

Oral Candidiasis in children and adolescents with cancer. Identification of *Candida* spp

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González-Gravina H, González-de Morán E, Zambrano O, Lozano-Chourio M, Rodríguez-de Valero S, Robertis S, Mesa L. Oral Candidiasis in children and adolescents with cancer. Identification of *Candida* spp Med Oral Patol Oral Cir Bucal. 2007 Oct 1;12(6):E419-23.
© Medicina Oral S. L. C.I.F. B 96689336 - ISSN 1698-6946

Received: 15-04-2006

Accepted: 3-06-2007

Indexed in:

- Index Medicus / MEDLINE / PubMed
- EMBASE, Excerpta Medica
- SCOPUS
- Indice Médico Español
- IBECs

ABSTRACT

Oral candidiasis represents a serious problem for children with cancer. The mortality rate of this infection has increased due to fungal septicemia, associated with a primary buccal infection. Objective: Identify the *Candida* spp. in buccal lesions of patients with cancer, establish the predominant species and correlate them to age and sex of the patient, clinical presentation, type of neoplastic disease and cytostatic therapy received. Study design: 62 patients, between 0-16 years, were investigated in a cross sectional study. Sample inclusion criteria: Patients with malignant neoplastic disease that were receiving cytostatic treatment and had suspicious lesions of oral candidiasis. Patients with antifungal therapy, active caries, calculus or intraoral appliances were excluded. A clinical evaluation was carried out. The lesion sample was taken and studied by direct exam and culture in CHROMagar-Candida and Sabouraud-Dextrose Agar with chloramphenicol. The identification of the isolated yeast was done by the filamentation test, carbohydrate fermentation and assimilation. Results: Most of the cases (69.35%) were positive to oral candidiasis, *C. albicans* was the most frequent species found, followed by *C. parapsilosis* (14.89%), *C. tropicalis* (12.77%), *C. krusei* (4.26%), *C. glabrata* (2.13%) and *C. lusitaniae* (2.13 %). In some cases more than one specie were isolated (9.30%). The most frequent location of the lesion was in the tongue (72.70%). The pseudomembranous candidiasis was the most frequent clinical presentation found (78.71%). There were not significant statistically differences with regard to sex and age of the patient, type of neoplastic disease and cytostatic agent received. Conclusion: The results suggest that oral candidiasis is a frequent complication in the pediatric oncological population, being *C. albicans* the main etiological agent, however, there is an important participation of other *Candida* species.

Key words: Oral Candidiasis, *Candida*, children, cancer.

INTRODUCTION

Oral candidiasis, a frequent and important nosologic entity of the buccal cavity; is caused by the pathogenic action of *Candida* species (1). There are diverse local factors that make the buccal tissues susceptible to *Candida* infection, such as acid saliva, xerostomia, night use of prosthetic dentures, tobacco, carbohydrate rich-diets and patients that receive radio- and chemotherapies in maxillofacial structures (2). The colonization for species of *Candida* genus in patients with cancer and their later dissemination, associated with long term episodes of neutrophenia, use wide spectrum antibiotics, treatment with corticosteroids and cytostatic substances, invasive surgical procedures (central and bladder catheters), xerostomia and prolonged hospitalization time (3). The mortality rate due to fungal septicemia, associated with a primary buccal infection, is a relevant problem in children with cancer (4). *Candida* species, existing in the mouth, have high probability to infect the digestive pathways and disseminate through the circulation, developing systemic infection that is life threatening for the patient (5). The detection and identification of *Candida* spp. involved in oral candidiasis are important for the treatment of patients with cancer (6). *C. krusei*, *C. glabrata*, *C. tropicalis* and *C. dubliniensis* species have shown their resistance to certain antifungal drugs (6-8). The proposal of this study was the identification and frequency of the isolated *Candida* species from buccal lesions in children and adolescents with cancer, according to age and sex of patients, clinical presentation, type of neoplastic disease and cytostatic therapy received.

MATERIALS AND METHODS

-Origin of the sample:

A cross sectional study was carried out in a sample (110 patients) in a given age (0–16 years). These patients were admitted to the Pediatric Oncology Unit at University Hospital of Maracaibo (40 patients) and the Oncohematology Unit at Pediatric Specialities Hospital (70 patients), Maracaibo, Venezuela, from February to May, 2003. Patients with a diagnosis of malignant neoplastic disease, under treatment with cytostatics agents and with oral candidiasis lesions were chosen for this study (62 patients). Patients that received antifungal therapy, ten days prior to the study, had either active extensive caries, dental calculus or intraoral appliances were excluded.

