effects of pathogens often depend on virulence factors. In H. pylori, strains expressing the virulence factors cytotoxin associated gene A (CagA) or vacuolating cytotoxin A (VacA), exemplify the role of virulence factors by increasing inflammation, and cancer rates.[14]

Material and methods

The present study was conducted in the Department of Dentistry Ananta Institute of Medical Sciences and Research Centre, Tehsil Nathdwara, Distt. Rajsamand, Rajasthan, India from July 2020 to June 2021, after taking the approval of the protocol review committee and institutional ethics committee. After taking informed consent detailed history was taken from the patient or the relatives if the patient was not in good condition.

Methodology

Study enrolled 24 patients (15 men and 9 women, aged 20-55years) 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 (6 men, 2 women), colon–rectum (4 men and 1 women), prostate (5 men), and breast (6 women). 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 at eight non-keratinized 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 procedures15 as well as the production of a set of metabolic enzymes (as tested with Rapid ID 32A and Rapid ID32 Strep).[16,17] With regard to bacterial counts, the results were expressed in MCF, equivalent to 1.5 × 108 cells/ml.

Results

Oral mucositis, according to WHO scores, involving nonkeratinized sites developed in 8 patients (33.33%) in the test group: 7 with Grade 1 and 1 with Grade 2. No ulcerations on the keratinized mucosa were scored. No mucositis developed in the control group. Table 1 shows 12 patients (50%) who developed plaque that consisted predominantly of saprophytic Gram-positive cocci (Streptococcus spp., Leuconostoc spp., Granulicatella spp., and Gemella spp.). The other 12 patients (50%) 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 (8.33%), while F. nucleatum was the most frequently found (16.67%). No significant differences were found in bacterial changes between t0, t1, and t2 in the test group. In the control group, the bacterial count remained unchanged during the observation period (Table 2) At t0, t1, and t2, differences in qualitative and quantitative variations between the two groups were not significant. (Table 3)

Table 1

SEX	BACTERIA	CHEMOTHERAPY	N (McF= 1.5 × 10^8 cells/ml)		
			t0	t1	t2
M	*Peptostreptococcus micros*	YES	0.3.5	0.3.5	0.4
M	*Actinobacillus spp.*	YES	0.3.5	0.3.5	0.4
M	*Streptococcus spp*	NO	4.5	0.5	0.5
F	*Granulicatella spp.*	YES	0.45	0.45	0.5
M	*Gemella spp.*	NO	0.5	0.45	0.5
M	*Leuconostoc spp.*	NO	1.5	1.5	1.5
F	*Fusobacterium Nucleatum*	NO	0.3	0.3	0.3
M	*Streptococcus spp*	YES	0.5	0.5	0.5
M	*Peptostreptococcus micros*	NO	0.4	0.4	0.4
F	*Porphyromonas gingivalis*	YES	0.3	0.3	0.3
M	*Actinobacillus spp.*	YES	0.3	0.3	0.3
F	*Streptococcus spp*	NO	0.5	0.5	0.5
F	*Peptostreptococcus micros*	NO	0.5	0.5	0.5
M	*Leuconostoc spp.*	YES	1.5	1.5	1.5
F	*Fusobacterium Nucleatum*	YES	0.4	0.4	0.4
F	*Streptococcus spp*	NO	0.5	0.5	0.5
M	*Streptococcus spp*	YES	0.5	0.5	0.5
M	*Porphyromonas gingivalis*	NO	0.4	0.4	0.4
M	*Gemella spp.*	YES	0.5	0.5	0.5
F	*Fusobacterium Nucleatum*	YES	0.4	0.4	0.4
F	*Gemella spp.*	YES	0.5	0.5	0.5
M	*Granulicatella spp.*	NO	0.5	0.5	0.5
M	*Leuconostoc spp.*	NO	1.5	1.5	1.5
M	*Fusobacterium Nucleatum*	YES	0.5	0.5	0.5

Table 2: Mean number of bacteria in the samples of test group

	T_0	T_1	T_2
Streptococcus spp	0.46	0.44	0.42
Gemella spp.	0.43	0.45	0.43
Leuconostoc spp.	0.45	1.5	0.42
Granulicatella spp.	0.45	4.43	0.22
Fusobacterium Nucleatum	0.44	1.5	0.31
Porphyromonas gingivalis	0.41	0.47	0.41

Peptostreptococcus micros	0.30	0.41	0.41
Actinobacillus spp.	0.42	0.37	0.46

Table 3: Cross-sectional analysis of mean bacterial counts between the two groups

Parameter	Test	Control
Streptococcus spp	0.42	0.45
Gemella spp.	0.43	0.41
Leuconostoc spp.	0.38	1.51
Granulicatella spp.	0.41	0.40
Fusobacterium Nucleatum	0.88	0.45
Porphyromonas gingivalis	0.45	0.25
Peptostreptococcus micros	0.39	0.29
Actinobacillus spp.	0.95	0.26

Discussion

Supragingival plaque is influenced by saliva and gingival fluid and allows the growth of aerobic and anaerobic organisms,[18] ultimately leading to complex microflora dominated by Gram-positive bacteria, particularly streptococci. This flora can be representative of the oral flora during chemotherapy,[19] 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.[19-21] In our pilot study, F. nucleatum was the most frequently found periodontal pathogen in dental plaque of patients undergoing chemotherapy. 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.[22] 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 Gram‐positive streptococci and staphylococci, whereas in studies of adults, most changes involved Gram‐negative organisms such as Enterobacteriaceae and Pseudomonas spp.[19] 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.[23] 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 6% of patients in the test group, in the remaining 94%, 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 (50%), the oral microflora consisted mainly of Gram‐positive cocci (saprophytic species of the oral cavity), while the remaining 50% 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 change in the intrinsic microhabitat of the oral cavity and more to a decrease in the efficiency of the immune response.[24] 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.[25] P. gingivalis was consistently associated with oral ulcerations in a study of hematopoietic stem cell transplant patients and had a positive predictive value.[26] P. gingivalis possesses several virulence factors such as fimbriae that enable the bacterium to attach and invade epithelial cells,[27] and a lipopolysaccharide capsule that is highly antigenic and can induce the production of pro‐inflammatory cytokines.[28] 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.

Conclusion

The present study 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.

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