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Frequency of Oral Mucositis and Microbiological Analysis in Children with Acute Lymphoblastic Leukemia Treated with 0.12% Chlorhexidine Gluconate

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In view of the morbidity potential of oral complications in patients with leukemia, this study evaluated the clinical and microbiological alterations that occur in the oral mucosa of children with acute lymphoblastic leukemia (ALL) undergoing antineoplastic chemotherapy and prophylactic administration of 0.12% chlorhexidine gluconate. The sample consisted of 17 children aged 2 to 12 years that underwent clinical examination of the oral mucosa for the detection of oral lesions. In addition, biological material was collected from labial and buccal mucosa for microbiological analysis. Oral mucositis was observed in only 5 (29.4%) patients. Microbiological analysis revealed a reduced number of potentially pathogenic microorganisms, such as coagulase-negative staphylococci (47%), *Candida albicans* (35.3%), *Klebsiella pneumoniae* (5.9%), enteropathogenic *Escherichia coli* (5.9%), and *Stenotrophomonas maltophilia* (5.9%). Patients with oral mucositis showed a higher frequency of coagulase-negative staphylococci (80%) when compared with patients with normal oral mucosa (33.3%). In conclusion, the results of the present study suggest that the prophylactic use of 0.12% chlorhexidine gluconate reduces the frequency of oral mucositis and oral pathogens in children with ALL. In addition, the present findings suggest a possible relationship between coagulase-negative staphylococci and the development of oral mucositis.

Key Words: acute lymphoblastic leukemia, children, chlorhexidine, mucositis, oral microbiota.

INTRODUCTION

Leukemia is a consequence of malignant transformation of a single hematopoietic progenitor cell. A sequence of molecular events disrupts the process of differentiation and limited proliferation that characterizes normal hematopoiesis and generates a leukemic clone capable of expanding by indefinite self-renewal (1). Acute lymphoblastic leukemia (ALL) is the most common type of pediatric cancer, accounting for nearly 30% of all pediatric cancers and 80% of childhood leukemias (2).

Oral mucositis is the most frequent complication of hematological cancer treatment, representing the main cause of pain (3-5) and a site favorable to the colonization and proliferation of bacteria (5,6). Studies have demonstrated an increase in the number and proportion of Gram-negative bacilli such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* and *Enterobacter* spp. in the oral cavity of oncological patients, which are detected in approximately two-thirds of all bacterial isolates (7,8). Gram-positive bacteria including coagulase-negative staphylococci and *Enterococcus* have also been identified in samples

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collected from immunocompromised patients (9,10). Moreover, immunocompromised patients frequently develop candidiasis whose pathogenesis is determined by factors such as immunological state of the host, alterations in the oral microbiota caused by antineoplastic chemotherapy, and resistance to *Candida* (11,12).

Chlorhexidine gluconate is a drug widely used in dentistry, which acts on the damaged mucosa by forming a protective barrier consisting of a whitish membrane that results from the coagulation of serum and salivary proteins, thus reducing the severity of oral ulcerations (13). In addition, chlorhexidine has recognized fungicidal and bactericidal properties (14), and it has been shown that preventive treatment with this drug and oral hygiene care reduce the occurrence of oral complications related to hematological cancer treatment (14).

In light of these findings and in view of the scarcity of data regarding the microbiota associated with the occurrence of oral mucositis in the pediatric cancer population, the aim of this study was to clinically evaluate oral mucosal alterations and to qualitatively analyze the oral microbiota in children with ALL undergoing antineoplastic chemotherapy and prophylactic treatment with 0.12% chlorhexidine gluconate.

MATERIAL AND METHODS

Sample Characterization and Clinical Evaluation

The sample consisted of 17 children with ALL aged 2 to 12 years, with no preference for sex or race, seen at the Infant Oncology and Hematology Center (COHI) of the Varela Santiago Children's Hospital, Natal, RN, Brazil. The study was approved by the Ethics Committee of the Federal University of Rio Grande do Norte, Brazil. The parents or responsible persons received information about the character and objectives of the investigation and signed a free informed consent form.

