Journal of Medical Microbiology (2014), **63**, 518–521 **DOI** 10.1099/jmm.0.060236-0

Paediatric oropharyngeal and cutaneous candidiasis with special reference to *Candida dubliniensis*

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Received 19 June 2013
Accepted 17 January 2014

Mucocutaneous and cutaneous candidiasis, though common in children, is often under-reported. The prevalence of *Candida dubliniensis* in causing these infections in this age group is also largely unknown. A prospective epidemiological cross-sectional study for candidiasis was performed in paediatric patients clinically suspected of candidiasis with oropharyngeal lesions (75 patients), cutaneous lesions (18 patients) and lesions at both sites (2 patients). *Candida* species were identified by conventional tests. For *C. dubliniensis*, chlamydospore production, growth on tobacco agar and growth at 45 °C were performed. Nine isolates were confirmed at a reference centre. The rates of candidiasis were 77.3 % (58 out of 75 patients clinically suspected of candidiasis) and 83.3 % (15/18) in oropharyngeal and cutaneous lesions respectively, and 1 of the 2 children with lesions at both sites was diagnosed as having chronic mucocutaneous candidiasis due to *C. dubliniensis*. The commonest species isolated was *Candida albicans*, in 41 (70.7 %) patients with oropharyngeal candidiasis and 11 (73.3 %) with cutaneous lesions; *C. dubliniensis* was isolated from 11 and 3 children respectively. In the paediatric population, *C. albicans* predominates in mucocutaneous and cutaneous candidiasis, with *C. dubliniensis* also contributing substantially.

INTRODUCTION

Candida spp. often affect the paediatric population in the form of oropharyngeal candidiasis (OPC), intertrigo and diaper dermatitis. The presence of OPC in a child is known to be a significant independent risk factor for predicting HIV infection. Persistent or recurrent candidal infection of skin and mucous membrane, and conditions like chronic mucocutaneous candidiasis (CMC), alert the physician to possible associated endocrinopathies or other conditions.

Inflammation of the skin may be a cutaneous manifestation of an underlying systemic disease. It is important to differentiate cutaneous candidiasis from other similar-looking conditions. This will ensure proper diagnosis and avoid inappropriate treatment with antifungals or steroid creams.

The recently identified species *Candida dubliniensis* has been reported to cause mucocutaneous and systemic infections in adults as well as the paediatric population (Blignaut, 2007). Although *C. dubliniensis* and *Candida albicans* isolates are both susceptible to azoles, fluconazole resistance has been observed in clinical isolates of *C. dubliniensis* from HIV-infected patients with prior exposure to fluconazole. Reviewing the Indian literature, there are at best very scanty published data on oropharyngeal and cutaneous candidiasis in general and on *C. dubliniensis* in particular in the paediatric age group.

Abbreviations: CMC, chronic mucocutaneous candidiasis; OPC, oropharyngeal candidiasis; PGIMER, Post Graduate Institute of Medical Education & Research.

METHODS

A prospective epidemiological cross-sectional study for candidiasis was performed for 1.5 years in paediatric (up to 12 years of age) patients with oropharyngeal and/or cutaneous lesions. In this study 95 children who were clinically suspected of having oropharyngeal and/or cutaneous candidiasis were included. The affected area was swabbed and smears were stained by Gram stain. Swabs were used for culture on Sabouraud's dextrose agar (SDA) slants with antibiotics. Primary smears showing inflammatory cells and predominantly yeast flora were considered as diagnostic of candidiasis.

Candida spp. were identified by conventional tests like germ tube, growth on cornmeal agar, sugar fermentation and assimilation. All germ-tube-positive and chlamydospore-producing *Candida* spp. were assessed for C dubliniensis. They were grown on tobacco agar kept at 28 °C (Khan *et al.*, 2004), growth or no growth at 45 °C was also noted (Kim *et al.*, 2003) On tobacco agar, *Candida dubliniensis* produced rough, yellowish-brown colonies with peripheral hyphal fringes and abundant chlamydospores after incubation for 48 to 72 h

at 28 °C. In addition, all the isolates formed abundant chlamydospores on the peripheral hyphal fringes after 24 to 48 h of incubation at 28 °C. In contrast, on this medium, all *C. albicans* isolates showed smooth, white-to-cream colonies without hyphal fringes or chlamydospores even after extended incubation for up to 10 days. Nine of the isolates thus identified as *C. dubliniensis* were sent to the Post Graduate Institute of Medical Education & Research (PGIMER), Chandigarh, for confirmation.

RESULTS

During the course of this study, 75 (78.9 %) patients with oral lesions, 18 (18.9 %) patients with cutaneous lesions and 2 (2.1 %) patients with both oral and cutaneous lesions were encountered. In patients with oral lesions, the incidence of candidiasis was found to be 77.3 % (58/75) and in patients with cutaneous lesions it was 83.3 % (15/17). Of the 2 patients with both oral and cutaneous lesions, 1 had infection with *C. dubliniensis* at both sites.

