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ORIGINAL ARTICLE

Persistent oral and urinary *Candida* spp. carriage in Italian HIV-seropositive asymptomatic subjects

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Key words

Candida spp. • Oral carriage • Urinary carriage • HIV-seropositive subjects

Summary

The aim of the present study was to ascertain frequency and persistence of Candida spp. oral and urinary carriage in asymptomatic, HAART-naive HIV-seropositive subjects who had not undergone therapy with antimycotic drugs, and whose CD4⁺ lymphocyte count was greater than 200/μl. Oral carriage was the most common Candida spp. carriage (63.0% of the subjects), while candiduria was more rarely observed (6.5%). C. albicans was recovered from the majority of the subjects examined (56.5%), followed by C. krusey (4.3%), C. tropicalis (2.2%) and C. dubliniensis (2.2%). C. albicans was also isolated from two urinary carriers (4.3%) and C. glabrata from another one (2.2%). The same C. albicans clone was repeatedly isolated from 14 out of 15 oral carriers while the same clone of C. dubliniensis was repeatedly isolated from one carrier, as shown by the persistence of RAPD fingerprint of serial isolates during one year of follow-up. Since persistence of Candida spp. carriage may influence the development of clinical candidiasis in immunocompromised hosts, monitoring of the carrier status could be useful for preventing clinical thrush in HIV-seropositive subjects.

Introduction

Asymptomatic oral *Candida* carriage has been shown to be much more common in HIV-seropositive subjects than in healthy subjects [1-3]. It is also known that oropharyngeal candidiasis is the most common opportunistic infection in AIDS patients [4, 5]. Although different species of *Candida*, such as *C. glabrata, C. tropicalis, C. krusey,* and *C. dubliniensis*, are at present recognized as increasing opportunistic pathogens specially in immunocompromised patients, *C. albicans* still remains the most common fungus isolated in humans [6-8]. The frequency of isolation of *C. albicans* and the clinical recurrence of oral candidiasis increase with advancing the HIV infection, so that up to 90% of infected individuals suffer from at least one episode of oropharingeal candidiasis during the course of the disease [2, 9]. Infections may be acquired from endogenous flora (e.g. commensal fungi that colonize oropharynx, genitourinary tract, gut, or skin) and clinical lesions of oropharyngeal or oesophageal candidiasis can develop as the HIV infection progresses [2, 10].
A prospective study conducted with HIV-infected subjects with and without symptoms of oropharingeal candidiasis revealed that although some patients maintained the identical *C. albicans* isotype on contiguous sequential visits, in others this occurred irregularly, yet periodically [11]. More recently, it has been reported that strains of *C. albicans* from AIDS patients with oral candidiasis were genetically less diverse than oral commensal isolates from patients with no signs of oral thrush at the time of the sampling, possibly because more resistant and hence stable strains were acquired during recurrent infections and multiple course of antimicrobial treatment [12]. The same studies indicate that *C. albicans* populations in patients with HIV exhibit genetic reshuffling. On the other hand, the persistence of the same DNA subtypes of *C. albicans* throughout each episode of infection during a long period of observation has been reported in four of five AIDS patients undergoing fluconazole therapy [9]. Despite exposure to antifungal drugs has no effect on the level of yeast carriage in HIV-positive patients [13], there is evidence that appropriate prophylactic use of antifungal agents reduce the clinical signs of candidiasis in patients with impaired immunitary conditions [14].
In Italy, isolation of *Candida* spp. from the oral cavity of patients infected by HIV has been reported by Campisi et al. (2002) [1] but the duration of the carriage status and the genotypes of the isolates have not been defined. Furthermore, no investigations on the status of urinary carriage have been reported in HIV-positive subjects. Therefore, the purpose of this study was to ascertain the frequency and the persistence of *Candida* spp. oral and urinary carriage in asymptomatic HIV-seropositive Italian subjects who had not undergone therapy with antimycotic drugs and with no prior history of thrush.

