Case Report

Pityriasis versicolor with a unique clinical appearance

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We experienced an atypical case of pityriasis versicolor with a unique clinical appearance and undescribed mycological features. Although Malassezia sp. was cultured from the keratotic material, the fungal elements observed in the material were not readily identified as Malassezia. The diagnosis was established with the aid of immunohistochemical and ultrastructural studies with the aetiological agent being identified as M. globosa.

Keywords Malassezia, Malassezia globosa, pityriasis versicolor

Introduction

Pityriasis versicolor is a superficial fungus infection caused by Malassezia furfur. Clinically, macular, erythematous, pigmented or hypopigmented lesions with fine scaling are present [1]. The diagnosis of this disease is established by detection of the pathognomonic fungal elements in a KOH preparation of skin scrapings [1]. The present paper describes an atypical case of pityriasis versicolor with a unique clinical appearance as well as undescribed mycological features of the fungus.

Case report

A 62-year-old Japanese female received cholecystectomy and noticed yellow-brown macular lesions on her chest. Four, well-demarcated, hyperkeratotic, unevenly yellow to brown-coloured, small macular exanthemas were observed on the middle part of her chest (Fig. 1). There was no subjective symptom.

Histopathological examinations

The hyperkeratotic skin lesion was biopsied. By haematoxylin–eosin stain, the horny layer showed marked thickening, partially with keratotic plugs in hair follicular pores (Fig. 2a), and contained numerous yeast cells and hyphae which were basophilic. The epidermis, except for the horny layer, showed no particular pathological findings. In the upper dermis, infiltration of a few mononuclear cells was seen. By methenamine–silver nitrate stain, many globose to subglobose fungal cells

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Histological findings. (a) Horny layer shows marked thickening (haematoxylin–eosin stain, × 18). (b) Many globose fungal cells of varying sizes and septate hyphae are seen among the horny cells in the entire horny layer (methenamine–silver nitrate stain, × 700).

Fig. 2

Mycological examinations

The keratotic material was removed and mounted in KOH and Parker ink. The fungal elements were composed mainly of two types of elements: globose to subglobose yeast cells and long septate hyphae. The yeast showed considerable variation in size (3–10 µm in diameter) and were crowded in the keratotic material (Fig. 3a). The hyphae varied from 1·6 to 4 µm in diameter and 40 to 70 µm lengths, and were mixed with the yeast cells (Fig. 3b). A few hyphae were approximately 15 µm in length.

The keratotic material yielded creamy colonies on Sabouraud glucose agar without cycloheximide when covered with a layer of olive oil at 24°C. By microscopic observation, the colonies consisted of globose yeast cells, varying in size from 1·4 to 4·3 µm in diameter, and occasionally with buds which were connected by a narrow base. No hyphae were seen. The morphology of the cultured fungus corresponded to the published descriptions of *Pityrosporum orbiculare* [2] which was first described by Gordon in 1951 [3] and is now known as *Malassezia globosa* [4].

Immunohistochemical examinations

Although *Malassezia* was obtained from the keratotic material, the morphological features of the fungus seen in the KOH and Parker ink preparation hardly seemed to coincide with those reported in the literature [1]. Namely, it is well known that the globose to subglobose yeast cells of *Malassezia* typically range from 2–8 µm in diameter [1]. The cells in culture show considerable variety in size [2]. The hyphae in pityriasis versicolor

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scales are usually short (10–40 μm) in length [1,5]. Furthermore, the organism in scrapings is easily stained deep blue with Parker ink [5] (Table 1). On the basis of our mycological findings, we were not certain whether the fungal elements observed in the skin lesion were identical to Malassezia. To identify the fungal elements, immunohistochemical examinations were carried out using specific antifungal antibodies; anti-M. globosa, anti-Candida albicans, anti-Aspergillus fumigatus, anti-Fusarium anthropi and anti-Trichosporon beigelii sera. The anti-M. globosa serum was produced by G. Midgley. Cultured cells of M. globosa were washed three times with sterile PBS and collected by centrifugation at 4000 g for 10 min at room temperature. The packed cells were combined with an equal volume of ballotini (80 mesh, Difco, East Molesey, UK) and suspended in sufficient PBS to fill the chamber in a Bead Beater (Biospec Products, OK, USA). This chamber was placed in a reservoir of ice and water and the suspension ground for four periods of 1 min separated by an interval of 1 min. The suspension of broken cells was centrifuged at 20000 g for 1 h at 4°C and the supernatant saved. This cytoplasmic extract (CE) was dialysed in Visking tubing (Scientific Industries International Inc, Loughborough, UK) against distilled water at 4°C for 24 h and passed through an assembly of filters (Millipore, Watford, UK), AP 25 0.1 to 0.9 μm. The cell wall was composed of electron-dense, multilayered and fibrillar structures. The outer surface of the cell wall was smooth, while its inner surface showed a sawtooth-like image in the sections. Rabbits were immunized with this purified extract combined with Freund’s complete adjuvant (Difco, East Molesey, UK). The obtained anti-CE sera with titres of 1:32 or higher were pooled and stored at −40°C. By immunocytochemistry, the antisera reacted with the cultured cells of M. globosa but not with those of C. albicans, A. fumigatus or Trichophyton mentagrophytes var. interdigitale. The generic specificity of the other antisera used were previously established by an absorption and dilution technique [6,7], although it was confirmed that only anti-A. fumigatus serum further reacted with Trichophyton rubrum.

