Oral bacterial community dynamics in paediatric patients with malignancies in relation to chemotherapy-related oral mucositis: a prospective study

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Abstract

The role of oral bacteria in the development of chemotherapy-related oral mucositis has not been fully elucidated. This study aimed to investigate oral bacterial community diversity and dynamics in paediatric patients with malignancies in relation to the occurrence of oral mucositis. Patients with malignancies (n = 37) and reference individuals without known systemic disorders (n = 38) were recruited. For patients, oral bacterial samples were taken from mucosal surfaces both at the time of malignancy diagnosis and during chemotherapy. If oral mucositis occurred, samples were taken from the surface of the mucositis lesions. Oral mucosal bacterial samples were also taken from reference individuals. All samples were assessed using a 16S ribosomal RNA gene 454 pyrosequencing method. A lower microbial diversity ($p < 0.01$) and a higher intersubject variability ($p < 0.001$) were found in patients as compared with reference individuals. At the time of malignancy diagnosis (i.e. before chemotherapy) patients that later developed mucositis showed a higher microbial diversity ($p < 0.05$) and a higher intersubject variability ($p < 0.001$) compared with those without mucositis. The change of bacterial composition during chemotherapy was more pronounced in patients who later developed mucositis than those without mucositis ($p < 0.01$). In conclusion, we found a higher microbial diversity at the time of malignancy diagnosis in patients who later develop oral mucositis and that these patients had a more significant modification of the bacterial community by chemotherapy before the occurrence of mucositis. These findings may possibly be of clinical importance in developing better strategies for personalized preventive management.

Keywords: 16S rRNA gene, 454 pyrosequencing, cancer, oral microflora, stomatitis
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Background

All forms of cytostatic therapy give rise to side-effects that have a major impact on the patients' quality of life during anticancer treatment. One of the side-effects is the inflammation of mucosal tissues, mucositis, which can involve the entire alimentary tract. Oral mucositis is one of the most frequently encountered forms and commonly occurs 7–10 days after the administration of cytostatic drugs. Oral mucositis presents as mucosal ulceration, bleeding and severe pain, which may require the use of opiates and parenteral nutrition. The mucositis-inflicted tissue damage also provides a port for the invasion of host endogenous bacteria into the circulation, causing bacteraemia and sepsis [1,2]. The patho-mechanisms of chemotherapy-related oral mucositis have not been fully

elucidated, although considerable progress has been made during the last decade in defining a cascade of destructive and inflammatory events [3]. However, beyond this paradigm, the association between mucositis and the commensal bacterial microflora is so far poorly understood [4,5].

It is now well recognized that the diversity of microorganisms colonizing the oral cavity has been greatly underestimated [6]. Most of the bacterial species cause no harm under healthy conditions. However, in patients with malignancies, the delicate homeostasis between host defence and commensal bacteria could be disturbed by the cancer itself, by the cancer-related secondary immunodeficiency, or by prophylactic antibacterials. The disrupted homeostasis might contribute to the oral mucosal tissue breakdown following chemotherapy. In addition, the chemotherapeutics can be bacteriostatic or bactericidal, thus affecting the oral bacterial community [4,7,8]. However, no clear pattern regarding the changes in the oral bacterial community and occurrence of oral mucositis can be discerned from the literature, most likely due to the limited number of studies published.

The impact of the bacterial community on the mucosal integrity during chemotherapy cannot be fully understood without comprehensive knowledge of the bacterial community composition. The conventional culture-based or biochemical methods can identify anticipated bacterial taxa, but lack the capacity to detect non-cultivable microorganisms and the possibility to address hitherto unknown taxa. However, modern molecular methods for identifying bacterial taxa have made it possible to assess a bacterial community with a reduction in bias experienced in culture-based methods [9], and furthermore, a massively parallel DNA sequencing technique, 454 pyrosequencing, has now greatly increased the capacity to detect bacteria of low abundance [10,11].

In this study, we employed 16S rRNA gene 454 pyrosequencing, in order to determine the diversity and relative abundance of oral mucosal bacterial taxa in paediatric patients with malignancies. The oral bacteria were assessed at the time of malignancy diagnosis prior to chemotherapy, during chemotherapy and at the time of mucositis in an attempt to follow the dynamics of the bacterial community in conjunction with chemotherapy-related oral mucositis.

Materials and Methods

Subjects

This study was designed as a prospective longitudinal cohort study. An ethics permit was granted by the Regional Ethical Review Board, situated at Karolinska Institutet, Stockholm, Sweden.

From November 2008 to December 2010, patients with newly diagnosed malignancies (n = 109) were enrolled from the Paediatric Cancer Ward, Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden. The exclusion criteria were as follows: (i) patients under 4 years of age or above 18 years of age, (ii) the treatment protocol did not include cytostatic drugs, and/or (iii) patients without national population registration number. Out of 60 patients that met inclusion criteria, 37 patients agreed to participate in the present study. Age and gender-matched children (n = 38), without any known systemic disorder and who had not been treated with antibacterials 3 months prior to the study, were recruited as reference individuals during their routine dental visit to the Division of Paediatric Dentistry, Department of Dental Medicine, Karolinska Institutet, Sweden. Assent and informed consent were obtained from all the included children and their parents, respectively.

For the patients, data regarding age, gender and diagnosis of malignancies were collected. Data including blood counts of neutrophils, leukocytes and thrombocytes, and levels of haemoglobin at the time of malignancy diagnosis, were extracted from laboratory test reports. Oral health status, including decayed, missing or filled teeth of permanent/deciduous teeth (DMFT/dmft) and gingival bleeding index (GBI) were assessed by the same dentist for all patients to avoid inter-examiner difference. Oral care instructions, including recommendation of a single 2.5 mg/mL benzydamine-based mouth rinse for the period of chemotherapy, were provided to the patients and parents. All patients were followed during the entire cytostatic treatment. One dose of the antibacterial cefotaxime was given intravenously to each patient as prophylaxis before placing a central venous catheter. The individual chemotherapeutic scheme and antibacterial agents used for treating infections were retrieved from medical charts and information regarding the use of the mouth rinse was gathered from the parents. The occurrence of oral mucositis was recorded and the grade of oral mucositis was scored using the World Health Organisation (WHO) system [12], which grades oral toxic effects into five levels: grade 0, no change; grade 1, soreness/erythema; grade 2, erythema, ulcers, can eat solids; grade 3, ulcers, requires liquid diet only; grade 4, alimentation not possible. A WHO grade > 1, which indicates ulcerative mucositis, was considered as occurrence of oral mucositis in the current study to avoid false-positive diagnosis.

For the reference individuals, data including age, gender and oral health status in terms of DMFT/dmft and GBI were recorded. The same professional performed the oral health evaluations for the patients and the reference individuals.