SHORT ARTICLE

Phenotypic characterisation of *Candida albicans* isolated from chronic hyperplastic candidosis

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The phenotypes of 35 *Candida albicans* isolates from 19 patients with chronic hyperplastic candidosis (CHC) and 35 isolates from 30 patients with non-CHC infections were compared. Typing was based on carbohydrate assimilation, chemical sensitivity and serology. Eight carbohydrate assimilation profiles were evident with the API-20C system and a single profile predominated for isolates from CHC (17 of 19 patients; 89%) and non-CHC (18 of 30 patients; 63%). Chemical sensitivity tests revealed four profiles with no significant difference between CHC and non-CHC isolates. Serotype A predominated for isolates from both CHC (15 of 19 patients; 79%) and non-CHC (25 of 30 patients; 83%) infections. Boric acid resistance was more prevalent in CHC isolates, although a significant difference was not apparent. In summary, there was no overall difference in the phenotypes of isolates from CHC and non-CHC patients, and clonal restriction of CHC isolates was not demonstrated.

Introduction

Chronic hyperplastic candidosis (CHC) is a form of oral candidosis that may show histological features of epithelial dysplasia and has an association with malignant change [1]. The incidence of this change has been reported to be as high as 40% for CHC [2] compared with 6% for other forms of oral leukoplakia [3]. The exact role of candida in CHC is unclear, but resolution of lesions and a reduction in the extent of epithelial dysplasia are known to occur following treatment with systemic antifungal therapy [4].

Previously, phenotyping has shown that *Candida* isolates from oral candidosis associated with HIV infection are restricted to specific strains [5, 6]. This clonal restriction has yet to be confirmed for CHC isolates, although different biotypes of *C. albicans* have previously been isolated from tissue from CHC lesional and adjacent normal mucosal sites [7]. The aim of this study was to compare the phenotypes of *C. albicans* isolates from CHC and non-CHC infections, in an effort to determine whether clonal restriction occurs in CHC.

Materials and methods

Isolation of candida

C. albicans (70 isolates) was isolated from the oral cavity of 49 patients (CHC, 19; non-CHC, 30) by oral rinse [8], imprint culture [9] or swab. Isolates were identified by CHROMagar[®] *Candida* (CHROMagar, Paris, France), germ-tube [10] and chlamydospore formation [11].

Phenotyping

Carbohydrate assimilation and chemical sensitivity profiles were obtained with the API-20C (bioMérieux, Basingstoke) and Microring YT (MYT; Medical Wire and Equipment, Corsham) systems, in accordance with the manufacturers' recommended protocols. Similarly, serological tests were done with IF6 antiserum (Iatron Laboratories, Tokyo, Japan) as recommended by the manufacturer. Sensitivity of isolates to boric acid (BDH, Poole 1.8 mg/ml) was based on the methodology of Williamson et al. [12].

Statistical analysis

Statistical analysis was performed with SPSS Version 6 (SPSS, Chicago, IL, USA). When multiple isolates were obtained from patients (either from different oral sites or on separate visits to the clinic), statistical comparison between CHC and non-CHC biotypes was

based on results obtained for the first isolate recovered (before treatment).

Results

Carbohydrate assimilation profiling of *C. albicans* with the API-20C produced eight profiles. Biotype B (API profile 2576174) predominated for *C. albicans* from both non-CHC (18 of 30 patients; 63%) and CHC (17 of 19 patients; 89%) infections. However, there was no significant difference in the isolation of this biotype from the two patient groups ($\chi^2 = 9.6$; $p = 0.21$).

Four MYT profiles were recorded, arising from differences in susceptibility to ethidium bromide (EB, 37 μ g) and 2,3,5-triphenyltetrazolium chloride (TTC, 150 μ g). The majority of *C. albicans* isolates tested (80%) were resistant to EB and sensitive to TTC. There was no significant difference between non-CHC and CHC isolates (Tables 1 and 2; $\chi^2 = 2.0$; $p = 0.58$).

Serotype A and B isolates occurred in 83% (25 of 30 patients) and 7% (2 of 30 patients) of non-CHC infections, respectively. Strains that could not be typed

(auto-agglutinating) were isolated from 10% of non-CHC patients. Similarly, serotype A and B isolates were obtained from 79% (15 of 19 patients) and 11% (2 of 19 patients) of CHC infections, respectively (Table 2). Isolates that could not be serotyped were found in 4 (21%) of the 19 CHC patients. There was no significant difference between the patient groups ($\chi^2 = 0.67$; $p = 0.72$) with respect to serotype.

The incidence of boric acid resistance was greatest amongst isolates from CHC patients (32% of CHC isolates were resistant compared with 16% from non-CHC patients) but this was not significant ($\chi^2 = 1.3$; $p = 0.25$).

