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CANDIDA IN SALIVA OF BRAZILIAN HEMOPHILIC PATIENTS

CANDIDA NA SALIVA DE PACIENTES HEMOFÍLICOS BRASILEIROS

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ABSTRACT

Hemophilia is a common hereditary hemorrhagic disorder, however little is known about the oral microflora of hemophilic patients. The aim of this study was to quantify the *Candida* and identify its species in non-stimulated saliva of hemophilic patients, and consider its relationship with clinical factors influencing *Candida* carriage. This study comprised evaluation of 86 hemophilic patients of the Hematology Center/UNICAMP and 43 healthy subjects as controls. All patients were submitted to anamnesis, intraoral examination and unstimulated saliva collection. *Candida* counts and species identification were performed in salivary samples. Candida was present in 64% of the hemophilic patients and in 44% of the healthy controls. *C. albicans* represented 65% and 68% of the isolated species, in hemophiliacs and control group respectively, and *C. tropicalis* was the second most common species in both groups. These results indicate that hemophilic patients carry *Candida* more frequently and in higher counts than healthy controls, independently of oral clinical parameter considered, as viral infections, complete dentures, transfusions of hemoderivatives, and salivary flow.
Uniterms: *Candida*; Hemophiliacs; Candidosis; Hemophilia; *Candida tropicalis.*

RESUMO

Hemofilia é uma alteração hemorrágica hereditária comum, entretanto pouco se sabe a respeito da microbiota oral destes indivíduos. O objetivo deste estudo foi quantificar a presença de *Candida* e identificar as suas espécies na saliva de hemofílicos, correlacionando os resultados com fatores clínicos que possam influenciar a presença deste fungo. Foram avaliados 86 hemofílicos do Hemocentro/UNICAMP e 43 indivíduos saudáveis. Todos os pacientes foram submetidos a anamnese, exame clínico intra-oral e coleta de saliva de forma não estimulada. A quantificação e identificação das espécies de *Candida* foram realizadas nas amostras de saliva. *Candida* estava presente em 64% dos hemofílicos e em 44% dos indivíduos saudáveis. *C. albicans* representou 65% e 68% das espécies identificadas, nos hemofílicos e grupo controle respectivamente, e *C. tropicalis* foi a segunda espécie mais comum. Estes resultados sugerem que pacientes hemofílicos albergam mais freqüentemente *Candida* na cavidade bucal e em maiores quantidades que os indivíduos do grupo controle, independentemente dos parâmetros clínicos analisados, como infecção viral, próteses dentárias, transfusões de hemoderivados e fluxo salivar.
Unitermos: *Candida*; Hemofílicos; Candidose; Hemofilia.

INTRODUCTION

Hemophilia is a hemorrhagic disorder caused by hereditary deficiency of factor VIII or IX, characterizing hemophilia A and B, respectively[2,5,14]. Hemophilia A is caused by reduction of the serum level or activity of factor VIII, an important protein in the activation of factor X in the blood clotting sequence. The disease shows an X-linked recessive pattern, affecting predominantly males. According to factor VIII activity detected in blood, hemophilia is classified in

mild (6 to 30% of activity), moderate (2 to 5%) and severe (less than 1%). Hemorrhagic episodes are related to the severity of the disease, although all patients can suffer hemorrhage following trauma or surgical procedures[2,5,13]. Hemophilia B is characterized by deficiency of factor IX with its pattern of transmission and clinical characteristics similar to hemophilia A[13].

Candida species have been found in the oral cavity of 25 to 50% of healthy subjects[1,12]. *C. albicans* is the most common, representing 60 to 90% of the isolated species[9,29]. Local and systemic factors can modify *Candida* carriage in the mouth as patients' age and gender, complete dentures, smoking, xerostomia, diabetes, drugs and genetic or acquired immunological deficiency[12,19].

