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ARTICLE

Significance of Oral Candida Infections in Children with Cancer

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Candidiasis is common in children with cancer, particularly during periods of severe immunosuppression and neutropenia. Our aim was to study the microbiological changes in the oral cavity of children with newly diagnosed cancer. The study group consisted of 30 consecutive children and adolescents, 16 with acute lymphoblastic leukemia and 14 with solid tumors. Oral cultures to detect fungi and bacteria were conducted for all patients before treatment, during and after neutropenic episodes. In 23 patients developing fever simultaneous throat, urine and blood sampling was carried out. No pathogens were found in the cultures taken before the outset (30 cultures) or after recovery from (30 cultures) the neutropenic episodes. In the 45 oral cultures taken during the neutropenic episodes 38 (84.4%) proved positive. Fungi were the most frequently isolated oral pathogens: 33/38 yeast and 6/38 bacterial infections were identified. There was no association between the underlying malignancy and the occurrence of the positive cultures. Of the 30 patients, all 23 (76.7%) who have developed moderate-to-severe neutropenia, developed oral fungal colonization or clinically obvious fungal infection at least on one occasion during the study. In addition to oral samples, fungi were identified in 9/23 pharyngeal swabs, 6/23 urine and 1/23 blood cultures. The initial fungal pathogen was exclusively (33/33) *Candida albicans*. In extended severe neutropenic states, *C. albicans* was replaced by non-albicans species (*C. kefyr, C. lusitaniae, C. sake, C. tropicalis*) in 5 patients between 4 to 6 days of the neutropenic episodes. Four of the non-albicans *Candida* strains were resistant to azole-type antifungal agents. Neutropenic episodes of children with cancer are associated with an increased risk of developing oral and even systemic infections with *C. albicans* that can be replaced by azole-resistant non-albicans strains in prolonged neutropenia contributing to morbidity of these patients. (Pathology Oncology Research Vol 12, No 4, 237–241)

Key words: child, cancer, oral infection, *Candida albicans*, non-albicans *Candida* strains

Introduction

Candidiasis, as an acute infectious complication, is common in children receiving treatment for cancer, particularly during periods of severe neutropenia.[14] Although *Candida albicans* is the most frequent *Candida* species, non-albicans *Candida* strains play an ever-increasing role.[3] Prevention of fungal colonization and control of local infection may be of critical importance in avoiding systemic candidiasis.[6] Our aim was to study the microbiological changes in the oral cavity during chemotherapy in children with newly diagnosed cancer.

Material and Methods

The study group consisted of 30 children and adolescents (15 girls and 15 boys, mean age 8.9 years, ranging from 2 to 24 years) with newly diagnosed cancer, admitted consecutively to the Hematology/Oncology Ward of the Department of Pediatrics of Medical and Health Science Center of University of Debrecen (MHSCUD). The underlying diseases were acute lymphoblastic leukemia (ALL) in 16 cases and solid tumors in 14 cases. Patients were treated according to standard proto-

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cols as accepted by the Hungarian Pediatric Oncology Group (HPOG).

Patients developing neutropenic episodes while on cytostatic chemotherapy were examined daily at least twice with respect to their physical condition by a pediatrician and at least once with respect to their oral status by one of the dentists participating in the study. Body temperature was taken at least three times, and complete blood count and differential count were determined at least once, daily. Additional imaging and laboratory investigation, including chest X-ray and other imaging techniques, ESR, CRP, electrolyte and blood gas analysis, liver enzyme and renal function tests were performed according to the patients' needs.

