

## RESEARCH ARTICLE

# Immunoexpression of Ki67 and p53 in the Dental Follicles of Impacted Teeth

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**Aim.** The purpose of this study was to evaluate the morphological aspects of dental follicles associated with impacted teeth and the immunoexpression of Ki 67 and p53, in the remaining epithelium from impacted third molars and canines. Another aspect was to identify the transformation of the remaining epithelium within the odontogenic lesions. **Material and method.** We included in the study a number of 100 dental follicles, of which only 62 met the inclusion criteria. The immunohistochemical reactions were carried out in the Immunohistochemistry Lab at the Department of Histology of the University of Medicine and Pharmacy of Tîrgu Mureş. We counted 100 cells from the remaining epithelial islands from which we determined the percentage of labeled cells for both markers. After this all data were subjected to a statistical analysis.

**Results.** Out of the 62 specimens 44 were from females and 28 from males. Positive results were obtained for the two markers but not for all cases. We have also found a positive correlation regarding the immunolabeling of the two markers ( $p=0.002$  in canines,  $p=0.0001$  in molars). Median values for ki67 were statistically significantly higher than the values for p53 for both types of dental follicles. **Conclusions.** Both markers were expressed in the remaining epithelial islands. The stronger expression of Ki67 in the follicles of impacted molars can demonstrate the higher frequency of odontogenic cysts in this area.

**Keywords:** dental follicles, impacted teeth, immunohistochemistry, Ki67, p53

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## Introduction

During tooth formation there are several successive stages that will most often culminate in tooth eruption (dental bud stage, cap stage and bell stage) [1]. In other situations the tooth remains impacted in the maxillary or mandibular bone. The teeth that are most commonly impacted are the mandibular third molars, followed by upper canines, upper wisdom teeth, lower canines, upper and lower premolars, upper and lower incisors [2,3].

An impacted tooth also retains the dental follicles which consists of condensed mesenchymal tissue that sometimes contains epithelial islands arising from the process of odontogenesis and often it is covered by small amounts of odontogenic epithelium [4]. These epithelial cells can maintain the ability of proliferation and differentiation, and may be the starting point of a dental pathology associated with impaction or cystic tumor pathology (follicular cyst, keratocyst, ameloblastoma). This is why some authors widely advocate the surgical removal of impacted teeth, to prevent the above mentioned complications [5].

Various immunohistochemical markers have been studied in the literature to assess the proliferating ability of the residual epithelium (p53, Ki67, MCM2, bcl-2).

p53 is an oncosuppressive protein with expression in the G1 phase of the cell cycle. In normal "unstressed" cells it has a short life span of about 20 minutes. In the cells that

are exposed to stress (proliferating cells) mutations can occur in the p53 gene, giving rise to a stable mutant protein that can be detected more easily in the nucleus using immunohistochemical methods [6,7].

Ki67 protein is present during all active phases of the cell cycle. Its immunohistochemical detection is used to evaluate the proliferative potential of healthy cells and also of pre-neoplastic or neoplastic cells, being an important prognostic factor in many tumor types. [8]

## Objectives

The purpose of this study was to evaluate and to compare the immunoexpression of p53 and Ki67 proliferation markers in the epithelial residues of the dental follicles of impacted molars and canines, to highlight the possible differences that could exist between the degrees of proliferation of epithelial cells remaining in the dental follicle in the two mentioned locations.

## Materials and methods

The study included 100 dental follicles selected from the archive of the Pathology Department at the Mureş County Emergency Hospital of Tîrgu Mureş, that were obtained from surgical removals of impacted teeth, performed at the Oral and Maxillofacial Surgery Clinic of Tîrgu Mureş.

The inclusion criteria for the study were as follows: dental follicles originating from patients with no other local inflammatory diseases or other pathologies and the existence of sufficient material for histopathological analysis.

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The size of the follicles assessed radiographically or by other imaging methods was not taken into account.

After applying these criteria only 62 follicles remained in the study, of which 42 derived from impacted molars and 20 from impacted canines. After harvesting and fixation in 10% formaldehyde, the operatory pieces were processed similarly being subjected to conventional histopathological methods. In each case the harvested material was taken up entirely from the blocks obtained by the infiltration with paraffin wax, and then 3-5 micrometer sections were prepared which were stained with hematoxylin and eosin.