Discussion

The present study compared *C. albicans* isolates from CHC and other forms of oral candidosis in phenotypic tests. The study was done because there is evidence suggesting that particular strains of *C. albicans* are associated with specific forms of oral candidosis [7, 13]. Previously, biotype differences between *C. albicans* from CHC lesions and adjacent normal

Table 1. Typing of 35 isolates of *C. albicans* from 30 patients with non-CHC candidal infection

Patient no.	Condition (isolation method)	Serotype	MYT profile	Boric acid sensitivity	API-20C profile
1	Oral candidosis (IC)	A	2 (123406)	S	B (2576174)
2	CAC (OR)	A	2	S	H (6776174)
3	Oral candidosis (OR)	A	2	S	B
4	Oral candidosis (IC)	A	2	S	B
5	Oral candidosis (IC)	A	2	S	B
6	Angular cheilitis	A	2	R	B
6	Angular cheilitis	A	2	R	B
7	Oral candidosis (IC)	A	2	S	B
8	Oral candidosis (IC)	A	2	S	B
8	Oral candidosis (IC)	A	2	S	B
9	Oral candidosis (OR)	A	2	S	B
9	Oral candidosis (IC)	A	2	S	B
10	Lichen planus (OR)	A	2	S	G (6576174)
11	Sore mouth (OR)	A	4 (103406)	R	D (2766174)
12	Dry mouth (OR)	A	3 (100406)	S	B
13	CAC (OR)	A	2	S	E (2776174)
14	CAC (OR)	A	2	S	B
15	Sore tongue (OR)	A	2	S	H
16	White lesion (OR)	U	2	S	B
17	Sore tongue (IC)	A	2	S	B
18	Sjögren's syndrome	A	2	S	A (2566174)
19	Oral candidosis (OR)	U	2	S	G
20	Sjögren's syndrome	A	2	S	B
21	Area of striae (IC)	A	2	S	B
22	Erythema of palate (IC)	A	2	S	G
23	Oral candidosis (OR)	A	1 (120406)	S	G
24	Sjögren's syndrome	B	2	S	B
24	Sjögren's syndrome	B	2	S	B
25	Intra-oral graft (IC)	A	2	S	B
25	Intra-oral graft (IC)	A	2	S	B
26	Burning mouth (OR)	A	3	S	H
27	Simple keratosis (IC)	B	ND	S	B
28	White lesion (IC)	A	2	S	E
29	Sublingual keratosis	U	2	R	B
30	CAC (OR)	A	3	R	B

CAC, chronic atrophic candidosis; IC, imprint culture; OR, oral rinse; U, auto-agglutinating; ND, not determined; S, sensitive; R, resistant.

Table 2. Typing of 35 isolates of *C. albicans* from 19 patients with CHC

Patient no.	Isolation method	Serotype	MYT profile	Boric acid sensitivity	API-20C profile
31	OR	A	2	R	B
32	IC	A	2	S	B
33	IC	A	3	S	E
33	IC	A	3	S	E
33	IC	A	2	S	B
34	IC	A	2	S	B
35	IC	A	2	R	B
35	IC	A	2	S	B
35	IC	A	3	R	B
36	IC	A	2	S	B
37	IC	B	2	S	C (2576175)
38	IC	A	1	S	B
39	IC	A	2	S	B
40	IC	A	1	S	B
40	IC	A	2	S	B
41	IC	A	2	R	B
41	IC	A	3	R	B
42	OR	U	2	R	A
42	OR	A	2	R	B
42	IC	A	2	S	B
42	IC	A	2	S	B
43	OR	U	3	S	B
44	IC	U	2	S	B
45	IC	A	2	R	B
45	IC	A	2	R	B
46	IC	A	2	S	B
46	IC	A	2	S	B
46	IC	A	2	S	B
46	IC	A	2	S	B
47	IC	U	2	R	B
47	IC	A	2	R	B
47	IC	A	2	R	B
48	IC	B	2	S	B
49	IC	A	3	S	F (6176174)
49	IC	A	3	S	F

IC, imprint culture; OR, oral rinse; U, auto-agglutinating; S, sensitive; R, resistant.

mucosa have been reported, as has variation between strains from speckled and homogeneous CHC lesions [7].

The API-20C is a standard method for yeast identification based on carbohydrate assimilation and has been used successfully in epidemiological studies [12–14]. In the present investigation, the most prevalent API-20C profile (biotype B) occurred in 89% and 63% of CHC and non-CHC patients, respectively. However, the increased incidence of biotype B in CHC patients was not significant. The predominance of biotype B is consistent with other studies, where it has accounted for 74.6% [12], 61.1% [15] and 57.8% [16] of oral *C. albicans* isolates.

The MYT is a commercial paper ring test used for the identification of yeast and is based on sensitivity to six chemicals. Although simple to use, the test proved to be limited as a typing tool, as only four profiles were evident for the 70 *C. albicans* isolates tested. Furthermore, a single profile (120406) accounted for 80% of the isolates. As with the API-20C, overall differences between CHC and non-CHC isolates were not evident.

C. albicans serotype differences have been associated

with type of candidosis [17] and the level of host immunocompetence [18]. In the present study, serotype A predominated for isolates from both CHC and non-CHC patients (80% of isolates), supporting the high incidence reported by Brawner [18]. Whilst serotyping was simple to perform and easy to interpret, its discriminatory power was low and typability restricted when auto-agglutinating strains were present.

The higher proportion of boric acid-resistant isolates from CHC (32%) compared with non-CHC patients (16%) was not significant ($p = 0.25$), but may warrant further investigation with increased numbers of isolates.

There are several considerations when comparing the biotypes of *C. albicans* from different forms of oral candidosis. Both local and systemic host factors have a significant role in the development of clinical candidosis and it is possible that candida grouped in this study as 'non-CHC isolates' could cause CHC in 'susceptible' individuals. Furthermore, whilst phenotypic characteristics are often simple to analyse, they may be unstable with changing environmental parameters such as those encountered within hosts or during laboratory culture [19]. With this caveat, based on the results from the present investigation, associa-

tions between *C. albicans* phenotype and CHC infection were not evident and clonal restriction was not demonstrated. Further investigation by genotypic methods is an area for future study and may provide a greater insight into those strains responsible for CHC infection.

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