

OCCURRENCE OF *PITYROSPORUM OVALE/ORBICULARE* IN SEBORRHEIC KERATOSIS AND MELANOCYTIC NAEVUS

BRÁZDIL J., BUČEK J., FEIT J.

2nd Department of Pathological Anatomy, Faculty of Medicine, Masaryk University, Brno

Abstract

Bioptic specimens from 224 melanocytic naevus lesions and 121 seborrheic keratosis lesions were stained with hematoxylin-eosin and examined under the light microscope. *Pityrosporum ovale/orbiculare* (*Malassezia furfur*) was found in 35% of melanocytic naevi and in 36% of seborrheic keratoses. The finding of *Pityrosporum ovale/orbiculare* was confirmed by PAS (periodic acid shift), methenamide silver staining and electron microscopy. The presence, number and location of *Pityrosporum ovale/orbiculare* microorganisms, the presence and degree of papillomatosis, the presence and degree of hyperkeratosis, lesion diameters and patients' age were recorded. The relation of papillomatosis presence to the finding of *Pityrosporum ovale/orbiculare* in melanocytic naevus and seborrheic keratosis was found. In 29 % of melanocytic naevi and 59 % of seborrheic keratoses, papillomatosis was present but not *Pityrosporum ovale/orbiculare* while, in 54 % of melanocytic naevi and 81 % of seborrheic keratoses, both papillomatosis and *Pityrosporum ovale/orbiculare* were demonstrated.

Keywords

Pityrosporum ovale/orbiculare, melanocytic naevus, seborrheic keratosis, papillomatosis, hyperkeratosis

INTRODUCTION

Pityrosporum ovale, *Pityrosporum orbiculare*, and *Malassezia furfur* were discovered independently, but later it was found that they represented different evolutionary stages of the same microorganism (1).

Pityrosporum ovale/orbiculare (PO) is a common skin saprophyte (2, 3). It is mainly found in seborrheic regions. It is also known as an opportunistic pathogen involved in pityriasis versicolor, seborrheic dermatitis, *Pityrosporum* folliculitis, confluent and reticulated papillomatosis (Gougerot-Carteaud) and some kinds of atopic dermatitis (4, 5, 6, 7, 8). Systemic infections caused by PO have also been described in patients with defects of the immune system (6). PO is also found in other skin lesions, especially in papillomatous lesions. Since only two studies concerned with this phenomenon have recently been published (9, 10), in this paper we describe the occurrence of PO in melanocytic naevi (MN) and

seborrheic keratoses (SK) and discuss the role of PO in the pathology and development of papillomatosis.

MATERIALS AND METHODS

A total of 224 MN and 121 SK specimens of bioptic materials provided by the 2nd Department of Pathological Anatomy, Faculty of Medicine, Masaryk University in Brno, were examined in the first quarter of 1998. The tissues were fixed with formalin, embedded in paraffin and stained with haematoxylin-eosin (HE). The following semiquantitative evaluation of histopathological features was used:

1. Frequency of PO: 0, none; +, rare (PO was found only in one place in the lesion and/or in about 50 cells); ++, intermediate; +++, massive (PO was found in more than 3 places in the lesion and/or in more than 200 cells).

2. Location of PO in the lesion: hyperkeratotic hair follicle (F) or hyperkeratotic lamellae on the epidermal surface (P).

3. Presence and degree of papillomatosis (magnification, x 3.2): 0, none; +, mild (up to 1/3 of the viewing field); ++, intermediate (1/3 to 2/3 of the viewing field); +++, pronounced (more than 2/3 of viewing field). (*Fig. 1*)

4. Presence and degree of hyperkeratosis (magnification, x 10): 0, none; +, mild (up to 1/3 of the viewing field); ++, intermediate (from 1/3 to 2/3 of the viewing field); +++, pronounced (more than 2/3 of the viewing field). (*Fig. 2*)

5. Diameter of lesions: +, up to 3 mm; ++, 3 to 6 mm; +++, 6 to 9 mm; +++++, larger than 9 mm.

Age of the patients at the time of biopsy was recorded.