Mouthrinsing with 0.12% chlorhexidine gluconate was prescribed to the patients for 1 min twice a day, 30 min after breakfast and after the last meal at night. The 0.12% chlorhexidine gluconate solution was administered for 10 days throughout the phases of chemotherapy.

Clinical examination of the oral mucosa was performed daily during the period of hospitalization of the patient for chemotherapy to evaluate the presence of oral mucositis.

Microbiological Evaluation

Material for microbiological analysis was collected from the labial and buccal mucosa during the intensification phase after the end of prophylactic treatment with 0.12% chlorhexidine gluconate. First, a sterile swab was passed to remove saliva and shaded cells. Next, a second swab was vigorously passed to collect material adhered to the mucosa. After collection, the swab was placed in 1.0 mL reducing saline solution (0.85% NaCl + 0.1% sodium thioglycollate).

The collected material was homogenized for approximately 30 s in a Vortex Mixer (Labnet International, Edison, NJ, USA) and then seeded onto the following culture media: mannitol salt agar, MacConkey agar, cetrimide agar, and Sabouraud agar (Difco Becton Dickinson, Franklin Lakes, NJ, USA). All plates were incubated under aerobic conditions for 24 to 48 h.

After incubation, the plates were examined regarding the presence of characteristic colonies of mannitol-fermenting and non-fermenting *Staphylococcus* (mannitol salt agar), fermenting and non-fermenting Gram-negative bacilli (MacConkey agar), *P. aeruginosa* (cetrimide agar), and yeast-like fungi (Sabouraud agar). Characteristic colonies of each microorganism were replated onto brain heart infusion (Difco Becton Dickinson) and incubated under the same conditions as used for isolation.

After incubation, the morphology and staining characteristics of the samples were determined by the Gram method modified by Kopeloff-Beerman and purity was confirmed by replating the samples onto the culture media used for primary isolation. None of the samples presented growth on cetrimide agar.

Staphylococci were characterized by traditional testing for free coagulase. All samples were coagulase negative and were stored for subsequent identification. Fermenting Gram-negative bacilli isolated on MacConkey agar were screened on triple sugar iron medium (Difco Becton Dickinson) and identified as *E. coli* and *K. pneumoniae* by the Mini-API identification system (bioMérieux, Craponne, France). *E. coli* was serotyped as classical enteropathogenic polyvalent B *E. coli*, serotypes O114, O125, O142 and O158, using polyvalent A-B-C serum (Probac do Brasil, São Paulo, SP, Brazil). The oxidative metabolism of non-fermenting Gram-negative bacilli was confirmed by the oxidation-fermentation test in Hugh and Leifson medium and the samples were characterized as *Stenotrophomonas*

maltophilia by the Mini-API system (bioMérieux). Yeast-like fungi were identified as *Candida albicans* by staining of the colonies on CHROMagar (Difco Becton Dickinson) and their identification was confirmed by the production of chlamydospores.

RESULTS

In the present study, only 5 (29.4%) of the 17 patients using 0.12% chlorhexidine gluconate developed oral mucositis of low severity, with a relatively similar distribution of cases according to the treatment phase (Table 1). Regarding anatomic location, oral mucositis affected the buccal mucosa in 2 cases, the gingiva in 2 cases, and the tongue in 1 case. None of the patients presented disseminated mucositis.

The microbiological analysis revealed a very low frequency of potentially pathogenic microorganisms in the oral mucosa of the patients. Among Gram-positive bacteria, coagulase-negative staphylococci were the most frequently isolated and were present in samples collected from 8 (47.0%) patients, followed by *C. albicans* identified in samples from 6 (35.3%) patients. Among the Gram-negative bacteria, enteropathogenic *E. coli*, *K. pneumoniae* and *S. maltophilia* were isolated in 1 (5.9%) patient each.

Analysis of the presence of potentially pathogenic microorganisms according to the presence or absence of oral mucositis disclosed a low frequency of enteropathogenic *E. coli*, *K. pneumonia,* and *S. maltophilia* in both groups (Table 2). Similarly, patients with normal oral mucosa and patients presenting oral mucositis exhibited similar frequencies of isolation of *C. albicans* (Table 2). Regarding coagulase-negative staphylococci, patients showing oral mucositis revealed a higher frequency of these Gram-positive bacteria when compared with patients with normal oral mucosa (Table 2).

