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Original Article

Study of the biologic behavior of odontogenic keratocyst and orthokeratinaized odontogenic cyst using TGF-alpha and P53 markers

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ABSTRACT

Odontogenic keratocyst (OKC) is an aggressive cyst, and its recurrence rate is higher than that of other odontogenic cysts. Orthokeratinized odontogenic cyst (OOC) is less aggressive than OKC, but bears the probability of carcinomatous changes. In this study, we evaluated the expression and intensity of P53 and TGF-alpha in order to compare the biologic behavior or probable carcinomatous changes of these two cysts.

In this cross-sectional study, 15 OKC and 15 OOC were stained immunohistochemically for P53 and TGF-alpha using the Novolink polymer method. Then, all slides were examined by an optical microscope with 400× magnification, and the stained cells in the basal and parabasal layers were counted. Finally, the results were analyzed by the Mann–Whitney and Wilcoxon tests (P-value < 0.05).

The difference between the expression of P53 and TGF alpha in the basal layer of OKC and OOC was not statistically significant (P-value > 0.05), but the expression of P53 and TGF-alpha in the parabasal layer in OKC was statistically higher compared to OOC ($P < 0.05$).

Considering the known role of P53 and TGF-alpha in malignant changes and the higher expression of P53 and TGF-alpha in OKC compared to those in OOC, the probability of carcinomatous changes was higher in OKC than in OOC.

Introduction

The term odontogenic keratocyst (OKC) was used for the first time by Philipsen in 1956 to define an odontogenic cyst with parakeratinized epithelial surface [20].

Despite its bland histology, OKC shows a more aggressive behavior and a higher expression rate, which distinguishes it from other odontogenic cysts that produce keratin. For a long time, many researchers supported the idea that the cyst has a neoplastic nature due to its biological behavior, based on a number of molecular and genetic pieces of evidence. It was found to be a neoplastic cyst or cystic neoplasm, in contrast to other odontogenic cysts, such as dentigerous and radicular cysts, which are reactive cysts. Therefore, in its recent classification, WHO refers to this lesion as a benign neoplasm entitled keratocystic odontogenic tumor (KCOT), because the epithelial surface of OKC shows a higher mitotic activity than other odontogenic cysts [1,4,13,16,17,20].

OKC may be found at any age, but most of the outbreaks or peak incidences of this type of cyst are registered in the second and third decade of life, and are also more common in males than in females. It happens in the mandible in 60–80% of the cases and has a special predilection for mandibular angle and ascending ramus [6,8,16].

Radiographically, OKC appears in the form of unilocular or multilocular radiolucency with specific borders and usually with sclerotic smooth margins [3,23].

The histopathologic characteristics of OKC are pathognomonic and include uniform thickness and rete ridgeless stratified squamous epithelium, corrugated superficial parakeratinization and also hyperchromatism, reverse polarization, and palisading arrangement of basal cells of epithelium [18–20].

In the past, OKCs were categorized into two types: parakeratinized and orthokeratinized. However, it was revealed that the orthokeratinized type not only lacks the typical characteristics of the parakeratinized type and is composed of orthokeratinized stratified squamous epithelium with a prominent granular layer, but also shows different biologic or clinical behavior in a way that its recurrence is very much lower than the parakeratinized type. This is the reason why nowadays the orthokeratinized type is considered a different cyst with different histopathological and clinical characteristics [4,21,24].

Previous studies on p53, such as those of Li et al. [12], Slootweg [22], LoMuzio et al. [15] and Baghaei et al. [2], and also of Li et al. [11] on TGF-alpha immunoexpression were conducted to investigate OKC and OOC. However, in all of these studies, both markers for

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P53 and TGF-alpha were not considered in the investigation of these two cysts.

Therefore, in this study, we compared OKCs and OOCs by investigating the extent of P53 protein and TGF-alpha expression. In doing so, we intended to ascertain the causes of the different biologic behavior patterns of these cysts.

It is noteworthy that P53 is a tumor suppressor gene. The major functional activities of the P53 protein are cell cycle arrest and initiation of apoptosis in response to DNA damage. Under physiologic conditions, P53 has a short half life (about 20 min). This type of P53 is named wild type. On the other hand, P53 mutations are common in a variety of cancers and play an opposite role as tumor suppressors. For example, the mutant type of P53 is an oncogene. Usually, in immunohistochemical staining, the mutant type of P53 is stained, which is why positivity of P53 can be an indication of tumorigenesis or cancer [10].

TGF-alpha is an oncogene structurally and biologically related to the epidermal growth factor (EGF) and is principally produced by malignant cells. Attachment of TGF-alpha to EGF receptor causes TGF-alpha proliferation. This marker is effective in malignant transformation of natural cells [4,10].

It is noteworthy that both markers can be found in many kinds of cancers, such as head and neck cancers, with higher expression than in normal tissues [10].