Several specimens were stained using the periodic acid shifts (PAS) technique. Two MN specimens were examined by electron microscopy. For this observation, the material was fixed with 3% glutaraldehyde, embedded in Epon and stained with 2% osmium. One specimen was impregnated with silver according to *Grocott (12)*.

The results were statistically evaluated using the test for the difference of two relative values (*11*).

RESULTS

Pityrosporum ovale microorganisms were present in groups or layers between hyperkeratotic lamellae of the epidermis or in the orifices of hyperkeratotic hair follicles (*Fig. 3*). They were seen as violet, circular or oval structures in HE stained preparations (*Fig. 4*). No PO hyphae were found. Positive results were obtained by the PAS and silver impregnation methods. Thick, dense walls with typical lace indentations on the cytoplasmatic side and less dense homogeneous contents were specific features of PO presence observed by electron microscopy.

The 224 MN lesions examined were compound or intradermal types. PO was found in 35% of them (78 cases). Out of these, 52% (41 cases) showed rare, 26% (20 cases) intermediate and 22% (17 cases) massive presence of PO.

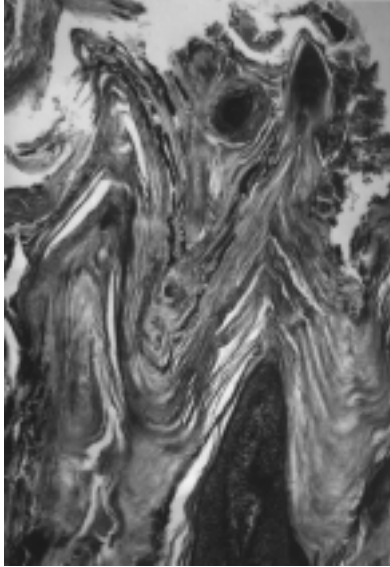


Fig. 1

Pronounced hyperkeratosis in seborrheic keratosis with intermediate papillomatosis; HE. Magnification, x 40.

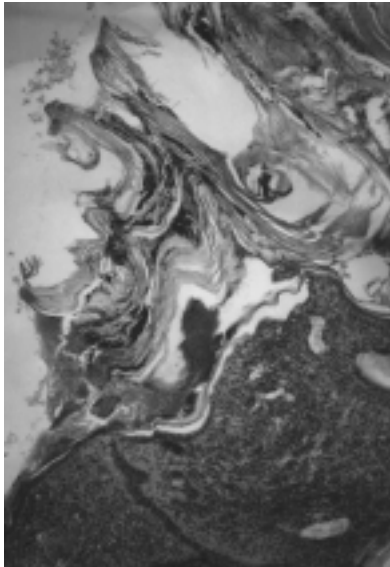


Fig. 2

Intermediate hyperkeratosis in melanocytic naevus with mild papillomatosis; HE. Magnification, x 40.

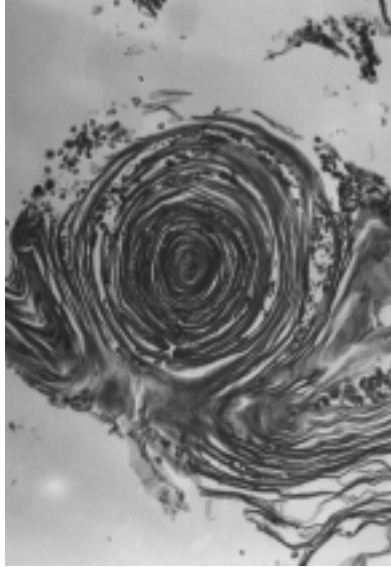


Fig. 3
Groups of *Pityrosporum ovale/orbiculare* between hyperkeratotic lamellae of the epidermis;
HE. Magnification, x 150.