Many studies have focused dental management in hemophilic patients, however few papers have dealt with oral microflora[17,25]. *Candida* carriage in these patients could be modified by administration of hemoderivatives or other drugs, and alterations in the immunological system as a consequence of infections, as HBV, HCV and/or HIV[4,24]. The aim of this paper was to quantify *Candida* in saliva and identify its species in normal and hemophilic patients, considering clinical factors that could influence *Candida* carriage in the oral cavity.

MATERIAL AND METHODS

A total of 129 patients were studied, 86 with hemophilia from the Hematology and Hemotherapy Center/UNICAMP, and 43 healthy subjects as control. All hemophiliacs were males, with a mean age of 27.5 years (range from 3 to 69 years). They were classified according to hemophilia type (A or B) and severity of the disease. Previous hemorrhagic episodes, blood or hemoderivatives transfusions, serological data, and occurrence of viral infections were considered. Control patients did not have any history of previous or present systemic disease. The male to female ratio was 1.0:0.8 (24/19), with a mean age 29.3 years old (range from 7 to 60 years).

All patients were submitted to physical intraoral examination and collection of unstimulated saliva for 5 min. Salivary flow was classified as reduced (< 0.2mL/min), normal (> 0.3 and < 0.4mL/min) and abundant (> 0.5mL/min), according to the criteria described by Screebny, et al.[23] (1989). Salivary samples were cultured in Saboraud dextrose agar with chloramphenicol for 48 hours, and *Candida* counts were expressed as CFU/ml. According to *Candida* count, each patient was considered "negative" (CFU/mL = 0), "carrier" (CFU/mL > 0 or < 400) and "positive" (CFU/mL < 400). *Candida* species identification was performed by germ tube and chlamydospores production, and carbohydrate fermentation and assimilation, according to Sandvén[22] (1990) and Larone[10] (1995).

Data were statistically analyzed by t test, chi-square, Wilcoxon, Kruskal-Wallis and two-tailed exact Fischer, using the EPI-INFO 6.04b program (CDC, USA), at a significance level of 5%.

RESULTS

Twenty-nine (34%) out of 86 hemophiliacs showed one or more viral infections diagnosed by serological tests (Table 1). Most of the patients with severe hemophilia A presented viral infections, and HCV was the most common, followed by HIV and CMV. Only one patient had HBV, and 8 presented simultaneous infections with two or more virus. Surprisingly no patient with severe Hemophilia B was infected, but 4 out of 8 with mild Hemophilia B were positive for HCV and one for HIV. Twenty-one patients had one viral infection and eight, two or more (HCV + HIV = 1, HCV + CMV = 2, HCV + HIV + HBV = 1, and HCV + HIV + CMV = 4). Smoking and drinking habits were higher in the control group, but use of drugs and prosthesis was similar in both groups (Table 2).

Mean salivary flow rates of both groups were statistically different (p = 0.0025), but within the range of normal values, 0.49±0.11mL/min and 0.41±0.21mL/min for the control and hemophilic groups, respectively. Nevertheless, in both groups, few patients had reduced salivary flow. *Candida*

TABLE 1- Distribution of 86 hemophilic patients regarding to type of hemophilia, severity of the disease and viral infections

Hemophilia	Viral Infection				
	HCV	HIV	HBV	CMV	Two or more viruses
Hemophilia A(68)					
Severe (n = 34)	16 (61.5%)	6 (75.0%)	0	6 (86.0%)	7 (87.5%)
Moderate (n = 18)	3 (11.5%)	0	0	1 (14.0%)	0
Mild (n = 16)	2 (8.0%)	1 (12.5%)	1 (100%)	0	1 (12.5%)
Hemophilia B(18)					
Severe (n = 5)	0	0	0	0	0
Moderate (n = 5)	1 (4.0%)	0	0	0	0
Mild (n = 8)	4 (15.0%)	1 (12.5%)	0	0	0
Total (n = 86)	26 (30.23%)	8 (9.3%)	1 (1.16%)	7 (8.13%)	8 (9.3%)

was not detected in saliva of 56% of control patients, and 36% of the hemophiliacs. On the other hand, 42% of the hemophiliacs were considered positive, but only 28% of the controls. Type and severity of hemophilia did not influence the presence of *Candida* in saliva (Table 3).