Materials and methods

This study is a descriptive analytic and cross-sectional study. Having achieved the consent of the research committee, a total of 30 cases, consisting of 15 OKC and 15 OOC, were used in this study.

Three to four micrometer sections from paraffin-embedded specimens were mounted on poly-L-lysine-coated glass slides.

After deparaffinization and rehydration in 5 steps of descending alcohol, the sections were incubated in citrate buffer in a microwave oven for 15 min for antigen retrieval. Afterwards, they were incubated in 0.5% H_2O_2 in methanol for 10 min to block endogenous peroxidase activity, and then rinsed with phosphate-buffered saline (PBS). In the next stage, the specimens were incubated for 1 h with the lyophilized monoclonal anti-P53 (NCL-P53-DO1, Novacastra, Germany) at a dilution of 1:50 and the lyophilized monoclonal anti-TGF-alpha (NCL-TGFα R1, Novacastra, Germany) at a dilution of 1:100. Immunocomplexes were subsequently treated with post-primary block and then detected by Novolink polymer (Novacastra, Germany) for 30 min, both incubated for 30 min at room temperature. After rinsing with PBS, the immunoreactivity was visualized by diaminobenzidine (DABO, DAKO, Denmark).

Sections were finally counterstained with hematoxylin, cleared and mounted with PV mount, and slides were blindly viewed independently by two oral pathologists using a light microscope (Olympus BX41TF, Tokyo, Japan).

Positive controls consisted of tissue specimen sections of breast carcinoma with known antigenic reactivity. A negative control was stained by omitting the primary antibody.

Specimen evaluation

The specimens were examined by a light microscope at 400× magnification. Cells with brown staining were counted in the basal and parabasal layers of each of the two cysts. The percentage of positive epithelial cells for each layer in 10 high-power fields of a microscope was determined and, with regard to cytoplasmic positivity for TGF-alpha and nuclear positivity for P53 antigen, classified at a scale ranging from 1 to 4: (+1) 0–25% positive cells; (+2) 26–50% positive cells; (+3) 51–75% positive cells; (+4) 76–100% positive cells.

Also, intensity of staining with P53 and TGF-alpha antigen was evaluated using the following method: (0) no staining and (+1), (+2), (+3), (+4) for very low, low, moderate, and high staining, respectively.

Finally, the SID (staining intensity distribution) score was calculated by multiplication of these two scores for each specimen [7].

The data were analyzed by means of statistical software SPSS 10 with Mann–Whitney and Wilcoxon statistical tests at a significance level of 0.05 for comparison of data between P53 protein and TGF-alpha.

Results

The expression of p53 protein was led to definite bright brown staining in the nucleus of the epithelial cells, but the expression of TGF-alpha was localized to the cytoplasm.

According to Wilcoxon's statistical test, the expression of P53 protein in basal and parabasal layers of OKC was not statistically significant ($P = 0.356$), but was statistically significant for TGF-alpha ($P = 0.041$).

The expression of P53 protein in the basal and parabasal layers of OOC was not statistically significant, but was near to significance ($P = 0.053$), and also was statistically significant for TGF-alpha ($P = 0.012$).

According to Mann–Whitney's statistical test, the following results for P53 protein were obtained:

The average expression of P53 protein in the basal layer in OKC was higher than in OOC, but this difference was not statistically significant ($P = 0.076$) (Fig. 1A,B, Table 1). In reality, if it was not statistically significant, the expression was not higher in the OKC.

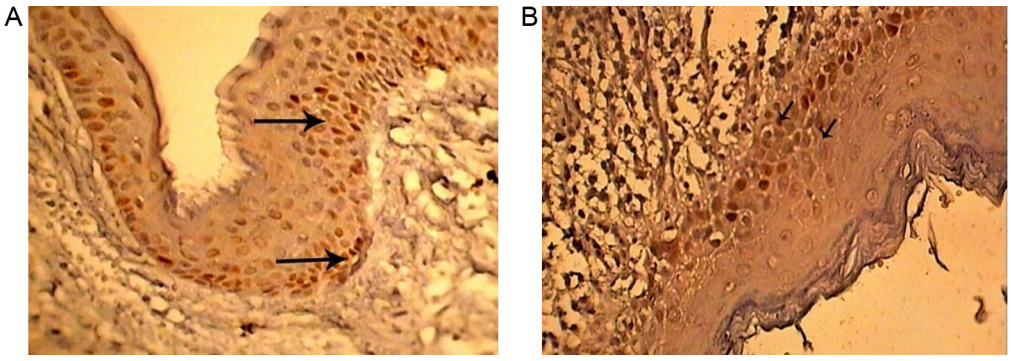

Figure 1. (A) P53 protein expression in the basal and parabasal layers of OKC (×400). (B) P53 protein expression in the basal and parabasal layers of OOC (×400).