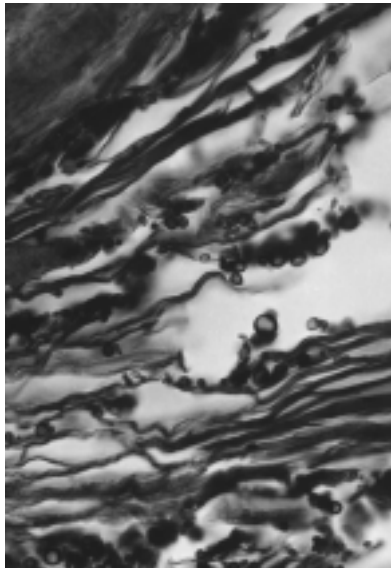


Fig. 4
Detail of a *P. ovale/orbiculare* cell; HE. Magnification, x 400.

Papillomatosis was found in 38% of all MN lesions. In MN lesions without PO presence (146 cases), papillomatosis was found in 29% (42 cases), while in MN lesions positive for PO (78 cases) the occurrence of papillomatosis was 54% (42 cases). This difference was significant ($P<0.05$). The relation between the frequency of papillomatosis and the presence of PO is shown in *Table 1*.

PO was found in 50% of the cases of papillomatous MN.

Papillomatosis was found in 18% of the cases in which PO was present in hair follicles; these cases of papillomatosis were classified as mild. The occurrence of papillomatosis was observed in 68% of the cases with hyperkeratotic lamellae on the surface of epidermis and all forms of papillomatosis, i.e., mild, moderate and severe, were present. This difference in the frequency of papillomatosis in relation to the location of PO was significant ($P<0.05$).

Hyperkeratosis was present in all the MN cases (100%) in which there were positive PO findings, but only in 71% of the MN cases without the presence of PO. The presence of PO in relation to the degree of hyperkeratosis is presented in *Table 2*.

PO was found in about 35% of MN lesions irrespective of their extent. There was no relation between PO presence and the age of patients with MN.

Out of 121 SK lesions examined, PO was found in 36% (43 cases). In this material, PO presence was rare in 40%, intermediate in 26% (11 cases) and massive in 34% (15 cases) of the cases.

The occurrence of papillomatosis was found in 59% (46 out of 78) of the SK cases where PO was absent and in 81% (35 out of 43) of the cases where PO was present. This difference was significant ($P<0.05$). The relationship between the occurrence of papillomatosis and the degree of PO presence is shown in *Table 3*. PO was found in 43% of the papillomatous SK cases. There was no case in which the presence of PO was limited to hair follicles.

Table 1
Presence of papillomatosis in relation to the amount of PO in melanocytic naevus

Presence of PO	Number of specimens	Presence of papillomatoses	
		Absolute numbers	%
Rare	41	18	44
Intermediate	20	13	65
Massive	17	11	65
Total presence	78	42	54
None	146	42	29

Table 2
Presence of PO in relation to the degree of hyperkeratosis in melanocytic naevus

Degree of hyperkeratosis	Number of specimens	Presence of PO cells	
		Absolute numbers	%
Mild	150	59	39
Intermediate	24	15	62
Pronounced	8	4	50

Table 3
Presence of papillomatosis in relation to the amount of PO in seborrheic keratosis

Presence of PO	Number of specimens	Presence of papillomatoses	
		Absolute numbers	%
Rare	17	13	76
Intermediate	11	9	82
Massive	15	13	87
Total presence	43	35	81
None	78	46	59

Table 4
Presence of PO in relation to the degree of hyperkeratosis in seborrheic keratosis

Degree of hyperkeratosis	Number of specimens	Presence of PO	
		Absolute numbers	%
Mild	59	12	20
Intermediate	34	19	56
Pronounced	28	12	43

Hyperkeratosis was present in all SK cases. The presence of PO in relation to the degree of hyperkeratosis is presented in *Table 4*.

