MIB-1 expression in odontogenic epithelial rests, epithelium of healthy oral mucosa and epithelium of selected odontogenic cysts: An immunohistochemical study

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Abstract. The aim of this study is to investigate the proliferative potential of rests of odontogenic epithelium found in follicles of unerupted teeth, epithelium of oral mucosa and epithelial linings of various odontogenic cysts. MIB-1 expression was studied in the rests of odontogenic epithelium (n = 10), healthy oral mucosa (n = 10), odontogenic keratocysts (n = 10) and other odontogenic cysts (n = 10) using an avidin–biotin peroxidase technique on paraffin sections. The number of positively stained cells was counted on 10 representative areas of epithelium using a ×40 objective. The average number of MIB-1 positive cells in each group was calculated. No MIB-1 positive cells were seen in the rests of odontogenic epithelium. The mean numbers of MIB-1 positive cells detected within the epithelium of oral mucosa, and of radicular and dentigerous cysts were similar. The number of MIB-1 positive cells was found to be increased in the presence of marked inflammatory cell infiltration. The highest number of MIB-1 positive cells was seen in the keratocysts. These findings suggest that removal of an unerupted tooth to prevent the possibility of neoplastic transformation of rests of odontogenic epithelium is not a justifiable rationale.

Key words: MIB-1; odontogenic epithelial rest; odontogenic cyst; immunohistochemistry.

Accepted for publication 6 September 2004
Available online 10 November 2004

Epithelial tumors and odontogenic cysts are derived from various types of epithelial cells in the oral tissues. These include remnants of odontogenic epithelium, the epithelial lining of dental follicles and surface epithelium lining the oral mucosa. Numerous studies have indicated that there are differences in the proliferation rates of various oral epithelial cells which may be important in the pathogenesis of epithelial tumors and odontogenic cysts.8,9,14–16,19,21,26.
The proliferative potential of rests of odontogenic epithelial cells found in dental follicles of unerupted teeth has not yet been investigated in detail. Uncertainty in this regard has been the foundation of one of the arguments in favor of surgical intervention for unerupted teeth.

The odontogenic keratocyst (OKC), exhibits more aggressive behavior as compared to other odontogenic cysts. This has led to a number of studies investigating possible differences among various cyst linings. Results of the latter studies indicate that the epithelial linings of keratocysts may have increased growth potential that is lacking in other types of odontogenic cyst linings. For this reason, the OKC has also been called a “cystic tumor” by some authors.

Immunohistochemical demonstration of proliferation-related markers is a valuable method for showing the proliferative potential of cells. A monoclonal antibody (MIB-1), which recognizes the epitope of Ki-67 antigen, is considered to be a reliable marker of proliferating cells. It is not expressed in quiescent cells.

The aim of this study is to investigate the comparative proliferation potential of rests of odontogenic epithelium found in dental follicles of unerupted teeth, epithelium of oral mucosa and epithelial linings of various odontogenic cysts. The proliferation potential of these respective epithelial cells may have some bearing on strategies taken for surgical intervention.

**Materials and methods**

Ten samples of dental follicles containing rests of odontogenic epithelium, 10 specimens of healthy oral mucosa, 10 cases of OKC and 10 cases of other odontogenic cysts (10 radicular, 10 dentigerous) constitute the material of this study. Oral mucosal samples were obtained during orthodontic or simple tooth extractions. Ten percent buffered formalin-fixed and paraffin-embedded tissue blocks of various odontogenic cysts and dental follicles were retrieved from the archives of The Department of Pathology of Gülhane Military Medical Academy. Four-micron thick sections were prepared. Slides were immunohistochemically stained with avidin–biotin peroxidase technique by using MIB-1 antibody (Neomarkers). DAB was used as chromogen and Mayer’s hematoxylin was the counterstain. The number of positively stained cells was counted on 10 representative areas of epithelium using a ×40 objective. The average number of MIB-1 positive cells in each group was calculated. The density of inflammatory cell infiltration in the surrounding tissues was semiquantitatively graded as “none”, “mild to moderate” and “intense”.