This investigation was carried out according to the norms proposed in the Declaration of Helsinki. The ethics committee of each one of the hospitals involved approved the development of this study. All the parents or legal guardians of the selected children signed a consent report after explaining the purpose of the study.

-Isolation and identification of *Candida* species

The samples were taken by scaling the lesions with a sterile wooden spatula, a direct exam was done to verify the microscopic characteristic of the *Candida* spp. (presence of pseudomycelia and blastoconidia) and were cultured on CHROMagar Candida (CHROMagar Candida, Paris, France) and Sabouraud Dextrose Agar with chloramphenicol for yeast isolation. The cultures were incubated at 37°C for 3 to 7 days.

The CHROMagar -Candida medium contains chromogenic substances that allow a presumptive diagnosis and identification of some species of *Candida* genus. It was prepared according to the manufacturer indications. The macroscopic study, after 72 hours, considered: number, consistency and color of the colonies. The isolates that developed a green color were identified presumably as *Candida albicans*, the blue ones as *Candida tropicalis* and the pink as *Candida krusei*.

The colonies developed on Agar Sabouraud Dextrose Agar were identified on the basis of their micromorphology on Rice Tween 80 (Dalmau Technique) and biochemical tests: carbohydrate fermentation by the Wickerham technique (glucose, galactose, lactose, maltose, sucrose, trehalose (2%), raffinose (4%)) and carbohydrate assimilation by the auxanographic technique in plaque (glucose, cellobiose, galactose, maltose, saccharose, lactose, sorbose, erythritol, trehalose, raffinose, inositol, xylose, melibiose, melezitose, rhamnose, mannitol), according to traditional methodology (9).

-Statistical analysis

Descriptive Statistics were used to present data in absolute and percent values, and summarized in tables. The Chi square test and the exact test of Fisher the tests were used for the statistical analysis. A 95% statistical confidence index was established ($p < 0,05$) and the statistical package used was SPSS 10.0 (SPSS Inc., Chicago, IL, USA).

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RESULTS

The samples studied (62 patients) showed that most of the patients were positive to oral candidiasis (43; 69.35%) both female (24; 38.70%) and male (19; 30.65%). The group of children (23 patients) between 7 and 12 years presented the highest number of positive cases (20 patients). The sample (8 patients) of the group between 0 and 2 years showed a relatively high number (6 cases) of oral candidiasis.

The distribution of *Candida* species isolated from oral candidiasis in children and adolescents with cancer, according to clinical presentation and type of cancer, are shown in Tables 1 and 2. *Candida albicans* was the most frequent species isolated in the malignant neoplastic disease.

DISCUSSION

Oral candidiasis represents a problem for children with cancer due to the possibility of a systemic infection that may be life threatening for the patient. It has been reported that the use of preventive therapy, before the establishment of chemotherapy, may decrease the incidence of oral candidiasis (10).

Most of the patients (69.35%) of the studied sample had oral candidiasis as has been previously reported (11). The highest incidence was observed in children with ages between 7 and 12 years old. This finding could be due to the mixed dentition and the concomitant presence of the salivary pellicle that could favor the adherence of *Candida* species

Table 1. Distribution of *Candida* species isolated from oral candidiasis in children and adolescents with cancer according to clinical presentation

<i>Candida</i> species.	Clinical Presentation					
	Pseudomembranous		Erythematous		Total	
	n°	%	n°	%	n°	%
<i>Candida albicans</i>	14	29.78	6	12.77	20	42.55
<i>Candida parapsilosis</i>	7	14.89	-	-	7	14.89
<i>Candida tropicalis</i>	5	10.63	1	2.13	6	12.76
<i>Candida krusei</i>	2	4.26	-	-	2	4.26
<i>Candida glabrata</i>	1	2.13	-	-	1	2.13
<i>Candida lusitaniae</i>	-	-	1	2.13	1	2.13
<i>Candida</i> sp	8	17.02	2	4.26	10	21.28
Total	37	78.71	10	21.29	47	100

P > 0.05

The sample was taken in Maracaibo, Zulia State, Venezuela. South America

Table 2. Distribution of *Candida* species isolated from oral candidiasis in children and adolescents with cancer according to type of cancer.