No relation was found between the presence of PO and the lesion size in SK.

In relation to age, PO was found in 50% of the materials obtained from patients between age 36 and 60 years and in 28% of those from patients over 60 years. This difference was significant ($P < 0.05$).

DISCUSSION

The incidence of PO on clinically normal skin has been reported to be 97% in the scalp, 80% on the back and 92% on the breast (2). These findings were completed with cultivation of the microorganisms, but this did not increase sensitivity of PO detection. Other studies (10, 12) as well as our study showed much lower incidence.

The presence of PO was observed by light microscopy in 35% of the 224 cases of MN examined in our study. A similar study (12) has shown about the same results (40%, 123 cases). In a study on papillomatous MN, PO findings were present in 62% of the lesions examined (200 cases) (10). In our observations, PO was present in 50% (84) of the papillomatous MN cases. This was different from the results obtained by *Bourlond et al.* who found PO in 85% (13) of their cases (9).

The occurrence of papillomatosis was observed in 29% of the specimens in which PO was absent, while it was seen in 54% of the materials with positive findings of PO. Mild forms of papillomatous MN showed rare findings of PO, while moderate and severe forms of papillomatosis were associated with intermediate or massive PO findings. Papillomatosis was found in 18% of MN cases where PO was found in hair follicles and was always manifested as a mild form.

Hyperkeratosis was present in 100% of the MN cases, regardless of their extent, that had positive findings of PO. No case in which PO was present in MN without hyperkeratosis was found. There was no relation between lesion size and the degree of PO presence. There was a relationship between the PO presence and patients' age: in older patients, there were fewer PO findings (13), which has been explained by a decrease in skin lipid substances occurring with increasing age. Similar results were obtained in our study: the presence of PO was recorded in 41% of the cases in the age group 26–50 years, and in 38% of the cases in the age group over 50.

PO was found in 36% of 121 cases of SK in our study. This finding confirms the results of other authors: 40% of 100 cases (13) and 45% of 64 cases (9). There are again differences in PO findings (9): 74% of 36 cases.

The occurrence of papillomatosis was observed in 59% (46 cases) of the SK cases in which no PO was found (78 cases) and in 81% (35 cases) of the SK cases with positive PO findings (43 cases).

PO was found in 43% of 81 cases in papillomatous SK. We could find a relationship between the increasing amount of PO and the occurrence of papillomatosis (*Table 3*).

Hyperkeratosis is a basic diagnostic sign for SK and was 'therefore' present in 100% of the lesions.

No relationship between the presence and amount of PO and the size of the lesion in SK was found.

A decrease in PO occurrence in relation to increasing age of the patients is more evident than in MN: 50% of the patients between 36 and 60 years and 28% of the patients over 60. This finding is interesting mainly because SK is a lesion found in older patients.

The results of our work show a relationship between the presence of papillomatosis in MN and SK, and the finding of PO in these lesions. Hyperkeratosis is a necessary prerequisite for the presence of PO in these lesions. The occurrence of papillomatosis in MN is more frequent in cases with superficial location of PO than in those with follicular location. The findings of PO in SK in patients 36 to 60 years old are more frequent than in patients older than 60 years.

PO was found only on the surface of the epidermis in all lesions examined for MN or SK. PO was never seen to be located intracellularly. In all the cases examined, PO was present in the oval form. No hyphal forms of PO were found, though a rare finding of hyphae is mentioned (9). No connection was found between the presence of PO and inflammatory changes in the lesion (10).

Papillomatosis offers favourable growth conditions for PO, especially in seborrheic regions, where the amount of lipids is higher on the surface of the epidermis. This location is most convenient for lipophilic PO.

Another way of explaining the relationship between the presence of PO and the occurrence and size of papillomatosis in MN and SK is to carry out a study with more cases, to study other hyperkeratotic lesions with papillomatosis, and to compare the presence of PO in seborrheic and other regions. A clinical study of the antimycotic treatment of these lesions and its clinical and morphological evaluation may also produce interesting results.