**Results**

The arithmetical mean number of MIB-1 positive cells in each group is shown in Table 1. No MIB-1 positive cells were seen in rests of odontogenic epithelium (Fig. 1) and the mean numbers of MIB-1 positive cells detected within the epithelium of oral mucosa and within the epithelium of radicular and dentigerous cysts were similar. MIB-1 positive cells were seen only in the basal epithelial cell layer. The number of MIB-1 positive cells was greater in association with significant inflammatory cell infiltration (Fig. 2). The highest number of MIB-1 positive cells were seen in the keratocyst group (Fig. 3), primarily in the basal and parabasal epithelial layers.

**Discussion**

In living organisms, there is continuous replacement of cells, with the exception of those in organs composed mostly of stable cells. The process of cell proliferation requires division of cells, which is under tight control of molecules expressed during the cell cycle. Disorganized and increased proliferation of cells occurs in various lesions, including cancer.

Growth rate is one of the determinants of tumor aggressiveness. There are several indicators used for determining cell proliferation status. These include the mitotic count, thymidine labeling index, bromodeoxyuridine incorporation, flow cytometry and immunohistochemical reactivity for various antibodies. Immunohis-

![Fig. 1. No MIB-1 positive cells were seen in the rests of odontogenic epithelium (×400).](image-url)
tochemical studies using proliferation markers have focused on antibodies like Ki-67, the Ki-67 clone MIB-1, and proliferating cell nuclear antigen (PCNA). These cell cycle-dependent antibodies are quite sensitive, and in particular the MIB-1 antibody has attained favor as a cell proliferation marker. MIB-1 is expressed in proliferating cells during the proliferating phase of the cell cycle, but is not expressed during the resting phase.

MIB-1 has been used to investigate the proliferation potential of normal tissues, as well as preneoplastic and neoplastic lesions\textsuperscript{6,8,9,17,21}. The identification of abnormally increased proliferation of precursor cells is an important aspect of neoplastic progression\textsuperscript{9}.

The lack of data concerning proliferation potential of odontogenic epithelial rests found in dental follicles of unerupted teeth is a source of controversy concerning surgical removal of unerupted teeth. However, it is also believed that cysts and tumors of the oral cavity could arise from remnants of odontogenic epithelium found in other oral tissues\textsuperscript{5,10,23}. A LDESPERGER et al.\textsuperscript{2} using PCNA, reported that cells of dental follicle epithelium in asymptomatic dental follicular specimens were apparently at rest. The results of our study demonstrate that odontogenic epithelial rests found in dental follicles of unerupted teeth contained no MIB-1 expressing cells. These findings suggest that removal of an unerupted tooth in order to obviate the presumed risk of neoplastic proliferation originating from the remnants of odontogenic epithelium may not be justified. However, there may still be other reasons for their removal.

It is known that the proliferative capacity of oral mucosal epithelium is notably dynamic in that it regularly regenerates itself on a relatively rapid basis. However, in the present study, epithelial cells from healthy oral mucosal specimens showed relatively rare MIB-1 expression. An increased proliferative marker expression was noted in the presence of heavy subepithelial inflammatory cell infiltration. It has been reported that chronic irritation such as that caused by chronic inflammation may stimulate proliferation of oral epithelial cells\textsuperscript{13,19,24}. In this study, the MIB-1 expressions of oral mucosal epithelium and of the epithelial linings of cysts were similar. A higher number of MIB-1 positive cells in odontogenic cyst epithelium and oral mucosal epithelium with subepithelial inflammatory cell infiltrates support the view that “the number of cycling epithelial cells may increase as a

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.jpg}
\caption{MIB-1 positive cells (arrows) were seen in inflamed odontogenic epithelium (×200).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.jpg}
\caption{Odontogenic keratocyst epithelium showing large numbers of MIB-1 positive cells (arrows) (×200).}
\end{figure}
consequence of inflammatory stimulation.

The growth rates of epithelial lining of various types of odontogenic cysts seem to differ. Keratocysts, known for their aggressiveness, contained significantly higher numbers of MIB-1 positive cells than the lining of the other odontogenic cysts.

Further investigations on genomic changes are needed to understand fully the proliferation potentials of different types of epithelial cells that participate in the formation of various odontogenic lesions.

References


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