Patients with oral candidiasis belonged to the following age groups: 35 (60.3 %) were 0–1 month, 6 (10.3 %) were 1 month to 1 year, 6 (10.4 %) were 1–4 years, 1 child was between 4 and 6 years (1.7 %) and 10 (17.2 %) were 6–12 years of age. With cutaneous candidiasis, the commonest age group involved was 1–4 years (9 children, 60 %) followed by 1 month to 1 year (4 children, 26.7 %). The neonatal and 4–6 years groups each contained 1 child (6.7 %). Of the 2 patients with both oral and cutaneous candidiasis, one was a 12 year old with oral as well as cutaneous candidiasis. This child was eventually diagnosed with CMC. This patient had had recurrent oral thrush since the age of 4 years with resultant hypertrophy of the tongue, angular chelitis and fissuring of the lips. Subsequently, he developed dystrophy of the nails and intertrigo in the webs of his fingers, with waxing and waning of symptoms. He was HIV negative. *C. dubliniensis* was isolated from this patient.

There were 40 neonates in the study, of which 36 had candidiasis. Thirty-five had oral candidiasis (97.2 %), which was found to be significantly more frequent in these children than cutaneous candidiasis, which was seen in only 1 patient (2.78 %) ($P<0.05$). Concerning predisposing conditions in the total 58 patients with OPC, 17 were tested for HIV antibody and 12 of them were HIV positive. Other predisposing conditions were intake of broad spectrum antibiotics for more than 5 days in 33 (56.9 %) patients, preterm birth in 28 patients (48.3 %), low birth weight in 26 (44.8 %), and malnutrition in 18 (31.03 %). Birth asphyxia was seen in 8 out of 35 (22.9 %) of the newborns in our study. History of vaginal infection in the mother during pregnancy was elucidated in only 1 (2.5 %) of the neonates.

In our study, association of skin maceration (in natural folds of skin) was seen in all the 15 patients with cutaneous candidiasis. Ten of these were on broad-spectrum antibiotics for >5 days' duration. Infrequent diaper change in 9 children and diarrhoea for >5 days in 5 were the other associated risk factors. Four children had been administered steroids for >5 days whereas 3 had malnutrition. Underlying multiple endocrine disorders, hypothyroidism, hypoparathyroidism and Addison's disease were encountered in the 1 patient with CMC. He had a hypertrophied tongue (Fig. 1)

Out of 53 patients with OPC, the predominant site was lingual in 48 and buccal in 5. OPC was of the pseudomembranous variety in 57 patients and erythematous in 1 patient.

Multiple site cutaneous candidiasis was seen in 2 patients, with areas like the periauricular region, axilla, neck fold and perineum being involved. The most common single site for cutaneous infection was perineal, in 11 out of 13 patients. The cutaneous lesions were erythematous and moist in all the patients, with satellite papules in 6 of them.

C. albicans was found to be the most common species causing OPC, in 41 out of 58 children (70.7 %), followed by *C. dubliniensis* in 11, *Candida tropicalis* in 5 and *Candida glabrata* in 1. In patients having cutaneous candidiasis, *C. albicans* was isolated from 11 out of 15 children (73.3 %), followed by *C. dubliniensis* from 3 and *C. glabrata* from 1 patient. *C. dubliniensis* was isolated from tongue and interdigital lesions from the case of CMC. Out of the 15 isolates of *C. dubliniensis* presumptively identified by their phenotypic characteristics, 9 were submitted to the Department of Medical Microbiology – Medical Mycology Reference Center, PGIMER, Chandigarh, India for confirmation.

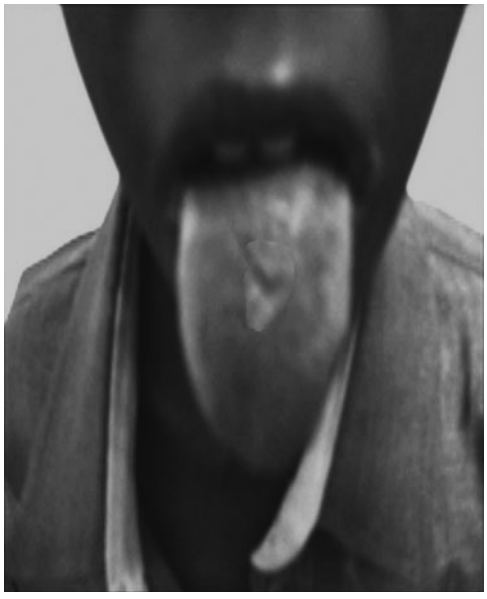

Fig. 1. Hypertrophied tongue in a boy with CMC.