Brázdil J., Buček J., Feit J.

VÝSKYT *PITYROSPORUM OVALE/ORBICULARE* U SEBOROICKÉ KERATOSY A MELANOCYTÁRNÍHO NĚVU

S o u h r n

224 melanocytárních nĚvů a 121 seboroických keratos bylo vyšetřeno ve světelném mikroskopu při barvení hematoxylin-eosin. *Pityrosporum ovale/orbiculare* (*Malassezia furfur*) bylo nalezeno v 35% melanocytárních nĚvů a v 36% seboroických keratos. Nález *Pityrosporum ovale/orbiculare* byl verifikován histologickými metodami a elektronovou mikroskopií. Ve vztahu k nálezu, množství a lokalisaci *Pityrosporum ovale/orbiculare* u dané lese jsme dále hodnotili přítomnost a velikost papilomatosy, přítomnost a velikost hyperkeratosy, velikost (průměr) lese a věk pacienta v době odběru biopsie. V naší práci byl nalezen vztah mezi přítomností

papilomatosa u melanocytárního névu a seboroické keratosy a nálezem *Pityrosporum ovale/orbiculare* u těchto lesí. V souboru bez nálezu *Pityrosporum ovale/orbiculare* se papillomatosa vyskytuje u melanocytárních névů ve 29% a u seboroických keratos v 59%. Naproti tomu ve skupině s pozitivním nálezem *Pityrosporum ovale/orbiculare* je papillomatosa přítomna u melanocytárních névů v 54% a u seboroických keratos v 81%.

REFERENCES

1. *Evans EGV*. Houby. Fungi. In: Greenwood D, Slack R, Peutherer J et al. Lékařská mikrobiologie [Medical Microbiology]. Praha: Grada, 1999 563–83.
2. *Roberts SOB*. *Pityrosporum orbiculare*: incidence and distribution on clinically normal skin. Br J Derm 1969 81: 264–9.
3. *Faergemann J, Alz R, Maibach HI*. Quantitative variations in distribution of *Pityrosporum orbiculare* on clinically normal skin. Acta Derm Venerol 1983 63: 346–8.
4. *Faergemann J*. *Pityrosporum* infections. J Am Acad Dermatol 1994 31: 18–20.
5. *Faergemann J*. *Pityrosporum ovale* and skin diseases. Keio J Med 1993 42: 91–4.
6. *Faergemann J, Maibach HI*. The *Pityrosporon* yeasts. Their role as pathogens. Int J Dermatol 1984 23: 463–5.
7. *Broberg A, Faergemann J*. A case of confluent and reticulated papillomatosis (Gougerot-Carteaud) with an unusual location. Acta Derm Venerol 1988 68: 158–60.
8. *Nordby CA, Mitchell AJ*. Confluent and reticulated papillomatosis responsive to selenium sulfidé. Int J Dermatol 1986 25: 194–9.
9. *Bourlond A, Votion V, Armijo F, Minne G*. *Pityrosporum ovale* dans les lesions keratosiques des zones seborrheiques. [Pityrosporum ovale in keratotic lesions of seborrheic regions]. Ann Dermatol Venerol 1984 111: 1081–5.
10. *Mittag H, Rupec M*. *Pityrosporum ovale/orbiculare* in papillomatous nevus cell nevi. Dermatologica 1991 183: 191–6.
11. *Reisenauer R*. Metody matematické statistiky a jejich aplikace. [Methods of Mathematical Statistics and their Application]. Praha: SNTL, 1970: 239pp.
12. *Schnborn Ch, Bolck F*. Histologischer Pilzbefall in Hauttumoren. Histological finding of yeasts in skin tumors. Mycose 1988 31: 411–7
13. *Bergbrant IM, Faergemann J*. Variations of *Pityrosporum orbiculare* in middle-aged and elderly individuals. Acta Derm Venerol 1988 68: 537–40