Decreased susceptibility of *Candida albicans* to azole antifungals: a complication of long-term treatment in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) patients

Riina Rautemaa1–3 *, Malcolm Richardson1,4, Michael Pfaller5, Pirkko Koukila-Kähkölä4, Jaakko Perheentupa6 and Harri Saxén6

1Department of Microbiology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland; 2Department of Bacteriology, Helsinki University Central Hospital HUSLAB, Helsinki, Finland; 3Department of Oral and Maxillofacial Diseases, Helsinki University Central Hospital, Helsinki, Finland; 4Department of Mycology, Helsinki University Hospital HUSLAB, Helsinki, Finland; 5Medical Microbiology Division, Department of Pathology, College of Medicine, University of Iowa, Iowa City, IA, USA; 6The Hospital for Children and Adolescents, Helsinki University Central Hospital and University of Helsinki, Finland

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**Background:** Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED, APS1) is an autosomal recessive disease exceptionally common in Finland. Most patients have chronic oral candidiasis from early childhood and this infection has been shown to be carcinogenic. Hence, patients receive repeated treatment and prophylactic courses of antifungals throughout life. In Finland, 92 patients have been diagnosed with APECED and 66 of them are currently alive. Our aim was to study the effect of long-term azole treatment on the candidal colonization of APECED patients and the influence on antifungal susceptibilities.

**Methods:** We evaluated the culture reports from 1994 to 2004 of 56 APECED patients followed in Helsinki University Central Hospital. *Candida albicans* strains of all 11 patients initially reported resistant (*n* = 27) and 12 patients reported susceptible (*n* = 16) to fluconazole were re-analysed for their susceptibility to fluconazole. Antifungal usage was analysed up to 30 years back.

**Results:** A total of 162 fungal cultures had been performed. Of these, 75% had been reported positive for *Candida* and 63% for *C. albicans*. Eleven patients (31.4%) had been reported to harbour at least once a *C. albicans* strain resistant to fluconazole. Re-analysis of the stored *C. albicans* strains originally reported to be resistant to fluconazole revealed a mean MIC of 19.5 mg/L.

**Conclusions:** Multiple courses (>6) of fluconazole annually and low dose prophylaxis are major risk factors for persistent colonization with *C. albicans* with decreased susceptibility in APECED patients.

Keywords: oral candidiasis, *C. albicans*, APS1, CMC

**Introduction**

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is a rare autosomal recessive disease caused by mutations of the AIRE (autoimmune regulator) genes.1 In Finland, 91 patients have been diagnosed with the disease since 1963 and 66 of them are currently alive. Most patients have chronic mucocutaneous candidiasis (CMC) from early childhood and oral CMC in APECED patients (APECED-CMC) is carcinogenic.2

Until the late 1980s, CMC in our patients was only treated when they had symptoms and prophylaxis was uncommon. In the early 1980s, mainly topical miconazole, pimafusin and natamycin were used for treatment. Systemic ketoconazole was only used during periods of severe symptoms but by the late 1980s low-dose intermittent prophylaxis became common.3 By the
mid-1990s, first itraconazole and soon thereafter fluconazole started to replace ketoconazole. The dose for fluconazole normally used for treatment was 1 mg/kg and for adults 50 mg daily. For prophylaxis 150 mg once a week or 100 mg twice a week was common. By the late 1990s, the response of many patients to the given antifungal treatment began to worsen and they suffered from a refractory candidal infection. Decrease in the susceptibility of the colonizing Candida albicans strains to azole antifungals was noted.

The occurrence of mucosal candidiasis refractory to fluconazole and due to C. albicans instead of non-C. albicans species is rarely reported. A decrease in in vitro susceptibility of C. albicans to azoles was initially reported in the late 1970s in patients with chronic (non-APECED) mucocutaneous candidiasis with repeated and prolonged courses of therapy. C. albicans with decreased in vitro susceptibility to azoles have been isolated from HIV patients. However, these interpretations are mainly based on MIC data from heterogeneous patient groups lacking clinical data. Long-term data of homogeneous patient groups with recorded antifungal usage does not exist. We report the usage of antifungals in 23 Finnish APECED patients over the past 30 years and the concomitant acquisition of decreased susceptibility to fluconazole in C. albicans.