Table 1. Distribution of patients according to the presence or absence of oral mucositis and treatment phase.

	Present		Absent	
	n	%	n	%
Induction	1	20.0	2	16.7
Consolidation	1	20.0	0	0.0
Intensification	2	40.0	8	66.6
Maintenance	1	20.0	2	16.7
Total	5	100.0	12	100.0

DISCUSSION

ALL is the most common childhood cancer in developed countries, accounting for one-third of all malignancies in this age group (1). Oral mucositis is the major complication in children with ALL, a consequence of cytotoxic chemotherapy, which leads to a high morbidity including pain, oral dysfunction, dysphagia, bleeding, systemic weakness and infection (3,4,15,16).

The development of oral mucositis may result from both direct and indirect effects of chemotherapy on cells (17). The direct effect is determined by interference of drugs in cell production, maturation and replacement, whereas the indirect effect is related to myelosuppressive action of drugs, which deregulates the immune system and repair process, increasing the risk of infection associated with oral mucositis (18).

In the present study, oral mucositis was observed

Table 2. Distribution of patients according to the presence or absence of selected oral microorganisms and the presence or absence of oral mucositis.

Microorganisms	Category	Oral mucositis			
		Present		Absent	
		n	%	n	%
Coagulase-negative staphylococci	Present	4	80.0	4	33.3
	Absent	1	20.0	8	66.7
	Total	5	100.0	12	100.0
Klebsiella pneumoniae	Present	1	20.0	0	0.0
	Absent	4	80.0	12	100.0
	Total	5	100.0	12	100.0
Escherichia coli	Present	0	0.0	1	8.3
	Absent	5	100.0	11	91.7
	Total	5	100.0	12	100.0
Stenotrophomonas maltophilia	Present	0	0.0	1	8.3
	Absent	5	100.0	11	91.7
	Total	5	100.0	12	100.0
Candida albicans	Present	2	40.0	4	33.3
	Absent	3	60.0	8	66.7
	Total	5	100.0	12	100.0

in only 5 (29.4%) patients and affected different sites, including the buccal mucosa, gingiva, and tongue. A noteworthy fact is that none of the patients evaluated in this study showed disseminated mucositis. These findings corroborate the relationship between prophylactic administration of 0.12% chlorhexidine gluconate and reduced frequency and severity of oral mucositis. Accordingly, Pinto et al. (14) observed that systematic preventive treatment with 0.12% chlorhexidine gluconate and oral hygiene care reduce the occurrence of oral complications in children with ALL undergoing antineoplastic chemotherapy.

The oral cavity is one of the most complex environments in the body, containing a wide microbiota with distinct subsets predominating at different habitats, many of them potentially pathogenic (6). In the present study, coagulase-negative staphylococci were the most frequent microorganism isolated from the oral mucosa of children with ALL. In addition, patients showing oral mucositis revealed a higher frequency of coagulase-negative staphylococci when compared with patients with normal oral mucosa, suggesting a possible relationship between these microorganisms and the development of mucositis.

Staphylococci represent a group of Gram-positive cocci. Most of these microorganisms are not pathogenic and reside mainly in the skin and mucous membranes of humans (19). However, there is a growing body of evidence to suggest that staphylococci can be frequently isolated from the oral environment of some specific groups such as children, the elderly and some groups with other systemic diseases such as those with rheumatoid arthritis and hematologic malignancies (20). In a systematic review conducted by Napeñas et al. (21), *Staphylococcus* was isolated from the oral microbiota of chemotherapy patients in 8 of the 13 analyzed studies. Despite of these findings, Smith et al. (20) further state that the role of *Staphylococcus* in the etiology of oral mucositis is controversial due to the diversity of the oral microbiota and the presence of this amphibiontic microorganism in the oral cavity of healthy patients.

