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Bacterial endotoxins and microorganisms in the oral cavities of patients on cancer therapy

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ABSTRACT

Objectives: This study investigated the presence of Streptococci, Staphylococci, aerobic gram negative bacteria (AGNB), *Candida* and bacterial endotoxins in the oral cavities of patients receiving chemo- and/or radiotherapy for cancer.

Methods: Samples of oral cavity rinse were collected from 100 patients on cancer treatment and 70 healthy individuals. Demographic and clinical data were recorded. Samples were cultured onto various agar plates for qualitative and quantitative analysis and tested for the presence of endotoxin. Results were analysed using the Mann-Whitney and chi-square tests.

Results: In cancer patients, *S. aureus* counts were high and 66.7% of patients on chemo- and radiotherapy carried these bacteria ($p = < 0.05$). The *Candida* carrier rate was significantly ($p < 0.01$) high in cancer patients (54%). No significant difference was found in the carrier rate of Streptococci and AGNB between the healthy and cancer group as well as between the cancer patients with chemo and radio- and chemotherapy alone. No significant difference was found in the level of endotoxin between the cancer patients and healthy individuals, and cancer patients with and without AGNB.

Conclusions: No differences in the prevalence of bacteria and bacterial endotoxins were found between the cancer patients and healthy individuals. Oral cavity endotoxins did not correlate with the carriage of AGNB. However, due to the high prevalence in cancer patients, the role of *Candida species* and *S. aureus* in the pathology may not be excluded.

1. Introduction

Chemo- and radiation therapy are the most widely used interventions for the treatment of cancer. Several adverse effects, including mucositis, are associated with these cytotoxic therapies. Oral mucositis occurs as a non-haematologic localised erythema which can progress into a deep ulcer causing discomfort, pain, significant morbidity and interruption in cancer treatment [1]. In addition, imbalance in the normal oral flora and establishment of extra-oral flora, due to oxidative stress, activation of the innate immune system and xerostomia, is caused by cancer therapy. During radiation therapy, an increase in aerobic gram negative bacteria, *Candida species* and lactobacilli have been reported [2–5]. During chemotherapy, an increase in the total number of species including Streptococci and Staphylococci and more complex microbiota in cancer patients have been reported [6]. These organisms can become established in the lesions of oral mucositis and they may cause pathology.

Aerobic gram negative bacteria (AGNB), which are common in the gut, are generally not found in the oral cavity. However, they have been isolated from diseased, as well as healthy individuals [7,8]. This colonization occurs as a result of denudement of oral mucosa from fibronectin, which sometimes exposes receptors which are required for the attachment of AGNB [9]. These bacteria produce endotoxins, which may penetrate into the submucosa, activate immune cells and further stimulate them to secrete pro-inflammatory cytokines. Little is known about salivary endotoxins in healthy individuals and cancer patients. Leenstra et al. [10] studied endotoxin levels in 15 healthy individuals and found mean endotoxin levels of 21 ng/ml. Millns et al. [11] studied 12 leukaemic children and healthy individuals and found 18.28 and 12.2 ng/ml of mean endotoxin respectively. They also suggested that there was no correlation between the carriage of AGNB and endotoxins. However, this study included only 12 cancer patients of which some carried AGNB and some did not carry these bacteria. The presence of uncommon gut flora such as AGNB, a change in the normal oral flora

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and endotoxins in the oral cavities of adult cancer patients on therapy, may cause pathology. This study investigated the presence of bacteria and their endotoxins, and *Candida* in the oral cavities of patients receiving different cancer treatment. In addition, an attempt was made to establish correlation between the presence of AGNB and endotoxins.

2. Materials and methods

2.1. Study population

One hundred patients diagnosed with any type of malignant solid tumour, who were scheduled for either radiation and chemotherapy or only chemotherapy at the Charlotte Maxeke Johannesburg Academic Hospital, were approached and asked to volunteer for the study. Ethical clearance (Certificate number: M160562) was obtained from The Committee for Research on Human Subjects (Medical), University of the Witwatersrand, Johannesburg. Informed consent was obtained from all individual participants included in the study. Patients with HIV, diabetes or oral prostheses, as well as smokers were excluded. Patients with haematological malignancies and head and neck cancers were excluded. Patients who were on antimicrobials and patients who had just begun their cancer treatment or were only on radiotherapy were also excluded. Patients on only chemotherapy and radiation and chemotherapy were included, as were patients who completed more than 2 cycles of chemotherapy. The demographic data and clinical parameters, such as the type of cancer, type of treatment and duration of treatment were recorded. Patients were examined for mucositis and the grade of oral mucositis was recorded [12]. Seventy healthy individuals were used as a control group. Individuals excluded from this group were those who had any systemic disease, had diabetes, were smokers, who wore oral prostheses and who were on antimicrobials. The study population and the group comparisons are described in Fig. 1.