Materials and methods

Isolation and identification of Candida spp. from patients

Swabs from the oral cavity and urine samples were collected from 46 HAART-naive HIV-seropositive sub-

jects (40 men and 6 women) confirmed by ELISA and Western blot, attendant at regular intervals the Communicable Diseases Division of a General Hospital in Catania (Sicily, Italy). At the time of the sampling, all subjects were asymptomatic for *Candida* infections and had their $CD4^+$ lymphocyte counts greater than 200/µl. The study was approved by the Bioethics Committee of the Hospital. All subjects provided informed consent before the recruitment. Oral swabs and urine samples were collected from 16 subjects at intervals varying from 1 to 6 months over one year time period. The swabs and 100 microlitre quantities of urine from each subject were plated, respectively, onto plates of CHROMagar *Candida* (Becton Dickinson, New Jersey, USA) and incubated at 37°C for 48-72 h.

Presumptive identification of isolates was attempted on the basis of the characteristic colouration of the colonies on CHROMagar *Candida*. All isolates were purified by subculturing on Sabouraud glucose agar (Oxoid, Hampshire, England) and identified to the species level by carbohydrate assimilation profiles obtained by two commercial systems: API ID 32 C and API 20 C AUX (both BioMérieux, Marcy-l'Etoile, France). The isolates suspected to be *C. albicans* were also studied for germ-tube formation in human serum incubated at 37°C for 2 h. Isolates showing carbohydrate assimilation profiles of *C. dubliniensis* were confirmed by intracellular β-glucosidase test, incubation at 42°C, and karyotyping [15, 16].

Genotyping of candida albicans and candida dubliniensis isolates

C. albicans and *C. dubliniensis* isolates from each carrier and their serial isolates were genotyped by RAPD fingerprinting, as previously described [17], in order to ascertain the persistence of the same genotype in the oral cavity and urine samples of the different carriers. The DNA extraction was performed in accordance with Hoffman's protocol (1993) [18]. The single repeat sequence $(GACA)_4$ was used as primer in the PCR experiments as described by Schonian et al. (1993) [19]. Briefly, 200 µM of each deoxinucleotide (Amersham Pharmacia Biotech, Uppsala, Sweden), 16 pmol primer, 25 ng template DNA, 2.5 U *Taq* DNA polymerase (Perkin Elmer Corp., Applied Biosystems, New Jersey, USA), 10 mM Tris/HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 3 mM Mg-acetate were added to a 50 µl reaction volume. The cycle parameters were 33 cycles of 20 s at 94°C, 1 min at 43°C, 20 sec at 72°C, followed by a final extension cycle of 6 min at 72°C, performed on a Hybaid thermocycler (Teddington Middlesex, United Kingdom). The RAPD products were separated by electrophoresis through a 1.2% agarose gel in 0.5 x TBE (89 mM Tris-borate, 2 mM EDTA) buffer at 2 Volts/cm for 16 h. The gels were stained with ethidium bromide and photographed. Lambda phage DNA digested with *Bst*E II was used for the DNA fragments size determination. RAPD fingerprints were analysed by the Gel Compare software (Applied Maths BVBA, Kortrick, Belgium).

Results

Out of 46 HIV-subjects, 29 (63.0%) were positive for oral carriage of *Candida* (twenty-eight men and one woman) and 3 (6.5%) for urinary carriage (two men and one woman). The number of yeast colonies in the plates inoculated with the samples varied from 10 to > 300, according to the different subjects. None of them had lesions presumptive for candidiasis at the time of the sampling or developed oral lesions nor genitourinary troubles during the twelve months of follow-up.

The most common species found in the oral cavity was *C. albicans*, isolated from 26 (56.5%) subjects, followed by *C. krusey* from two subjects (4.3%), *C. tropicalis* from one (2.2%) and *C. dubliniensis* from another one (2.2%). One of the two *C. krusey* isolates was recovered from a carrier of *C. albicans* (mixed culture). Species isolated from urines were *C. albicans* (two subjects, 4.3%) and *C. glabrata* (one subject, 2.2%). *C. albicans* was cultured from both oral and urinary samples of the carrier woman.

Sixteen of the positive subjects under study (15 men and the positive woman) were submitted to further oral and urine sampling during one year of follow-up. Two to six serial isolates were obtained from the 14 men positive for *C. albicans* and two isolates each from the oral swabs and urines from the positive woman, for a total of 43 serial isolates of *C. albicans*. Furthermore, six serial isolates at two months intervals were obtained from the oral carrier of *C. dubliniensis*.