Oral cultures were obtained at least 3 times in each patient: before initiating intensive cytostatic therapy (30 cultures) to determine the characteristic microflora of the patient, during treatment-related neutropenic episodes (30 cultures) to investigate the microbiological changes and identify the dominant pathogen if existed, and after recovery from neutropenia (30 cultures) to examine the effectiveness of treatment. In cases of prolonged neutropenia (>7 days) sampling was repeated at least weakly (15 additional cultures). Altogether 105 oral cultures were conducted. In 23 patients developing fever in association with moderate-to-severe neutropenia simultaneous blood, throat and urine culture samples were obtained. Oral candidiasis was diagnosed, and mucositis was scored according to Epstein.[5,6]

In febrile patients, empiric antibiotic therapy (ceftazidime, w/o amikacin) was started immediately and modified if necessary according to the result of microbiological cultures or the condition of the patients. Systemic antifungal therapy (fluconazole 6 mg/kg/die) was introduced if fungal infection was diagnosed by clinical signs or microbiological samples, or empirically, if febrile neutropenia lasted over 7 days despite of antibiotic treatment. Fluconazole was changed for amphotericin B (starting dose 0.5 mg/kg/die to be escalated up to 1.0 mg/kg/die in 5 to 7 days) if culture results proved fluconazole-resistant strains.

Oral cultures were obtained by swabbing the palatal and buccal mucosa using Transystem Standard Tube (Copan Italia, Brescia, Italy). The specimens were inoculated on blood agar, chocolate agar, eosin-methylene blue agar and Sabouraud-dextrose agar (SD) plates (Scharlau Chemie, S.A, Barcelona, Spain). Yeasts growing on Sabouraud agar were identified using CHROMagar Candida (Becton Dickinson, Franklin Lakes, NJ, USA) and API ID32C (BioMerieux, Hazelwood, MO, USA). The determination of antifungal susceptibility was performed using Etest (AB Biodisk, Solna, Sweden).

Throat samples were cultured similarly as oral samples. Blood samples were incubated using BACT/Alert (Organon Teknika, Cambridge, UK) automatic device. Urine samples were cultured on Uricult Plus Plates (Orion Diagnostica, Espoo, Finland).

Results

Of the 30 patients 7 developed mild, 12 moderate and 11 severe neutropenia during the treatment periods. In patients with mild neutropenia absolute neutrophil count (ANC) varied from 1.1 to 1.9 G/L (mean 1.5 G/L). Neutropenic episodes lasted from 5 to 7 days (mean 6 days). No patients developed fever (axillary temperature <38.0 °C) and no significant oral lesions were noticed. In patients with moderate neutropenia ANC was between 0.6 to 1.0 G/L (mean 0.8 G/L). Neutropenic episodes lasted from 5 to 26 days (mean 16 days). Fever was registered in all 12 children. Ulcerative mucosal lesions (grade 2 and 3) were found in 3 children. Clinical signs of oral candidiasis were observed in 7 patients. In patients with severe neutropenia ANC varied from 0.2 to 0.5 G/L (mean 0.4 G/L). Neutropenic episodes lasted from 19 to 40 days (mean 29 days). Each patient was febrile, 8/11 patient had severe ulcerative mucositis (grade 3) and 9 had clinical signs of oral candidiasis.

Six patients (1 with moderate and 5 with severe neutropenia) developed signs of fungal esophagitis, otherwise no severe organ manifestation was observed. Fungemia (*C. albicans*) was identified in 1 sample and bacteremia was identified in 13 samples obtained from patients with moderate-to-severe neutropenia. Eight patients (2 with moderate and 6 with severe neutropenia) developed clinical signs of septicemia associated with hemoculture-proven fungal and bacterial infections (*Table 1*). Every patient survived and recovered from the infectious complications.

No dominant pathogens were found in cultures taken before and after neutropenic episodes (data not shown). From the analyzed 45 oral cultures taken during the neutropenic episode, 38 (84.4%) contained dominant pathogens. No changes to the initial oral microflora were observed in patients with mild neutropenia (7 cultures). All 38 positive cultures were obtained from patients having developed moderate-to-severe neutropenia. All 23 patients with moderate-to-severe neutropenia, i.e. 76.7% of study patients, were noted with at least one positive oral culture during the time of observation.

The most frequently isolated pathogens were yeasts (33/38). Bacteria were found in 6/38 cultures (*Table 1*). There was no association between the type of cancer and the occurrence of the positive cultures (data not shown).

Out of the 38 positive oral cultures, 12 were obtained from children with moderate neutropenia. The distribution of these positive samples was as follows: 10/12 yeast, 2/12 bacteria (*Table 1*). *C. albicans* was detected only in 1/12 samples obtained from the pharynx and none from the urine or blood samples in addition to the positive oral samples. Pathogenic bacteria were found in 2/12 samples taken from the pharynx, 1/12 from the urine and 5/12 from the blood.