About 73% of the HCV-positive patients and 87.5% of the HIV-positive patients were *Candida* carriers/positive in saliva. To assess the influence of these viral infections on *Candida* carriage in saliva, patients affected by each of the infections (HIV, HCV, HBV, and CMV) were excluded and *Candida* carriage was analyzed in the remaining individuals. Results showed no statistically significant differences between groups and in the hemophilic group as a whole. On the other hand, when considering only hemophiliacs with viral infections, they carried more *Candida* in saliva when compared to control and hemophilic group without viral infections (Table 4). The influence from complete dentures and salivary flow on salivary *Candida* carriage in hemophilic patients was also analyzed, and exclusion of one or both of these factors did not interfere with *Candida* counts (Table 5).

Regarding *Candida* species, *C. albicans* corresponded to 68 and 65%, *C. tropicalis* 37 and 51%, and *Candida* sp 26 and 9% in the control and hemophilic groups, respectively. *C. krusei* (1 patient) and *Rhodotorula rubra* (1 patient) were also isolated from hemophiliacs (Table 6).

DISCUSSION

Hemophilia is a hereditary hemorrhagic disorder, characterized by deficiency in factors VIII or IX, which are fundamental glycoproteins in blood clotting[2,26]. Some primary or secondary hemophilia-related factors, as viral infections and transfusion therapies, can interfere with the immune system and predispose hemophiliacs to alterations in oral microflora and, consequently, to oral infections[6,8,21].

The present results showed that *Candida* was more frequently found in the saliva of hemophilic patients (64%) than in controls (44%). It was also noticed that, although the frequency of *Candida* carriers/positives was higher in hemophiliacs, there were no cases of clinical oral candidiasis.

TABLE 2- Distribution of 86 patients and 43 healthy individuals regarding to smoking and drinking habits, complete dentures, and drug use

Parameter	Control (43) n (%)	Hemophiliacs (86) n (%)	p value
Smoking	7 (16.3%)	3 (3.6%)	p= 0.015
Drinking	4 (10.3%)	0 (0.0%)	p= 0.011
Prosthesis	1 (2.4%)	7 (8.9%)	NS
Drugs	2 (4.9%)	2 (2.4%)	NS

TABLE 3- Distribution of control and hemophilic patients regarding to salivary *Candida* carriage

Type of hemophilia	Salivary Candida carriage			Mean CFU
	Positive (CFU[1]/mL <400)	Carrier (CFU/mL>0<400)	Negative (CFU/mL= 0)	
Hemophilia A (68)				
Severe (n=34)	16 (47%)	7 (20.5%)	11 (32.5%)	3.899 ± 2.615
Moderate (n=18)	8 (44%)	4 (22%)	6 (33%)	3.255 ± 1.806
Mild (n=16)	5 (31%)	5 (31%)	6 (38%)	2.717 ± 0.832
Hemophilia B (18)				
Severe (n=5)	2 (40%)	2 (40%)	1 (20%)	3.723 ± 2.760
Moderate (n=5)	2 (40%)	1 (20%)	2 (40%)	5.403 ± 3.719
Mild (n=8)	0	3 (37%)	5 (63%)	4.633 ± 2.481
Total (n = 86)	33 (38.4%)	22 (25.6%)	31 (36%)	3.653 ± 2.275
Control (n = 43)	12 (28%)	7 (16%)	24 (56%)	2.558 ± 0.910

1 CFU – colony forming units in $\log_{10}$/mL

Patients affected by severe hemophilia A or B need more frequent transfusional therapies and could be more susceptible to infections[8,13]. However, in the present patients, severity of hemophilia did not correlate with *Candida* counts in saliva, indicating that transfusions did not significantly impair immune defense to *Candida.*

Reduced salivary flow has been considered the most important factor leading to *Candida* carriage and development of candidosis[7,15,19]. In the present study, the mean salivary flow rates of both groups, although different, were within normal range. Therefore the increased salivary *Candida* carriage in hemophiliacs cannot be explained simply considering salivary flow.