The participants were asked to rinse their oral cavity with 10 ml of sterile distilled water and expectorate into a specimen jar.

2.2. Microbiological analysis

The oral cavity rinse samples were vortexed and serially diluted to obtain 1:10 to 1:1000 dilutions. One hundred microliters of the concentrated sample as well as each dilution was spread on to Mitis salivarius agar (Streptococci), Baird Parker agar (*Staphylococcus aureus*), Chromagar (*Candida species*) and MacConkey agar (AGNB) for the quantitative and qualitative analysis of microorganisms. All the plates were incubated at 37 °C for 48 h. Mitis salivarius agar was incubated under CO_2. Colony counts were performed, multiplied by the dilution factors and expressed as colony forming units (cfu/ml). In addition, 0.5 ml of oral rinse was added into Brain Heart Infusion broth (BHI) and this was incubated at 37 °C for 24 h as an enrichment step to allow low numbers of AGNB to be detected. This BHI broth was further cultured onto MacConkey agar. Cultures of *Candida species* and AGNB were purified by further culturing and identified using a biochemical reaction based API® 20 C AUX (bioMérieux, France) and API® 20 E (bioMérieux, France) technique respectively.

One millilitre of oral rinse from all cancer patients and healthy individuals were stored in Eppendorff tubes at −20 °C for the endotoxin assay.

2.3. Endotoxin assay

The Hycult Biotech Limulus Amebocyte Lysate (LAL) assay was used to detect endotoxins in the oral rinse samples collected from 60 cancer patients and 16 healthy individuals. The detection range was 0.04 EU/ml to 10 EU/ml of endotoxin. The test is based on the intravascular coagulation in the American horseshoe crab (Limulus Polyphemus) formed by bacterial endotoxins which can be detected upon cleavage of chromophore, p-nitroaniline (pNA). This reaction can be stopped by the addition of acetic acid. *E. coli* endotoxin (Lot number 19819K0316-A) was used as a positive standard and with each test run, a standard curve was generated. The mean absorbance of the zero standard was less than 0.1 OD and the mean absorbance of the 10 EU/ml standard was higher than 0.6 OD at 405 nm. A standard curve was obtained by plotting the absorbance (linear) versus the corresponding concentrations of the *E. coli* standards.

The oral rinse that was stored at −20 °C in the Eppendorff tubes were warmed back to room temperature. Samples were centrifuged at 5000 rpm for 5 min, similar to a method described by Leenstra et al. (1996) [10]. The supernatants were collected (200 µl) into another Eppendorff tube. These were placed on a heating block at 75 °C for

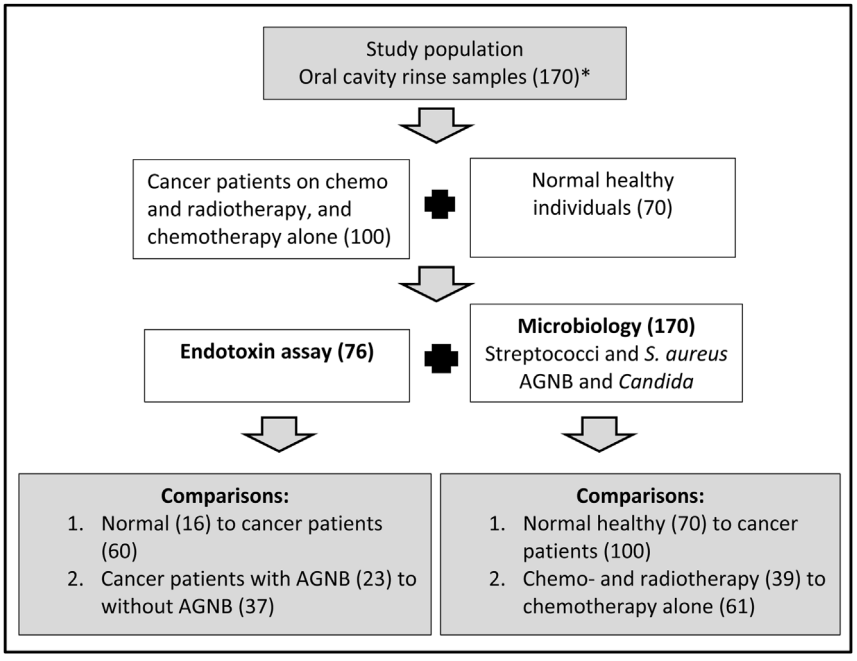

Fig. 1. Study population and design. *In brackets is the number of patients analysed.