Twenty-six out of the 38 positive oral cultures were obtained from children with severe neutropenia. The distribution of these samples was as follows: 23/26 yeast, 4/26 bacteria (*Table 1*). In addition to positive oral samples, yeasts were found in 8/11 of the pharyngeal swabs, 6/11 from the urine and 1/11 from blood culture samples. Bacteria were detected in 3/12 samples obtained from the pharynx, 5/12 from the urine and 8/12 from the blood.

Initially, *C. albicans* was detected in each of the 33 positive oral cultures. Non-albicans *Candida* species (2 *Candida kefyr*, 1 *Candida lusitaniae*, 1 *Candida sake* and 1 *Candida tropicalis*) were observed exclusively in patients with severe neutropenia and were always preceded by *C. albicans* infection, 4 to 6 days before the identification of the non-albicans strains.

As expected, all *C. albicans* strains were susceptible to fluconazole and itraconazole, in addition to amphotericin B. Among the non-albicans *Candida* strains we found 1 fluconazole- and 3 itraconazole-resistant strains (MIC values were 64 and 1 mg/L, respectively). All non-albicans *Candida* species were susceptible to amphotericin B.

Discussion

Cancer and cancer treatment profoundly impairs oral health. Oral complications, presenting as mucositis, xerostomia, bleeding and infections are three times more common in children than in adults.[13] The clinical diagnosis of oral infections may be difficult due to reduced inflammatory responses in the immunocompromised host.

In this study we have assessed 105 oral swabs of 30 consecutive children with newly diagnosed cancer. Of the 38 positive cultures 33 (86.8%) were positive for fungi and 6 (15.8%) revealed pathogenic bacteria, indicating that fungal pathogens are about five times as common as bacteria in the oral cavity of children with cancer. Altogether 23 of 30 patients (76.7%) were diagnosed on at least one occa-

Table 1. **Pathogenic microorganisms found in cultures obtained from children with cancer**

Type of culture	*Degree of neutropenia*	*Isolated pathogenic microorganisms*	
		yeasts	*bacteria*
Oral (105)	moderate (12)	*C. albicans* (10) *Klebsiella pneumoniae* (1)	*Staphylococcus aureus* (1)
	severe (11)	*C. albicans* (23) *C. kefyr* (2) *C. lusitaniae* (1) *C. sake* (1) *C. tropicalis* (1	*Klebsiella pneumoniae* (4)
Pharyngeal (23)	moderate (12)	*C. albicans* (1)	*Pseudomonas aeruginosa* (2)
	severe (11)	*C. albicans* (8)	*Pseudomonas aeruginosa* (1) *Klebsiella pneumoniae* (2)
Urine (23)	moderate (12)	none	*Escherichia coli* (1)
	severe (11)	*C. albicans* (6)	*Escherichia coli* (2) *Enterococcus faecalis* (3)
Blood (23)	moderate (12)	none	*Staphylococcus epidermidis* (2) *Staphylococcus haemolyticus* (1)[1] *Staphylococcus coag. neg.* (1) *Klebsiella pneumoniae* (1)[1]
	severe (11)	*C. albicans* (1)[1]	*Staphylococcus epidermidis* (2)[1] *Staphylococcus haemolyticus* (3) *Staphylococcus coag. neg.* (2)[1] *Klebsiella pneumoniae* (1)

Numbers in parentheses indicate the number of culture samples (column 1), the number of patients with neutropenia (column 2) or the number of identified microorganisms (columns 3-5) specified in the cultures. Numbers in upper indices (columns 3 and 4) indicate patients with fungemia and bacteremia and with clinical signs of septicemia.

