

Prevalence of yeasts in the oral cavity of children treated with inhaled corticosteroids

Prevalência de leveduras na cavidade bucal de crianças tratadas com corticosteróides inalados

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ABSTRACT: The aim of this study was to observe the prevalence of *Candida* spp. in the oral cavity of children undergoing treatment with inhaled corticosteroids. Thirty children treated with inhaled corticosteroids and thirty control children were studied. Saliva samples were collected through oral rinses with phosphate buffered saline (PBS). The samples were plated on Sabouraud's dextrose agar and incubated at 37°C for 48 h. After this period, the number of colony-forming units per ml (cfu/ml) of saliva was calculated. The isolates were identified by phenotypic characterization. *Candida* spp. was isolated from 43.33% of the samples of children treated with corticosteroids, with a mean of 780 cfu/ml of saliva, and from 30% of the samples of the control group, with a mean of 560 cfu/ml of saliva. No significant statistical difference was observed between the groups. *C. albicans* was the prevalent species in both groups, followed by *C. guilliermondii*, *C. parapsilosis* and *C. stellatoidea*. Furthermore, *Rhodotorula rubra* and *C. lusitaniae* were also isolated from the treated group. We concluded that there was no significant increase in the prevalence and number of *Candida* spp. in the oral cavity of children treated with inhaled corticosteroids.

DESCRIPTORS: *Candida*; *Candida albicans*; Adrenal cortex hormones; Asthma.

RESUMO: O objetivo deste trabalho foi verificar a prevalência de *Candida* spp. na cavidade bucal de crianças tratadas com corticosteróides inalados. Foram estudadas 30 crianças usuárias de corticosteróides inalados e 30 crianças controle. A coleta de saliva foi realizada através de enxágües bucais com solução fisiológica tamponada (PBS). As amostras foram semeadas em ágar Sabouraud dextrose e incubadas a 37°C por 48 horas. A seguir, foi realizada a contagem de unidades formadoras de colônias por ml (ufc/ml) de saliva, e as cepas isoladas foram identificadas por caracterização fenotípica. Foram encontradas leveduras do gênero *Candida* em 43,33% dos pacientes usuários de corticosteróides, com média de 780 ufc/ml de saliva, e em 30% do grupo controle, com média de 560 ufc/ml de saliva, não ocorrendo diferença estatisticamente significante entre os grupos. *C. albicans* foi a espécie prevalente em ambos os grupos, seguida por *C. guilliermondii*, *C. parapsilosis* e *C. stellatoidea*. No grupo usuário de corticosteróide, verificou-se também a presença de *Rhodotorula rubra* e *C. lusitaniae*. Concluiu-se que não houve aumento significante na presença e na quantidade de leveduras do gênero *Candida* na cavidade bucal de crianças tratadas com corticosteróides inalados.

DESCRIPTORIOS: *Candida*; *Candida albicans*; Corticosteróides; Asma.

INTRODUCTION

Candida species are human pathogens related to superficial lesions, especially in the oral mucosae. The isolation of *Candida* from the oral cavity does not imply a disease, since the prevalence of this fungus in healthy adults is from 3 to 48%, and the average prevalence in children is from 45 to 65%¹⁷. *Candida albicans* is the most frequently isolated species from the human oral cavity, but other species have also been isolated, including *C.*

glabrata, *C. tropicalis*, *C. kefyr*, *C. parapsilosis*, *C. krusei* and *C. guilliermondii*²⁵.

The mechanism by which these fungi can cause oral candidosis include their ability to adhere to mucosae, to form pseudohyphae and to secrete histolytic enzymes^{3,13}. The defense of the oral cavity against *Candida* is attributed to nonspecific immune mechanisms such as mucosae integrity, leukocytes, macrophages and saliva components,

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and to specific immune mechanisms, including antibodies and cell-mediated immunity¹⁷.

Oral candidosis has been described as one of the side effects of the therapy with inhaled corticosteroids^{4,12,19,23}, which have anti-inflammatory properties and are often used for the symptomatic treatment of asthma in adults and children^{5,23,24}. The incidence of oropharyngeal candidosis in patients treated with inhaled corticosteroids varies between 0 and 77%, mainly because of the different diagnosis criteria used^{4,22,27}.