DISCUSSION

Advances in medical technology and the increased survival of immunocompromised patients have led to a general increase in candidiasis. However, microbiological studies on oropharyngeal and cutaneous paediatric candidiasis still remain an under-reported entity in the Indian literature. In Indian studies, prevalence of oral candidiasis has been reported in 2 to 3.2 % of neonates and cutaneous candidiasis in 2 % of them (Dash *et al.*, 2000; Gupta *et al.*, 1996). Understandably, this was the most common age group with OPC in our study of children with clinically suspected candidiasis. Cutaneous candidiasis, however, was found in significantly lower numbers than OPC in infants. Felea *et al.* (2004) have reported a *Candida* culture positivity of 85 % in children manifesting clinical symptoms of candidiasis. From cutaneous lesions in children, in the diaper area an 80 % rate of *C. albicans* infection has been reported by some authors; in other body areas the rate was 33 % (Leyden & Kligman, 1978). López Martinez & Ruiz-Maldonado (1982), while studying 140 children (0–20 months) with diaper rash, found that *Candida* spp. were present in 65.7 % of cases. Diaper use and wetting of the perineal area are more common for children younger than 4 years, and this was the age group most commonly affected by cutaneous infection by *Candida* spp. Diarrhoea as a contributing factor was seen in a third of the children in the age group 1–4 years, probably as a result of bottle or spoon feeding predisposing them to unhygienic conditions and *Candida* infection. The skin of an infant is more vulnerable to infections than that of an adult due its thinness, weaker intracellular attachments and less sweat and sebaceous secretion (Dash *et al.*, 2000). CMC has been reported by Bhowate & Dubey (2004) and Sathish Kumar *et al.* (2005) in a 12-year-old girl and an 11-year-old boy, respectively; however, these children did not appear to have any endocrinopathies. Myhre *et al.* (2004) reported the association between CMC and endocrinopathies. The authors have suggested investigation of the link between hypothyroidism and CMC. Gupta *et al.* (1996), while studying the clinical profile and risk factors for OPC in sick newborns, found that birth asphyxia was a significant factor responsible for thrush in newborns. Malnutrition as a risk factor for OPC was also reported in 48.4 % of malnourished Nigerian children (Jabra-Rizk *et al.*, 2001). HIV positivity is a known risk factor for development of OPC. Lal & Chussid (2005) reported that 20–70 % of children with HIV infection or AIDS had signs of oral candidiasis.

A twofold increase in the recovery of *C. albicans* from skin has been noted earlier in patients on long-term antibiotics (Honig *et al.*, 1988). In our study, the association of candidiasis and antibiotics was found in 66.67 % of cases. *C. albicans* is by far the commonest species reported by most workers, in OPC as well as in skin infections. It has been reported in 64 % of children with oncohaematological diseases, and is followed by *Candida krusei*, in a study in Chennai (Kumar *et al.*, 2005). In a recent study, in Brazilian children with HIV infection, *C. albicans* was the commonest species isolated. An increase in non-*albicans* species of *Candida* in children being treated with protease inhibitors was reported by Melo *et al.* (2009). In South African institutionalized HIV-infected children, *C. albicans* (40.4 %) and *C. dubliniensis* (26.3 %) were the most frequently isolated species in OPC (Blignaut, 2007). In our study, though *C. albicans* still was the commonest species isolated, *C. dubliniensis* was the second most predominant species in causing skin candidiasis and OPC. *C. dubliniensis* is being increasingly reported in non-HIV situations and at sites other than oropharyngeal (Alvarez *et al.*, 2009). In our study, 4 of the total 15 strains of *C. dubliniensis* were from cutaneous sites. To the best of our knowledge, the present report of *C. dubliniensis* causing CMC is the first record of its occurrence in India.

Tobacco agar, originally described as the medium of choice to differentiate *Cryptococccus* spp. colonies from those of *Candida* spp. is now being used increasingly to differentiate *C. dubliniensis* from *C. albicans* (Tendolkar *et al.* 2003). Tobacco agar provides a reliable test to differentiate *C. dubliniensis* from *C. albicans* and provides a presumptive identification of *C. dubliniensis*. Akgül & Cerikçioğlu (2009) have recently reported that the reliability of growth on tobacco agar makes it an important test to provide presumptive identification of *C. dubliniensis*.

In neonates in a tertiary care hospital, oral lesions due to *Candida* spp. were found to be more common than cutaneous lesions. In toddlers, on the other hand, cutaneous candidiasis predominated. Broad-spectrum antibiotic usage was a common predisposing factor. Lingual and perineal sites were more frequently affected. *C. albicans* was the commonest cause followed by *C. dubliniensis*; the latter species was also isolated from CMC. Thus a novel and potentially troublesome fungus like *C. dubliniensis* also appears to share a substantial quantum of infection in children.

ACKNOWLEDGEMENTS

We wish to acknowledge the help extended by Dr Arunaloke Chakrabarti and his team in confirming the identity of the isolates submitted to the PGIMER, Chandigarh, India.

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