Deparaffinized sections of the biopsied specimen were immunohistochemically stained by the fluorescent antibody [8] and/or indirect immunoperoxidase methods [6]. By both methods, all the fungal elements in the tissue preparations were positively stained with the anti-M. globosa antibody. Fungal elements were not stained by any of the other reagents.

**Table 1** Morphological comparison between M. globosa and the fungal cells in the horny layer in the present case

<table>
<thead>
<tr>
<th>M. globosa cells</th>
<th>Fungal cells in the horny layer in the present case</th>
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</thead>
<tbody>
<tr>
<td>Globose cells</td>
<td>Size: 2–8 μm, uniform or varying†</td>
</tr>
<tr>
<td>Thickness of cell wall</td>
<td>0–1–0–2 μm‡</td>
</tr>
<tr>
<td>Length of hyphae</td>
<td>Short (10–40 μm§)</td>
</tr>
<tr>
<td>Stainability with Parker ink</td>
<td>Easily stained¶</td>
</tr>
<tr>
<td></td>
<td>Thickness of cell wall: 0–1–0–9 μm</td>
</tr>
<tr>
<td></td>
<td>Length of hyphae: Long (40–70 μm)</td>
</tr>
<tr>
<td></td>
<td>Stainability with Parker ink: Hardly stained</td>
</tr>
</tbody>
</table>

†The sizes of the cells are relatively uniform in the horny layer in vivo (personal communication from Dr Y. Soh), while they vary in culture [2]. ‡This value is quoted from [10]. §This finding is quoted from [1]. ¶This finding is quoted from [5].

Ultrastructural examination

A part of the biopsied specimen was double-fixed in glutaraldehyde and osmium tetroxide, dehydrated and embedded in Epon 812. Ultrathin sections were double-stained with 2% uranyl acetate and Reynolds’ lead citrate [9], and observed by JEM 1010B transmission electron microscope (TEM).

Except for the deepest of a few cell layers of the horny layer, the horny cells showed loosely packed keratin filaments in the cytoplasm and lacked desmosomal contacts. Fungal elements were seen in the intercellular space as well as in the cytoplasm of the horny cells. The cell wall of the fungal cells varied from 0.1 to 0.9 μm. The cell wall was composed of electron-dense, multilayered and fibrillar structures. The outer surface of the cell wall was smooth, while its inner surface showed a sawtooth-like image in the sections.

Fluorescent dye (Blankophore-P) stain

To determine the viability of the fungal elements in tissue, the fluorescent dye Blankophore-P (Bayer Co., Köln, Germany) was applied. Deparaffinized sections of skin were stained with Blankophore-P [11], and then the
Fig. 4 Ultrastructural findings of the horny layer. The horny cells (K) show loosely packed keratin filaments in the cytoplasm and lack desmosomal contacts. Fungal cells (F) in the horny layer possess protrusions (arrowhead) from the inner surface of their cell wall (uranyl acetate–lead citrate stain, \( \times 10000 \)).

fluorescence of 20 randomly chosen yeast cells was measured. As a control, a biopsy of normal skin obtained from a healthy individual was similarly processed and the fluorescence of 10 randomly chosen saprophytic yeast cells of *Malassezia* in the follicular infundibulum was measured. The intensity of fluorescence in the present case (average value = 765 ± 58) was about 90% of that of the control (average value = 848 ± 50). A significant difference between the two values was confirmed by paired \( t \)-test (\( P < 0.005 \)), suggesting a significant decline of viability of the fungal cells in the present case.

**Discussion**

In the present case, a well-demarcated, hyperkeratotic lesion was observed on the chest. Although *Malassezia* was obtained and cultured from the keratotic material, the fungal elements observed in the keratotic material appeared atypical. However, by immunohistochemical examination, the fungal elements in the hypertrophic horny layer were shown to have the same antigenic property as that of the *M. globosa* extract. Moreover, by TEM, the fungal elements in the horny layer possessed protrusions from the inner surface of their cell wall, which are one of the ultrastructural characteristics of the genus *Malassezia* [2,10]. The vital fluorescent dye demonstrated a definite decline of viability of the fungal elements in the horny layer. Therefore, there is a possibility that the morphology of the fungal elements, such as variation in size of the yeast cells and hyphae in the horny layer, might be attributable to the extremely thickened horny layer or to their low viability.

**References**