

症 例

Morphological detection of fungi in dental caries and marginal periodontitis : Report of two cases

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Abstract : The presence of fungi was histologically detected in marginal periodontitic and dental carious lesions in two cases. Two teeth with dental caries and marginal periodontitis were extracted and fixed with formalin. These teeth were decalcified and prepared for histological examinations. Grocott stain disclosed the brown pseudohyphae of *Candida albicans* with plaques in carious lesions and on the surface of dental roots with periodontitis. These morphological findings showed that *C. albicans* was present in not only marginal periodontitis but also in dental caries lesion in some conditions.

Key words : Dental caries ; periodontitis ; fungi infection ; *Candida albicans*

Introduction

Periodontitis and dental caries are considered to be infectious diseases from a bacteriological and immunological perspective¹⁾. The causative factor for dental caries is generally considered to be the presence of *Streptococcus mutans* in the dental plaque²⁾. Periodontitis is attributed to gram-negative anaerobic bacilli as *Porphyromonas gingivalis* and *Actinobacillus*

*actinomycetemcomitans*¹⁾. More than four hundred species of oral flora have been detected in the oral cavity^{1, 3)}. Fungi were also found less frequently^{4~11)}. On the other hand, the morphological features of fungi in dental carious or periodontal lesions have so far not been reported except a few reports concerning periapical periodontitis^{10, 12, 13)} and a recent report of marginal periodontitis¹⁴⁾. Thus, we performed a morphological examination of dental plaque in dental

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caries and marginal periodontitis, and the following findings were obtained.

Case 1

The patient was a 47-year-old female with the chief complaint of severe mobility of the mandibular left cuspid and hypersensitivity to cold water of it. An X-ray photograph indicated that the mandibular left cuspid with a carious lesion could not be saved because of severe marginal periodontitis. Abundant dental plaque appeared from neck to the root and a histopathological examination of the tooth was conducted.

Case 2

The patient was a 71-year-old female who complained of mobility of the maxillary and mandibular right first premolars and a strong odor emanating. Although defects were present from the second premolar to the second molar on both sides of the mandible, the patient had not worn a partial denture for over three years. The mandibular right first premolar was extracted.

Informed consent was obtained from patients of case 1 and case 2, whose teeth were used for histological study. The extracted teeth for treatment of periodontitis were fixed in 10% neutral phosphate-buffered formalin for three days. The teeth were then immersed in Plank-Rychlo decalcifying solution for a few days, then they were embedded in paraffin wax, and cut into 4- μ m thick sections, deparaffinized, and dehydrated in an ethanol series. Periodic acid-Shiff (PAS) and Grocott-Gomori methenamine-silver (Grocott) stain for fungi was then performed.

Grocott stain indicated the presence of dark-brown pseudohyphae of fungi within

the plaque found in the carious cavity (Fig. 1 A, C) and on the surface of the root of tooth of case 1 (Fig. 1 B, D). PAS stain also showed fungal pseudohyphae. Since the morphology of pseudohyphae with a constricted septum resembled the typical appearance of *Candida albicans* seen in control chronic gastric ulcer (Fig. 1 E). Pseudohyphae of *C. albicans* were also observed in the periodontium (Fig. 1 F) in case 2.

Discussion

The present cases showed the histopathological features of *C. albicans* in addition to other microbes in carious cavities and on the root in periodontal deep pockets. Recently, there has been increasing interest in the presence and role of yeast as pathogen of oral diseases²⁾. Yeast constitutes a part of the normal microbial flora of the oral cavity²⁾, and *C. albicans* is the most common yeast isolated from the oral cavity of either the patients with^{4, 8)} or without^{5, 14)} periodontitis, and medically compromised adults^{10, 11, 15)}. *C. albicans* has been isolated by culture techniques from dental plaque of dental carious lesion⁸⁾, subgingival flora^{4~6)}, infected root canals of apical periodontitis^{9, 10)}, and periodontal pockets^{4, 7)}. This microorganism can grow structurally by budding a unicellular form or in hyphal form, and shows interconversion in different conditions^{10, 13, 16)}. To date, a few histological examinations regarding the presence of fungi in dental carious lesion^{13, 16)} and in chronic marginal periodontitic lesion and subgingival biofilms¹⁴⁾ have been conducted by transmission and scanning electron microscopic examinations and immunohistochemistry. The electron