Studies on the deposition of corticosteroid sprays on the tissues revealed that approximately 10% of the inhaled medicine remains in the lungs. The remaining percentage is deposited on the oropharyngeal mucosa, where the drug can cause many effects before being absorbed¹². The local side effects of inhaled corticosteroids, besides oropharyngeal candidosis, include cough, hoarseness, dysphonia and perioral dermatitis^{2,4}. Candidosis probably occurs because these substances cause immunosuppression, which interferes with the cell-mediated immunity and cause an increase in the quantity of glucose in the saliva, which, in turn, can stimulate the growth of *Candida*⁴.

Toogood *et al.*²² (1980) reported cultures positive for *Candida* spp. in 60% of the patients treated with inhaled corticosteroids. The incidence of oral candidosis was between 4 and 13% in adults and 1% in children and these rates varied according to the dose and frequency of use of the medication. On the other hand, Shaw, Edmunds²⁰ (1986) isolated *Candida albicans* from the oral cavity of 29% of the healthy children studied and of 45% of the children treated with inhaled beclomethasone. These data suggest a high incidence of *Candida* in users of inhaled corticosteroids regardless of the dose used. Therefore, the purpose of this work was to observe the prevalence of yeasts of the *Candida* genus in the oral cavity of children treated with inhaled corticosteroids, comparing the results with a control group.

MATERIALS AND METHODS

Sixty children, between 4 and 12 years old, were included in the present study. Thirty of these children were users of inhaled corticosteroids for the treatment of symptomatic asthma (Table 1). None of the selected patients were diabetic or used antibiotics or antifungal for a minimal period of 60 days before the collection of the samples. The patients were under treatment at the São José

TABLE 1 - Chemical names and daily dose of inhaled corticosteroids used by the patients selected for this study.

Chemical name of the corticosteroid	Dose/day	Number of patients
Fluticasone propionate	100 µg	4
	250 µg	2
	500 µg	1
Beclomethasone dipropionate	150 µg	1
	200 µg	2
	400 µg	12
	800 µg	4
	1,200 µg	1
Budesonide	400 µg	3

Clinic, São José dos Campos, SP, Brazil. The adult responsible for the child was informed about the purpose of the present study and in case of agreement, signed the informed consent form. This work was previously evaluated and approved by the Research Ethics Committee of the School of Dentistry of São José dos Campos, São Paulo State University.

Saliva samples were collected through oral rinses with a 10 ml sterile physiological solution (0.85% NaCl, Synth, SP, Brazil) and buffered with a phosphate solution (PBS – 0.1 M and pH 7.2) for 30 seconds. Each sample was centrifuged for 10 minutes (2,000 x g) and the supernatant was discarded. The pellet was resuspended in 2.5 ml of PBS (Sigma, SP, Brazil) and agitated in a mixer (Vortex, Marconi, Piracicaba, SP, Brazil) for 30 seconds.

An aliquot of 0.1 ml of the suspension was plated in Sabouraud dextrose agar (Difco, Detroit, USA) with chloramphenicol (Carlo Erba, Rio de Janeiro, Brazil; 0.1 mg/ml), incubated at 37°C for 48 hours, and at room temperature for five extra days. The yeast colonies were counted and the number of colony-forming units per ml (cfu/ml) of saliva was calculated. Yeast colonies were confirmed microscopically by Gram-stained smear and subcultured in Sabouraud dextrose agar for identification. The samples were identified by observing the germinative tubes in sterile rabbit serum, the presence of hyphae and chlamydospores in

cornmeal Tween 80 agar (Difco, Detroit, USA) and carbohydrate fermentation and assimilation^{18,26}. The urease test was used for the identification of *Rhodotorula*¹⁰.

Data were analysed statistically by chi-square (χ^2), Mann-Whitney and Pearson's correlation tests, with a 5% significance level.

RESULTS

Candida spp. was isolated from the oral cavity of 43.33% of the patients treated with corticosteroids and of 30% of the control group. However, no significant statistical difference was observed between the groups (Table 2).

In relation to the isolated species, *C. albicans* was the most frequently found species in both groups (55% of the isolated species in the group treated with corticosteroids and 38% in the control group). No statistically significant difference was observed in the proportion of *C. albicans* and the other species between the studied groups (Table 3).

The other isolated species were *C. guilliermondii*, *C. parapsilosis* and *C. stellatoidea*. Among the children users of inhaled corticosteroids the presence of *C. lusitaniae* and *Rhodotorula rubra* was also observed.

There was no statistical significant difference between the mean of cfu/ml of saliva in the group treated with corticosteroids (780 cfu/ml) and that in the control group (560 cfu/ml) (Mann-Whitney's test, $p = 0.251$).