**Patients and methods**

We evaluated the culture reports from 1994 to 2004 for 56 APECED patients followed in Helsinki University Central Hospital. The use of antifungals was analysed based on the patient records up to 30 years back. All available C. albicans strains reported to have decreased azole antifungal susceptibility on one occasion or more were re-analysed for their susceptibility to fluconazole by the CLSI microdilution method (M27-A2). Yeasts had originally been tested for their susceptibilities to fluconazole by Etest (AB Biodisk, Solna, Sweden), C. albicans strains from age- and sex-matched APECED patients reported to be susceptible to azole antifungals were re-analysed as controls. Also, a database search on all reported C. albicans strains with a decreased azole susceptibility in years 1999–2004 was performed. The results were analysed using the Mann–Whitney t-test and the Fisher’s exact test. Differences between the two cohorts were considered significant if P < 0.05.

**Results**

**Fungal culture reports**

The hospital database search of years 1999–2004 revealed ~3000 cultures positive for yeasts annually. All samples reported as C. albicans with a decreased susceptibility to fluconazole were found to be from APECED patients. For the 56 APECED patients, a total of 162 fungal cultures had been done. Forty-two patients (75%) had been reported positive for Candida and of them 35 (63%) for C. albicans. Eleven patients had been reported at least once with a C. albicans strain resistant or of decreased susceptibility to fluconazole. Seven patients had been reported with a non-C. albicans strain but only two of them with decreased fluconazole susceptibility.

**Patient demographics**

The median age was 32.5 years (SD ± 13.3 years) for the 56 APECED patients in 2004, and their median age at onset of CMC was 4.3 years (SD ± 5.1 years). The median age at onset of oral candidiasis was 5.6 years (SD ± 7.5) for the group of patients colonized with Candida strains reported resistant to fluconazole and 5.3 years (SD ± 3.6) for the patients with susceptible Candida strains.

**Analysis of antifungal usage**

The antifungal usage of the 11 patients reported with C. albicans resistant or of decreased susceptibility to fluconazole (resistant group) and 12 age- and sex-matched patients colonized with fluconazole-susceptible C. albicans (susceptible group) were analysed up to 30 years back (Figure 1). The patients in the resistant group had more years on ketoconazole (mean 6.5 versus 2.0 years) and miconazole (mean 10.9 versus 7.2 years) but fewer fluconazole years (mean 2.8 versus 4.8 years) by first report of resistance than the patients in the susceptible group by 2005. The difference was statistically significant only for ketoconazole (P < 0.05). Nine of the 11 patients (81.8%) in the resistant group had been on all three azoles in contrast to seven (58.3%) in the susceptible group. Patients in the resistant group had received significantly more often multiple courses (>6 per year or continuous prophylaxis) of ketoconazole, miconazole and fluconazole than patients in the susceptible group (P < 0.05 for all comparisons). All nine patients in the resistant group (81.8%) who had been prescribed fluconazole had been on multiple courses, on average for 3.4 years, in contrast to two patients (16.6%) in the susceptible group, on average for 3.5 years. Six of the patients with fluconazole-susceptible C. albicans had been on one to three courses of fluconazole annually on average for 6 years (range 1–9 years).

**Antifungal susceptibility testing**

Twenty-five of the 27 strains isolated from the resistant group had initially been reported to be resistant or of decreased susceptibility to fluconazole. Re-testing revealed MICs between 4 and 32 mg/L (mean 19.5 mg/L; SD ± 10.7 mg/L) for the resistant strains, and between 0.12 and 2 mg/L (mean 0.30 mg/L; SD ± 0.46 mg/L) for the susceptible strains (Figure 2). C. albicans with decreased susceptibility for fluconazole had been isolated repeatedly over the years from 9 of the 11 patients in the resistant group. Five of them had become colonized with the strains with decreased susceptibility already in the mid-1990s.