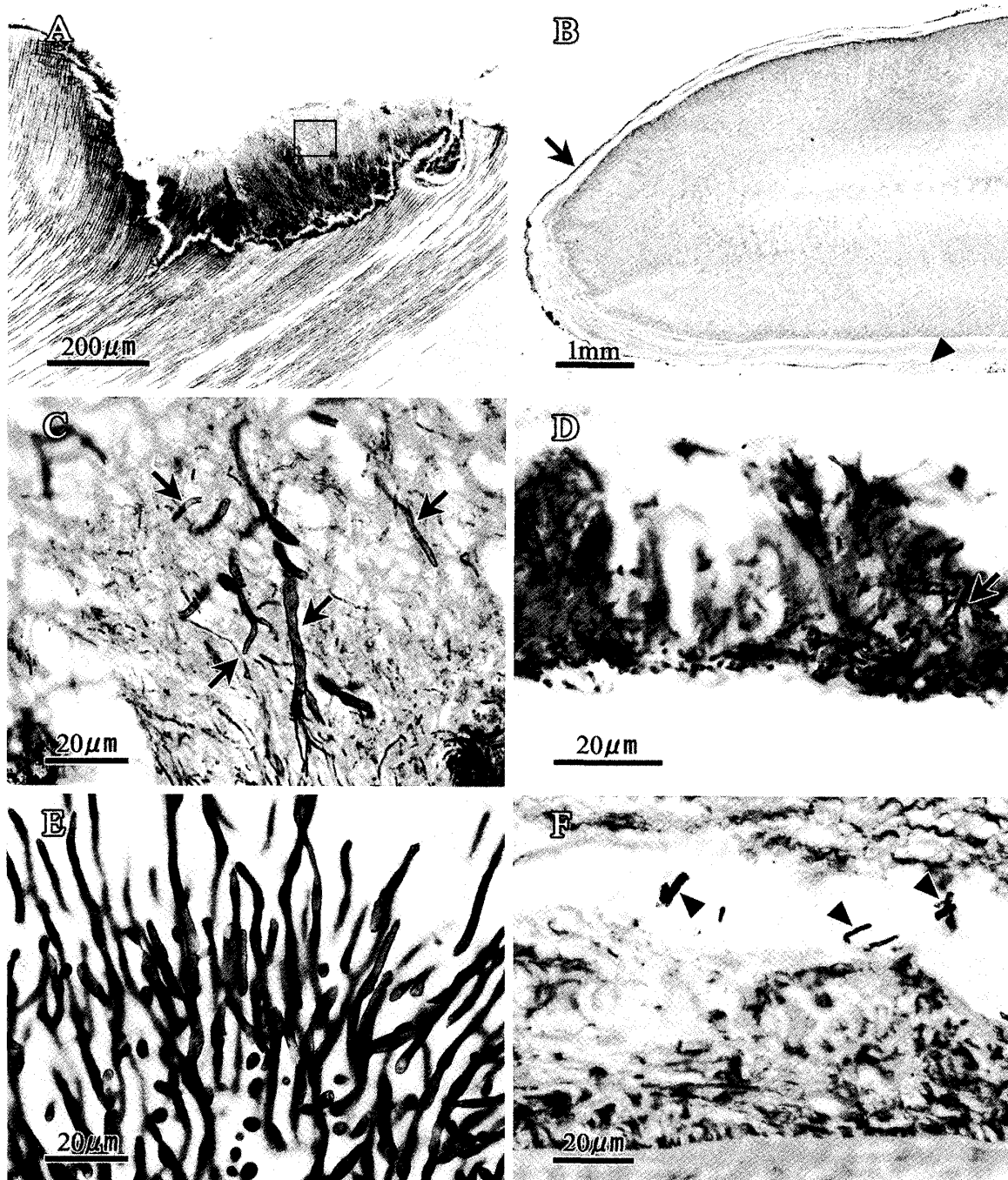


Fig 1. Histopathological findings (Grocott stain)
 Case 1 (A, C) ; Case 2 (B, D, F) ; Positive control ; *Candida albicans* observed in a chronic gastric ulcer (E)

(A) Carious lesion of dentin in Case 1.
 The area indicated by the quadrilateral is magnified in (C).

(B) Surface of apical root in Case 2. The area indicated by an arrow is magnified in (D) and the area indicated by an arrowhead is magnified in (F).

(C) An enlargement of the area indicated by the quadrilateral in (A). Pseudohyphae (arrows) of *C. albicans* can be seen in bacterial flora covering the surface of the carious cavity.

(D) An enlargement of the area indicated by an arrow in (B). Pseudohyphae (an arrow) of *C. albicans* can be seen on the surface of the apical root.

(E) A typical appearance of *C. albicans* in the ulcer floor of a chronic gastric ulcer.

(F) An enlargement of the area indicated by the tip of the arrowhead in (B). Pseudohyphae (arrowheads) of *C. albicans* can be seen in the periodontium.

microscopic examination found the yeast form of fungi, which had numerous budding forms at apical foramen of root-filled.^{12, 13)} On the other hand, Grocott stain in the present study clearly demonstrated pseudohyphae with constricted septum of *C. albicans* in the dental plaque in the carious cavity. Hyphal formation is one of virulence factors of *C. albicans*¹⁰⁾, because hyphal extension was observed to penetrate into the dentinal tubules¹⁶⁾. Furthermore, *C. albicans* possessed the high ability to dissolve hydroxyapatite to a greater