Analysis of the *C. albicans* serial isolates using RAPD fingerprints with primer $(GACA)_4$ originated 15 different patterns, while a further distinct pattern was obtained from the *C. dubliniensis* isolates. The same RAPD pattern was shown by the oral and urinary isolates from the carrier woman. RAPD fingerprintings of 41 *C. albicans* serial isolates from 14 out of 15 carriers showed persistence of the respective patterns throughout all the follow-up period. Only in one carrier the second isolate differed from the first one. The six isolates of *C. dubliniensis* obtained during the one year of follow-up also maintained the same RAPD pattern.

Discussion and conclusions

Asymptomatic oropharyngeal carriage of *Candida* spp. is a common finding in HIV-infected patients. Costa et al. (2006) [3] found that out of 99 HIV-positive patients studied, 62 (62.6%) had positive culture for *Candida* (oral carriage). Similar prevalence had previously been reported by Campisi et al. (2002) [1], who found an oral *Candida* carriage rate of 61.9%, by Gugnani et al. (2003) [20], who discovered a proportion of 65.3% in HIV-seropositive subjects, and by Sanchez-Vargas et al. (2005) [21] that reported a *Candida* isolation rate of 66.7% in HIV/AIDS patients. A similar high rate of oral carriage of *Candida* (63.0%) was detected in the HIV-positive subjects monitored in our study. Moreover, according to the cited studies, the most common species

isolated in the oral cavity of the HIV-positive subjects examined was *C. albicans* (87.0%).
Despite the high prevalence of *C. albicans*, some other species which have been generally isolated at minor frequency have proved to be present in the oral cavity of the immunocompromised patient population [16, 21-24]. In our study, *C. dubliniensis*, in particular, showed to be a persistent commensal in one subject, while some other species (*C. krusei*, *C. glabrata* and *C. tropicalis*) were occasionally isolated.
Yeast colonization on oral mucosa is considered the precondition of oral candidiasis and early detection of oral carriage of *Candida* spp. is seen to be important for identification of patients with the propensity for rapid progression of HIV infection. Despite it has been shown that the status of oral *Candida* carrier is not associated with the number of $CD4^+$ cells or the viral load [1, 3, 25, 26], little is known about the changes, if any, which may occur in *C. albicans* when asymptomatic HIV-positive subjects develop clinically significant candidiasis. Also studies on molecular epidemiology of oral *Candida* spp. colonization have not completely elucidated what happens to *Candida* spp. strains carried by HIV-infected subjects in the transition from a healthy carriage status to a disease status. The combined results of a number of studies monitoring HIV-infected subjects have shown different scenarios ranging from maintenance of genetically invariant strains to replacement of commensal strains by unrelated strains [2]. In a preceding study on the discriminatory power of some phenotypic and molecular typing methods, RAPD *C. albicans* fingerprinting was proved highly discriminatory and suitable for epidemiological studies [17]. Therefore, the persistence of the same RAPD patterns in the serial isolates throughout the follow-up period, as observed in 14 out of 15 *C. albicans* and in one *C. dubliniensis* carriers, suggests that the same strain can colonize for a long time the oral mucosa of HIV-infected subjects.
To our knowledge, up to now no systematic investigations on the frequency and persistence of candidal urinary carriage in asymptomatic HIV-positive subjects have been conducted. In our study, urinary carriage was detected in only three subjects (6.5%), two of which were colonized by *C. albicans* and the third one by *C. glabrata*. One of the two *C. albicans* urinary carrier was also oral carrier and his isolate belonged to the same RAPD type of the *C. albicans* isolate cultured from the oral swab. Further epidemiological investigations are needed to better determine the frequency of urinary candidial carriage and to understand its role in developing opportunistic infections in the urinary tract.
In conclusion, a study of HIV-positive subjects asymptomatic for candidiasis over a 1-year period allowed us to monitor the dynamics of *Candida* spp. oral and urinary colonization. Since persistence of carriage may influence the development of clinical candidiasis in HIV-positive subjects, monitoring the carrier status could be useful for preventing *Candida* spp. infections by topical or systemic antifungal drugs when $CD4^+$T count falls under 200 cells/µl. Therefore, systematic oral and urinary examination for HIV-positive subjects should be recommended together with the survey of their immune status for better managing opportunistic infections in these patients.

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