In the group of patients treated with inhaled corticosteroids, no correlation was observed between the number of colony-forming units of *Candida* per ml of saliva (cfu/ml), dose of medication and time of use (Table 4).

TABLE 2 - Proportion of patients of the corticosteroid-treated and control groups that is positive or negative for *Candida* spp.

	Corticosteroid-treated	Control	Total
<i>Candida</i> spp. (+)	13	9	22
<i>Candida</i> spp. (-)	17	21	38
Total	30	30	60

No statistically significant difference was observed between the groups (χ^2 test; $p = 0.284$).

DISCUSSION

Among the children included in the control group, 30% were positive to *Candida*. This observation is in accordance with the findings of Shaw, Edmunds²⁰ (1986), who reported a percentage of 29% children. On the other hand, Berdicevsky *et al.*¹ (1984) observed 45-65% of *Candida*-positive children. Ollila *et al.*¹⁴ (1997), studying children with similar ages in relation to those included in the present work, reported 19% of *Candida*-positive individuals.

When data of the two studied groups were compared, it could be observed that among patients treated with inhaled corticosteroids the percentage of *Candida*-positive patients was higher (43.33% and 30%, respectively). Nevertheless, no statistically significant difference could be detected between the groups.

In the present study, 43.33% of the patients treated with inhaled corticosteroids were positive to *Candida* in the saliva. Previous studies regarding the incidence of *Candida* in inhaled corticosteroid-treated patients reported divergent results. Shaw, Edmunds²⁰ (1986) observed a higher incidence of *Candida* in the group of children treated with inhaled corticosteroids in relation to the control ones (43% and 29%, respectively). Knight, Fletcher⁸ (1971) also observed higher prevalence among patients treated with corticosteroids (prednisone). On the other hand, Lenander-Lumikari *et al.*¹¹ (1998) did not detect any difference between counts of *Candida* in asthmatic adults users of inhaled corticosteroids.

Considering the isolated species, *C. albicans* was the most prevalent one in both groups. This observation agrees with the findings of several previous studies^{6,7,15,26,28}. No significant statistical difference was observed between the proportion of

TABLE 3 - Percentage of *Candida albicans* and other species isolated among the patients of the corticosteroid-treated and control groups.

	Corticosteroid-treated	Control	Total
<i>C. albicans</i>	11	5	16
Other species	9	8	17
Total	20	13	33

No statistically significant difference was observed between the groups (χ^2 test; $p = 0.353$).

TABLE 4 - Number of colony-forming units of yeasts per ml of saliva (cfu/ml), dose and time of treatment with corticosteroids.

Cfu/ml of yeasts	Dose/day (µg)	Time of treatment (months)
0	100	4
0	150	2
400	500	24
0	400	7
0	400	3
1,700	100	18
0	100	9
0	250	12
0	400	2
200	400	4
100	100	24
200	400	4
2,400	1,200	96
100	400	2
0	400	5
1,500	800	3
200	400	6
14,700	400	2
0	400	6
1,300	800	6
400	250	5
0	400	2
0	400	8
0	800	4
0	400	4
200	200	6
0	200	12
0	400	2
0	800	48
0	400	2

No statistical significance was observed between the cfu/ml and dose/day ($r = 0.110$; $p = 0.562$) and between cfu/ml and time of treatment ($r = 0.042$; $p = 0.824$).

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C. albicans and others species between the studied groups.

A higher diversity of *Candida* species was observed in the group of children under inhaled corticosteroid treatment. In this group, isolates of *C. guilliermondii*, *C. parapsilosis*, *C. stellatoidea* and *C. lusitaniae* were also found. Besides, *Rhodotorula rubra* strain was also isolated from these patients. These results are in accordance with previous studies that reported higher prevalence of non-*albicans Candida* species among patients with other predisposing factors to candidosis^{7,9}.

Patients using inhaled corticosteroids are considered at risk for candidosis by many authors^{4,12,16,19,23}. The increase in the risk to candidosis has been related to the number, frequency of inhalations²¹ and delivery of the drug without spacer devices¹⁶. In this study, no significant increase in *Candida* spp. levels in the oral cavity of children treated with corticosteroids was observed.

CONCLUSIONS

- No significant increase in *Candida* spp. in the oral cavity of children treated with inhaled corticosteroids was observed.
- *C. albicans* was the most frequently isolated species, in both control and corticosteroid-treated groups.
- Among the children users of inhaled corticosteroids, no correlation was observed between the number of colony-forming units of *Candida* per ml of saliva (cfu/ml) and the dose of medication and time of treatment.

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