extent by removing calcium ions¹⁷⁾. These findings may indicate that *C. albicans* in the dental plaque had thus penetrated into below dentinal tubules and decalcified the infected dentin. Namely, *C. albicans* may be involved in pathogenesis of dental caries by acceleration of decalcification¹⁸⁾.

The fact that *C. albicans* was isolated from apical periodontitic lesions^{4, 7)} and the electron microscopic presence of yeasts at the apical foramen¹²⁾ of teeth with fillings led us to think that yeast may have caused periapical inflammation which did not respond favorably to conservative root canal therapy¹⁰⁾. *Candida* spp. were detected at only a rate of a little less than 20% of the subjects in the periodontal pockets^{6, 7)}. As a result, it was recognized that this rate of prevalence was not suitable for *Candida* spp. as a causative factor of periodontitis. On the other hand, *C. albicans* has several virulence factors¹⁰⁾ such as the proteolytic activity, hyphal formation, which penetrates into the dentinal tubules¹⁶⁾, and adhesive activity. Therefore, it may be thought that *C. albicans* is also capable of modulating the periodontitic state by virulence factors.

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齶窩および歯根膜内に組織学的に *Candida albicans* がみられた二症例

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抄録：辺縁性歯周炎と齶蝕病巣内の真菌を組織学的に検出した。辺縁性歯周炎と齶蝕に罹患した二例の抜去歯をホルマリン固定し脱灰後、パラフィンに包埋して、組織学的検索を行った。Grocott 染色により齶窩および根面と歯根膜組織内に黒褐色に染まる菌糸を検出した。それらの形態はカンジダの菌糸に酷似していた。この結果から、ある種の条件下で、カンジダが辺縁性歯周炎の歯周組織や齶蝕歯の齶窩内に存在することがあきらかとなった。

キーワード：真菌, *Candida albicans*, 齶蝕, 慢性辺縁性歯周炎