**Discussion**

The occurrence of mucosal candidiasis that is refractory to therapy with fluconazole and due to C. albicans instead of a non-C. albicans species is rarely reported. In our hospital during 1999–2004, all samples reported as C. albicans with decreased susceptibility to fluconazole were found to be from patients with APECED. Eleven of our 56 APECED patients had been reported to harbour a C. albicans strain resistant or with decreased susceptibility to fluconazole representing 31.4% of APECED patients reported with C. albicans. The incidence is very high...
Azole-resistant *C. albicans* infection in APECED

Figure 1. The years of usage of azole antifungals of the 23 APECED patients in the two patient groups (resistant group, left-hand panel; susceptible group, right-hand panel). Patients colonized with a *C. albicans* strain with decreased susceptibility to fluconazole had a history of more usage of ketoconazole.

Figure 2. The fluconazole MICs of the *C. albicans* strains isolated from 23 Finnish APECED patients using a broth dilution technique as detailed in the CLSI document M27-A2. The MICs (mg/L) were significantly higher in the resistant group (left-hand panel). The colonization with the resistant strains seems to be persistent. Patient 5 was given eradication treatment for 1 week with intravenous liposomal amphotericin B and became colonized with a susceptible strain thereafter. Omitting all azoles since 2001 has not resulted in return of susceptible *C. albicans*.
compared with any other patient group with chronic oral candidiasis.3,8

Analyses of medical records of 23 Finnish APECED patients up to 30 years back show that the amount of fluconazole used rather than the years of usage seems to correlate with the development of clinical resistance of C. albicans to fluconazole. Also, liberal previous use of ketoconazole was found to be a risk factor for decrease in fluconazole susceptibility. Two patients reported with a fluconazole-resistant strain had according to patient records never been exposed to fluconazole but only to ketoconazole and miconazole.

Re-analysing the stored C. albicans strains originally reported to be resistant or of decreased susceptibility to fluconazole revealed MICs between 4 and 32 mg/L with a mean MIC of 19.5 mg/L. According to the currently used definitions, these values encompass the designations susceptible or susceptible, dose-dependent.9 However, the original reporting of resistance did accord with clinical resistance. This observation raises the question of whether the current designations are wholly applicable to APECED-CMC. C. albicans strains from the 12 age- and sex-matched APECED patients originally reported to be susceptible to azole antifungals revealed MICs between 0.12 and 2 mg/L with a mean MIC of 0.30 mg/L and would be defined as susceptible. Patients in this group responded well to treatment with fluconazole.

Colonization with C. albicans with decreased susceptibility to fluconazole was persistent. For 9 of the 11 patients, C. albicans with elevated MIC of fluconazole was isolated repeatedly and in 5 patients colonization had evolved already by the mid-1990s. Despite the cessation of the use of azoles and favouring topical polyenes since the year 2001 the strains have persisted. The more resistant C. albicans strains seem to replace the susceptible strains in the normal oral flora in a similar way to that seen with methicillin-resistant Staphylococcus aureus in skin flora.

The patient series in this study is unique in covering up to 30 years of antifungal usage and 10 year antifungal susceptibility data for 23 APECED patients. Due to the carcinogenicity of APECED-CMC and the life-long need for treatment, the risk of selection of azole-resistant Candida strains must be considered. The use of topical polyenes or chlorhexidine should be preferred. The susceptibility of the colonizing strains should be followed up and antifungal treatment should be based on this data. Up to three courses of a therapeutic dose of fluconazole can probably be used annually without risk for selection and development of resistance. All patients with APECED require good oral hygiene as dental plaque has been shown to be a major source of yeasts in the oral cavity.10

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Transparency declarations
None to declare.

References