Detection of *Candida albicans* by Polymerase Chain Reaction from Formalin-Fixed Paraffin-Embedded Tissue Specimens

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**Introduction**

Of the several species of Candida, *Candida albicans* plays a major role in oral candidiasis. It can also be a potent causative factor for the development of leukoplakia and median rhomboid glossitis\(^1\). Rapid identification of *Candida albicans* is important for the appropriate treatment of its lesions. The purpose of this study was to present a method to identify the species of *Candida albicans* by PCR, using formalin-fixed paraffin-embedded tissue specimens.

**Materials and Methods**

We studied six biopsy specimens that were suspicious of fungal infection by histological observation. The histological diagnoses of five cases were leukoplakia, aspergillosis, palatal ulceration, median rhomboid glossitis and moderate dysplasia. Five normal mucosae were also investigated. All specimens were fixed in 10% formalin, embedded in paraffin and stained with hematoxylin and eosin, periodic acid-Schiff reaction (PAS), and Grocott staining. Immunohistochemical staining was conducted using an anti-*Candida albicans* mouse monoclonal antibody (Chemicon International, MAB 806, 1 : 1000). All procedures were performed according to the common protocol of pathological laboratories.

DNA was extracted from the paraffin-embedded tissues using DEXPAT (TaKaRa) according to the manufacturer’s protocol. We used primers that were designed on a highly conserved region of fungal 18S rDNA gene (universal fungal primers; forward primer : CGAATCGCATGGCCTTG, reverse primer : TTCTCAGGCTCCCTCTCC) and primers which were designed on the variable region (D1/D2) at the 5’end of the large 26S rDNA gene of *Candida albicans* (Candida specific primers; forward primer : TGGTGCTCTCTGGGCGCG, reverse primer : AAAGATCACATTATGCGCCAACATCCTAGGTAA). Thirty five cycles of PCR were conducted on the DNA samples extracted from paraffin-embedded tissues under conditions of 94°C for 1 min, 55°C for 1 min and 72°C for 3 mins. Electrophoresis of the PCR products was performed through 2.5% agarose gels, staining with ethidium bromide and visualization with ultraviolet light. The DNA was transferred to hybond N+ nylon membranes (Amersham) and subjected to Southern hybridization.

In order to make the hybridization probe, *Candida albicans* was incubated and its DNA was extracted by
Table 1 Results of special stains, immunohistochemistry and PCR

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Site</th>
<th>Histological diagnosis</th>
<th>PAS/Grocott stain</th>
<th>Immunohistochemistry</th>
<th>PCR (common fungi)</th>
<th>PCR (C. albicans)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67</td>
<td>F</td>
<td>tongue</td>
<td>leukoplakia</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>M</td>
<td>maxillary sinus</td>
<td>aspergiliosis</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>61</td>
<td>M</td>
<td>palate</td>
<td>ulcer</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>M</td>
<td>tongue</td>
<td>median rhomboid glossitis</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>67</td>
<td>M</td>
<td>tongue</td>
<td>moderate dysplasia</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>71</td>
<td>M</td>
<td>buccal mucosa</td>
<td>leukoplakia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

the standard extraction method. PCR was performed on the *Candida albicans* DNA and the amplified products were ligated into a TOPO PCR vector (Invitrogen). The inserts were verified by DNA sequencing using a BigDye terminator sequencing kit (Applied Biosystems). The Candida specific fragment was labelled with 32P-dCTP and hybridized with the PCR products on the membrane. The radioactive signal was visualized using a BAS 2000 system (Fuji Film).

**Results and Discussion**

Five samples of normal mucosa did not show any PCR amplification. Table 1 shows the clinical data and the results of special stains, immunohistochemistry and PCR.

We selected cases that demonstrated apparent fungal hyphae from microscopical observation. The fungal hyphae in Cases 3 and 6 were positively stained with anti-*Candida albicans* antibody, but the other cases showed no immunoreactivity to the antibody and we could not identify the fungal species.

We performed PCR using two primer sets, the universal fungal primers and Candida specific primers. The former can reportedly detect the presence of a broad range of fungal species and the latter specifically amplifies *Candida albicans* DNA fragment\(^2,3\). PCR with the universal fungal primers revealed the presence of fungi in all cases (Fig. 1), which was compatible with PAS and Grocott staining results. PCR with the Candida specific primers showed obvious amplification in Cases 3, 4 and 6 (Fig. 2 a), which were confirmed to be derived from *Candida albicans* DNA by Southern hybridization (Fig. 2 b). The results suggested that the infectants were definitely *Candida albicans*. Immunohistochemical detection in Case 4 was negative for *Candida albicans*, but PCR demonstrated its presence. Cases 1, 2 (aspergillosis) and 5 were positive with universal fungal primers and negative with Candida primers, which implicated infection with fungal species other than *Candida albicans*. Although the severity of the fungal infection was varied in the studied specimens, it was difficult to make a quantitative evaluation of the infectant, because the amplification efficiency greatly depends on the sample's condition and purity of the DNA, rather than the amount of target DNA.

Fungal hyphae can be distinguished by special staining such as PAS or Grocott staining, but the correct identification of the species should be done with a microbiological study. Immunohistochemical staining can be performed on formalin-fixed paraffin-embedded specimens and is helpful for the purpose\(^4\), but the sensitivity is sometimes inadequate. The PCR technique is a simple and objective alternative to identify fungi\(^5\). In this study, we showed that PCR is highly useful to detect *Candida albicans* in routine pathological specimens in terms of its sensitivity, specificity and objectivity. The whole procedure takes only a few hours, meaning that it can be rapidly conducted along with other pathological inspections to collect indispensable information on the type of infectant.
Fig. 1 Result of PCR with the universal fungal primers. Amplification of 138 bp product is seen in all the specimens (Cases 1-6). Marker: pUC mix marker (Fermentas).

Fig. 2 Result of PCR with the Candida specific primers. Cases 3, 4 and 6 show 178 bp product (a), which was confirmed to be derived from Candida albicans by Southern hybridization (b). Marker: pUC mix marker (Fermentas).

References


