Colonization of neonate skin by *Malassezia* species: Relationship with neonatal cephalic pustulosis

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Background: Colonization of neonate skin by *Malassezia* species and their causative role in neonatal cephalic pustulosis is unclear.

Objectives: We sought to determine the skin colonization by *Malassezia* in healthy newborns, and to investigate its association with neonatal cephalic pustulosis.

Methods: Samples for *Malassezia* colonization were taken from cheeks and scalps of 104 neonates between 24 and 72 hours after birth, and again 2 or 4 weeks later. Pustules were sampled with concomitant nonlesional skin cultures if neonatal cephalic pustulosis was diagnosed.

Results: *Malassezia* colonization increased significantly with age of the neonate (5% at the first week, 30% at 2-4 weeks). In all, 26 patients were given the diagnosis of neonatal cephalic pustulosis during follow-up. No correlation was found between the severity of the disease and *Malassezia* isolation. Skin colonization of patients with neonatal cephalic pustulosis (20.8%) was not higher than colonization of healthy newborns (37%).

Limitations: Not all of the neonates were examined by the authors at the second visit.

Conclusions: *Malassezia* colonization increases after the first week of life. No correlation was found between neonatal cephalic pustulosis and *Malassezia*. (J Am Acad Dermatol 2007;57:1012-8.)

Malassezia are lipophilic members of the microflora of human skin and that of many other warm-blooded animals. The causative role of these organisms in pityriasis versicolor has been well documented since 1846, when they were first described in the lesions of this infection. They are known to be associated with many of the diseases affecting the skin, and potentially fatal venous catheter-related fungemia in premature neonates, particularly in those receiving intravenous lipid emulsion.[1,2]

Newborn skin is sterile at birth, but resident flora may be detected within the first hours of life. Both the age at which neonates become infected and the route by which healthy neonate skin is colonized with *Malassezia* is unclear. Most of the studies about this subject have included small numbers of children with wide ranges of age, have not used suitable isolation media, and have generally been performed in intensive care departments in neonates with numerous systemic problems. As such, these studies documented results that differed considerably, ranging from no colonization by *Malassezia* to substantial colonization.[3-7]

Aractingi et al[8] first described neonatal cephalic pustulosis in 1991, in a 20-day-old boy who developed a pustular eruption of his face, neck, and scalp. Diagnostic criteria of the disease were described by Rapelanoro et al[9] as age at onset, cephalic localization, direct microscopic examination that is positive for *Malassezia*, elimination of other causes of neonatal pustulosis, and positive response to

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Supported by Nobel and İlsan İltaş Drug Companies and Hacettepe Scientific Research Unit.
Conflicts of interest: None declared.
Presented as a free communication at the 13th Congress of European Academy of Dermatology and Venereology, Florence, Italy, on November 17-21, 2004.
Accepted for publication February 20, 2007.
Reprints not available from the authors.
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Published online September 24, 2007.
0190-9622/$32.00

doi:10.1016/j.jaad.2007.02.030

topical ketaconazole therapy. The causative role of *Malassezia* in this common neonatal dermatosis is unclear, because none of the authors has been able to find the causative agent in all of his or her patients.

The aim of this study was to observe skin colonization by *Malassezia* species in healthy newborns, to evaluate the factors affecting the colonization, and to discern its relationship with neonatal cephalic pustulosis.

METHODS

First visit

We investigated 104 healthy newborns, 24 to 72 hours after birth, who were seen in our maternity department and research hospital between October and December 2003. Demographic characteristics of the newborns, antimicrobial drug use, and family history of both seborrheic skin and atopy were recorded. Complete skin examinations of the newborns were performed and samples for colonization were taken from scalps and cheeks. Mothers were informed about the clinical appearance of neonatal cephalic pustulosis and were then asked to call the researchers if pustular lesions had appeared. In addition, mothers were called every week to question them about their neonates' skin lesions and to encourage their continuing cooperation in the study.

The study protocol was approved by the institutional review board, and all parents gave written informed consent before study-related procedures were done.

Second visit

Parents were called every week for 4 weeks and asked whether their babies had pustular lesions or not. Whenever a pustular lesion was recognized, the mothers were asked to come to our clinic. If they were not able to come to the hospital, they were visited in their homes. Mothers of the babies who had no pustular lesions were invited for a second hospital visit 2 to 4 weeks after birth. In all, 50 of 104 neonates, whether they had pustulosis or not, were clinically examined at the second visit. The rest of 54 neonates had only telephone calls because their mothers were not willing to come to the hospital. The type of feeding and skin care each child received was recorded and complete skin examinations were performed. Patients with nonfollicular pustules located on the face and neck before the age of 1 month were given the diagnosis of neonatal cephalic pustulosis after the elimination of other causes of neonatal pustulosis by clinical and laboratory investigations. Tzanck smear, direct microscopy of potassium hydroxide preparations, Gram stains, and bacterial cultures had been performed in case of suspicious clinical conditions, such as erythema toxicum neonatarum, bacterial and candidal folliculitis, and herpes simplex infections. Clinical stages of neonatal cephalic pustulosis were reported as mild (≤ 5 pustules), moderate (6-10 pustules), and severe (>10 pustules). Pustules were sampled with concomitant nonlesional skin cultures (scalp and cheek). Neonates without pustules were also sampled on the cheek and scalp 2 to 4 weeks after birth.

Sampling and microbiological evaluation

Each colonization sample from newborns was taken by using a sterile cotton swab moistened with saline solution, which was applied to a 4-cm^2 area of the cheek and scalp, both of which had not been disinfected. Pustule samples were obtained using a microlance or a sterile moistened skin swab.

All samples were cultivated on modified Dixon agar (36 g malt extract, 6 g peptone, 20 g desiccated ox-bile, 10 mL Tween 40, 2 mL glycerol, 2 mL oleic acid, 12 g agar, 1 L distilled water, pH: 6) and Sabouraud's dextrose agar culture media, at the patient's side, to minimize contamination and to obtain maximum positive culture results with this fastidious yeast. The agar plates were incubated at 32°C and evaluated for the existence of growth every day for a total of 7 days. Identification of isolated yeast was based on morphologic and physiologic tests, namely Tween assimilation profiles, catalase reaction, and the ability to grow at 40°C.[10-13]

Catalase test. One drop of hydrogen peroxide solution was applied onto a yeast colony, which was on a glass slide. Production of gas bubbles was considered a positive catalase reaction.

Use of Tween 20, 40, 60, and 80. *Malassezia* yeast suspensions were prepared in Sabouraud's dextrose broth, which were adjusted to McFarland No. 2 turbidity, mixed with Sabouraud's dextrose agar, and poured into plates. After solidification, each plate was divided into 4 sections, and wells 3 mm in diameter were opened in the middle of each section. A total of 5 μL of each Tween compound (Tween 20, 40, 60, and 80) was applied into the wells. The plates were incubated at 32°C for 7 days and were examined each day for the existence of any growth around the wells that contained Tween compounds.

Growth at 40°C. Isolates were inoculated onto modified Dixon agar and incubated at 40°C for at least 2 days and then examined for the existence of any growth.

The isolates were identified according to the criteria defined by Guillot et al[11] and shown in Fig 1.