Several systemic factors have been related to oral candidiasis, such as immunosuppression, HIV and other viral infections[3,4,16,27]. From 86 hemophilic patients, 8 were HIV-positive, and of these, 7 were *Candida* carriers/positives in saliva. All 8 patients were using anti-retroviral therapies without clinical disease. It is well known that HIV is an important factor for *Candida* colonization of the oral cavity[18,28]. HCV and CMV infections did not influence salivary *Candida* carriage. In the present sample, only one HBV patient was positive to salivary *Candida*. When only individuals with viral infections were analyzed, about 73% of hemophiliacs were *Candida* carriers/positive in saliva. This clearly indicates that other infections are positively related to the presence of *Candida* in the mouth. Nevertheless, the relatively small number of patients studied

TABLE 4- Influence of viral infection in salivary *Candida* carriage in hemophiliacs with and without viral infections and control group

Group	Positive (CFU/mL <400)	Carrier (CFU/mL >0<400)	Negative (CFU/mL = 0)
Hemophiliacs with viral infections (n = 29)	15 (51.7%)	6 (20.6%)	8 (27.5%)
Hemophiliacs without viral infections (n = 57)	21 (37%)	13 (23%)	23 (40%)
Total (n = 86)	33 (38.4%)	22 (25.6%)	31 (36%)
Control (n = 43)	12 (28%)	7 (16%)	24 (56%)

TABLE 5- Influence of oral prosthesis (complete dentures) and reduced salivary flow in salivary *Candida* carriage in hemophilic patients

Parameters	Salivary Candida carriage		
	Positive (CFU/mL <400)	Carrier (CFU/mL >0<400)	Negative (CFU/mL = 0)
All hemophiliacs (n = 86)	33 (38.4%)	22 (25.6%)	31 (36%)
Excluding oral prosthesis (n = 79)	31 (39%)	18 (23%)	30 (38%)
Excluding reduced salivary flow (n = 72)	28 (39%)	18 (25%)	26 (36%)
Excluding both factors above (n = 52)	20 (39%)	10 (19%)	22 (42%)
Excluding both factors above and viral infections (n = 40)	14 (35%)	7 (17.5%)	19 (47.5%)

TABLE 6- *Candida* species found in control and hemophilic patients

Candida species	Control (n = 43) n (%)	Hemophiliacs (n = 86) n (%)
C. albicans	13 (68%)	36 (65%)
C. tropicalis	7 (37%)	28 (51%)
Candida sp	5 (26%)	5 (9%)
C. krusei	0	1 (2%)
Rhodotorula rubra	0	1 (2%)

should be considered, and in some cases the viral disease was under control, as in HIV patients.

C. albicans is the most common oral *Candida* species, representing 60 to 90% of the isolates in normal and immunocompromised individuals[8,20]. The present results revealed that *C. albicans* and *C. tropicalis* were the most common species in controls and hemophiliacs. When considered the 40 hemophilic patients without any local or systemic factors (viral infections, complete dentures, and salivary flow), 21 were *Candida* carriers/positives in saliva and, from these, 16 (76%) were positive for *C. albicans* and 10 (47%) for *C. tropicalis*, showing that former group carried more *Candida* than the control.

It is interesting to note the high frequency of *C. tropicalis* in patients of both groups, not reflecting the literature data[1,11]. This can be interpreted as a regional higher incidence of *C. tropicalis* in the present population and should be better clarified.

CONCLUSION

In summary, in the present study, hemophiliacs showed higher salivary *Candida* counts and higher number of individuals carriers/positives for *Candida* when compared to controls. Viral infections facilitated the presence of *Candida* in the mouth, nevertheless hemophiliacs without any viral infections significantly carried more *Candida* than controls. No factor could be found, inherent or not to hemophilia, that could significantly contribute to the increased salivary *Candida* counts in these patients, and this deserves further studies.

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