Involvement of fungi in the development of oral mucositis has been subject of speculation and remains marginally controversial. Candidiasis is a common finding among patients receiving head and neck radiation or myeloablative chemotherapy. Thus, it is not unexpected to isolate *Candida* from patients with mucositis, probably as a coincident condition, rather than causal (6). In the present study, *C. albicans* was isolated from samples of only 6 (35.3%) patients and no relationship could be established between the presence of this microorganism and the development of oral mucositis. Similarly, Epstein et al. (12) studied 115 patients undergoing hematopoietic cell transplantation and observed a lower frequency of *C. albicans* in the oral mucosa of patients using chlorhexidine as topical antifungal agent. In addition, the authors observed no correlation between *Candida* colonization and presence or severity of mucositis.

The oral bacterial infections can be due to opportunistic enterobacteria, such as *Pseudomonas* and *E. coli* (22). In immunocompromised patients, both the amphibiontic and the opportunistic microbiota can become pathogenically harmful and cause severe infections that may reach the bloodstream and lead to the occurrence of generalized infections (21). In the present study, it was observed a low frequency of Gram-negative bacteria in collected material, with enteropathogenic *E. coli* and *K. pneumoniae* being isolated from samples of 1 (5.9%) patient each. Similar results were found by Sheehy et al. (10) in a study about the oral microbiota of children undergoing liver transplantation, with *Enterobacteria* being isolated only rarely. Coherently, according to Napeñas et al. (21), most of the oral bacterial changes noted in pediatric patients undergoing chemotherapeutic regimens involve Gram-positive streptococci and staphylococci, whereas in adult patients, most changes involve Gram-negative organisms such as *Enterobacteriaceae* and *Pseudomonas* sp.

S. maltophilia is an opportunistic pathogen highly resistant to antibiotics which has gained importance over the last years in bacterial isolates obtained from clinical material. This microorganism has been associated to the development of bacteremia in patients with hematologic malignancies and neutropenia (23). In our study, one sample (5.9%) was positive for *S. maltophilia*. Labarca et al. (23) studied *S. maltophilia* infection in patients undergoing allogenic bone marrow transplant and observed that severe neutropenia and severe mucositis may favor infection by this bacterium through impairment of host defenses.

In conclusion, the results of the present study revealed a very low frequency of potentially pathogenic microorganisms in the oral mucosa of the children with ALL, a fact that can be explained by the prophylactic use of chlorhexidine in combination with daily oral hygiene care. Patients showing alterations in the oral mucosa exhibited a higher frequency of coagulase-negative

staphylococci when compared with patients with normal oral mucosa suggesting a possible relationship between these microorganisms and the development of oral mucositis.

RESUMO

Tendo em vista o potencial de morbidade das complicações orais em pacientes com leucemia, este estudo avaliou as alterações clínicas e microbiológicas que ocorrem na mucosa bucal de crianças com leucemia linfoblástica aguda (LLA), submetidas à quimioterapia antineoplásica e administração profilática do gluconato de clorexidina 0,12%. A amostra foi constituída de 17 crianças de 2 a 12 anos, as quais foram submetidas a exame clínico da mucosa oral para a detecção de lesões bucais. Além disso, foi coletado material biológico das mucosas labial e jugal para análises microbiológicas. A mucosite oral foi observada em apenas 5 (29,4%) pacientes. A análise microbiológica revelou a presença de um número reduzido de microorganismos potencialmente patogênicos, como estafilococos coagulase-negativos (47%), *Candida albicans* (35,3%), *Klebsiella pneumoniae* (5,9%), *Escherichia coli* enteropatogênica (5,9%) e *Stenotrophomonas maltophilia* (5,9%). Pacientes com mucosite oral apresentaram uma maior freqüência de estafilococos coagulase-negativos (80%) quando comparados aos pacientes que exibiam mucosa oral normal (33,3%). Em conclusão, os resultados do presente estudo sugerem que o uso profilático do gluconato de clorexidina 0,12% reduz a freqüência de mucosite oral e de patógenos orais em crianças com LLA. Além disso, os presentes achados sugerem uma possível relação entre estafilococos coagulase-negativos e o desenvolvimento de mucosite oral.

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