sion with oral colonization and/or infection. It has been found that oropharyngeal colonization of Candida species may increase the risk of systemic infection especially when oral ulcers develop during the neutropenic episodes.[5] Risk factors for systemic fungal infection also include the use of broad-spectrum antibiotics and steroids.[6] All of our patients exhibiting oral fungal infections received empiric antibiotic treatment, and corticosteroids were applied as part of the induction and post-consolidation treatment protocols in children with acute lymphoblastic leukemia and lymphoma. Well-designed, large studies, utilizing reliable microbiological or histopathological methods estimated the frequency of fungal colonization and infection in pediatric oncologic patients. In the classical study from the St. Jude Children's Research Hospital, Hughes analyzed 109 fatal cases of systemic candidiasis by complete autopsy.[10] He identified fungal lesions in the oral cavity of 26% of the deceased children. *Antemortem* cultures identified *Candida* strains in 69% of throat and 23% of nasopharyngeal samples within 2 months to the fatal outcome. In addition to case reports and small-scale studies, 3 groups of investigators assessed oropharyngeal fungal colonization and infections of 42, 26 and 36 children, respectively, with newly diagnosed leukemia and lymphoma.[7,8,15] One study analyzed yeast colonization and infections in 64 pediatric hematopoietic stem cell transplant recipients.[9] Similar to our results, reported rates of fungal colonization and infections varied from 35% to 69%.

Regarding microbiological speciation, Gozdasoglu et al. identified exclusively *C. albicans* from 36 surveillance cultures and Stinnett et al. identified 2 *C. tropicalis*- and 1 *Rhodotorula rubra*-positive samples in addition to 37/40 *C. albicans*-positive ones.[8,15] Even in the St. Jude study investigating fatal cases of advanced pediatric cancer patients, *C. albicans* was the predominant pathogen.[10] These observations are in contrast with our results identifying 5/33 (15.2%) non-albicans *Candida* strains of the positive fungal cultures. Four of the 5 non-albicans *Candida* strains identified in this study were resistant to azole-type antifungal agents. A similar impact of non-albicans *Candida* strains in children with cancer has only been reported in hematopoietic stem cell transplant recipients.[9] In adults with advanced neoplastic diseases, an increasing importance of azole-resistant oral fungal pathogens has already been acknowledged.[1-3] Interestingly, in our cases, colonization with *C. albicans* always preceded that of with non-albicans *Candida* strains and occurred only in patients with severe, long-lasting neutropenia.

Lack of the use of appropriate microbiological culture techniques may result in an underestimation of fungal colonization. Among the 30 patients enrolled in this study, 11 (36.7%) had ulcerative mucositis and 16 (53.5%) had clinical sign of candidiasis. Stinnett et al. identified only 15% of the leukemic children presenting with clinical signs of oral fungal infections, whereas 46% of their patients proved colonized with fungi when investigated by culture methods.[15] Similarly, in an early study of pediatric leukemia patients, the reported incidence of symptomatic oral fungal lesions was 21%.[11]

Neutropenia has been recognized as one of the major risk factors of developing nosocomial fungal infections both in the oral cavity and in other organs, in particular deep-sited lesions. We detected oral yeasts only in association with moderate-to-severe neutropenic episodes of children with cancer, and oral *Candida* colonization and infections occurred in 100% of these patients. In addition to oral candidiasis, 1 patient with moderate and 5 with severe neutropenia (20.2%) developed signs of fungal esophagitis. One patient (3.3%) with severe neutropenia was found with fungemia accompanied by clinical signs of septicemia. Prevalence rates of fungemia were reported to occur in 0-6% of children with cancer, representing a rare but severe complication in pediatric oncology.[8, 9,10,12]

In conclusion, oral fungal infections develop frequently in children with cancer, in particular in patients with prolonged, severe neutropenic episodes. The pathogenic agents colonizing the oral cavity may induce symptomatic infections, often systemic ones, and may promote the development of infections with drug-resistant strains. Regular dental check-up examinations, oral microbial surveillance and application of professional oral hygienic measures in children with cancer may decrease the incidence, duration and severity of infectious complications. Early introduction of systemic antifungal treatment in pediatric cancer patients with microbiologically proven oral yeast colonization and infections seems to be a prudent approach.[4] Further studies are required to study the impact of local or systemic anti-fungal prophylaxis on fungal morbidity in children with cancer who develop neutropenia.

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