Type of Cancer*	Species of <i>Candida</i>							Total
	<i>C. albicans</i>	<i>C. parapsilosis</i>	<i>C. tropicalis</i>	<i>C. krusei</i>	<i>C. glabrata</i>	<i>C. lusitaniae</i>	<i>C. sp</i>	
Retinoblastoma	2	-	-	-	-	-	-	2
Acute Lymphoid Leukemia	13	5	2	1	-	1	8	30
Rabdomyosarcoma	1	1	-	-	-	-	-	2
Meduloblastoma	1	-	1	1	-	-	-	3
Acute non Lymphoid Leukemia	-	-	1	-	1	-	-	2
Grade I Astrocytoma	1	-	1	-	-	-	-	2
Total	20	7	6	2	1	1	10	47

*It was identified 1 only case in several types of cancer, i.e. Epidermoma Anaplastic and Ewing's Sarcoma (*C. albicans*); Non-Hodking's Lymphoma(*C. parapsilosis*); Neuroblastoma (*C.tropicalis*); Osteosarcoma and Ovary Tumor (*Candida* sp). The sample was taken in Maracaibo, Zulia State, Venezuela. South America.

to the epithelial surface (12). Also the physical conditions of these children may contribute to an unsuitable buccal hygiene. The prevalence of positive cases in female population as previously observed (13,14), did not represent any significant difference.

The pseudomembranous clinical presentation was mainly observed (78.71%) in the positive cases while the erythematous ones were lower (21.29%). The relationship between clinical presentations and the isolated *Candida* spp is shown in Table 1. *C. albicans* was predominantly isolated (42.55%), as was observed previously (6, 15, 16), followed by *C. parapsilosis* (14.89%), *C. tropicalis* (12.76%), *C. krusei* (4.26%), *C. glabrata* and *C. lusitaniae* (2.13%). These findings have been previously reported (6,17,18). The high frequency of these species may be related to the patients' immunosuppression status and the empirical treatment with antibiotic and wide spectrum antimycotics that may cause mutation phenomenon (19, 20).

This finding may explain the reason why some isolated *Candida* species (21.28%) were not identified by the used methodology. It is important to note that two different species were identified in four cases of oral candidiasis, i.e., *C. albicans* and *C. tropicalis* (2 cases); *C. albicans* with *C. krusei* (1 case) and *C. tropicalis* and *C. parapsilosis* (1 case).

After chemotherapy, candidiasis pseudomembranous was the most frequent clinical presentation in oral candidiasis in children and adolescents with cancer, as has been previously observed (21-23). The clinical presentations were observed mainly in the tongue (72.70%) followed by the inside cheeks (12.70%), soft palate (10.90%) and lips (3.60%) as was observed previously (24) but differ in the preferential location, hard palate, found in the buccal cavity (25). It is important to note that the positive cases (90.70%) were presented as non painful lesions.

The distribution of *Candida* species according to type of cancer (Table 2), showed that the patients that suffered acute lymphoid leukemia presented the highest number of positive cases, in accordance with some reports (26, 27) but differed from the predominant oral candidiasis in patients with acute myeloid leukemia and sarcoma (4, 11). The incidence of oral candidiasis in patients with leukemia may be due to the alteration of the mediated-immunity by cells in this pathology and the processes that participate in the maturation of lymphocyte and plasmatic cells, generating serious alterations in the mediated-immunity by antibodies (humoral). These conditions favour the susceptibility to fungal infections (28). *C. albicans* was presented as the most frequent specie, either in the acute lymphoid leukemia or in the rest of the oncological pathologies evaluated in this study; similar results have been reported (21).

The combination of cytostatic treatments and isolated *Candida* spp. showed not significant statistically evidences. However, the combination methotrexate-purinethol presented the highest number of cases of *Candida* species. This behavior may be due to the oral complications of the pediatric patients with malignant neoplastic pathologies and the potential immunosuppressive effect of therapies used in

this disease that has a direct cytotoxic effect on oral mucosa (29,30). The epithelial cells of the buccal mucosa are very sensitive to chemotherapeutic drugs, they inhibit the cellular cycle, attacking the tissues with more mitotic activity. Nevertheless this cellular inhibition is unspecific, therefore, tissues of rapid renovation like the gastrointestinal mucosa are equally affected (31).

There are certain specific toxic drugs that affect the buccal mucosa, such as methotrexate, doxorubicin, cyclophosphamide and vincristine, which are frequently used in oncological therapies protocols for infants patients (19,20). The cytotoxic result of these drugs: are epithelial atrophy and necrosis, favoring the colonization by fungus, bacteria and viruses.

Oral candidiasis is a frequent complication in pediatric oncology. *C. albicans* is the main etiologic agent, however, there is an important participation of other *Candida* species.

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