Cases were evaluated by two pathologists and those that met the inclusion criteria were subjected to immunohistochemical analysis. IHC staining was performed on 0.3-0.4  $\mu$  thick sections, on polysine slides (Thermo Scientific). The antibodies selected were: Ki 67 (Clone MIB-1, 1: 100, DAKO) and p53 (clone 318-6-11, 1:50, DAKO). Antigen retrieval was achieved by using the HIER (Heat Induced Epitope Retrieval) method. The detection system used was Ultra Vision LP Detection System HRP Polymer (LabVision), the chromogen used was diaminobenzidine and hematoxylin counterstaining for nuclear reaction. Negative control reactions were obtained by the omission of primary antibody application. The positive control reactions were represented for Ki67 by the sections collected from the normal vaginal mucosa and p53 by the squamous epithelium from tonsils.

Immunohistochemical reactions were performed in the Laboratory of the Histology Department at the University of Medicine and Pharmacy of Târgu Mureş.

Interpretation of results based on immunohistochemical reactions was performed by two pathologists. To assess the proliferative index 100 cells were counted in the epithelial islands and the odontogenic epithelium found in the structure of follicles, and the percentage of labeled cells was identified for each marker. The results were entered in a database and were subjected to a statistical analysis. For this purpose the MedCalc Software was used, version 12.5.0.0. Student test, Chi square test and Mann Whitney test were applied, followed by Spearman's rho. The level of statistical significance was set at 0.05. The study was approved by the Ethics Committee for scientific research at the Emergency County Hospital of Târgu Mureş, Romania no.4194/ 2015 and by the Ethics Committee at the University of Medicine and Pharmacy of Târgu Mureş, Romania no.30/2015.

## Results

The results regarding the *age of patients* included in the present study reveal a significantly higher mean age ( $p=0.004$ ) calculated for the impacted canines compared to the mean age obtained for impacted molars (Table I).

In this study 32 cases of impacted molars were from patients aged between 10 and 20 years and only 10 cases were recorded from patients older than 20 years. In the group of impacted canines patients had a uniform distribution

**Table I. Statistical analysis regarding the age of patients**

	canine	molar
Number of values	20	42
Minimum	14,00	14,00
Median	19,50	18,50
Maximum	57,00	54,00
Mean	28,20	19,33
Std. Deviation	17.19	6.230

in terms of their age, 10 cases were diagnosed between the ages of 10 and 20 years, and 10 cases after the age of 20. There was a statistically significant correlation ( $p=0.0479$ ) between the incidence of impacted teeth and the age of patients.

Distribution of cases by sex showed greater frequency in women, both for impacted canines and impacted molars (16, and 28 cases, respectively) than in males (4 cases, and 14 cases, respectively). The application of the Chi square test did not reveal any statistically significant value ( $p=0.43$ ), thus no gender differences were obtained based on the type of the affected tooth.

## Results regarding immunohistochemical reactions

Of the 20 follicles originated from impacted canines 16 cases showed positive nuclear reaction for Ki-67, while 12 cases demonstrated positive reaction for p53. Of the 42 follicular bags derived from impacted wisdom teeth, 40 indicated positive reaction for Ki67, while 28 specimens had positive reaction for p53 (Figure 1).

The difference in median percent values between the two markers identified in the two types of dental follicles was assessed with the Mann Whitney test and a significant value ( $p=0.05$ ) was obtained only for Ki67. For p53 we obtain statistically insignificant value ( $p=0.2$ ).

To check the correlation between the two variables, Ki67 and p53, the Spearman's rho was performed. Both for the dental follicles of impacted canines and molars there was a positive and statistically significant correlation ( $p=0.002$  for canines and  $p=0.0001$  for molars) (Figure 2,3).

When testing the correlation between age and sex of patients with impacted teeth and the immunexpression of the two antibodies there were no statistically significant values between the two studied markers (Table II).

## Discussion

Dental inclusion is a dental anomaly characterized by the retention of a tooth in the maxillary or mandibular alveolar bone beyond the normal period of eruption [1]. The most commonly impacted tooth is the wisdom tooth, followed by canines, premolars and supernumerary teeth. Very often this pathology drives the patient to present to an oral and maxillofacial surgeon.

Previous studies showed a very large percentage regarding the incidence of impacted teeth in the general population, varying between 14% and 96%. Of these, approximately 95-98% is represented by wisdom teeth, 50% being partially erupted, and the rest of them remain impacted

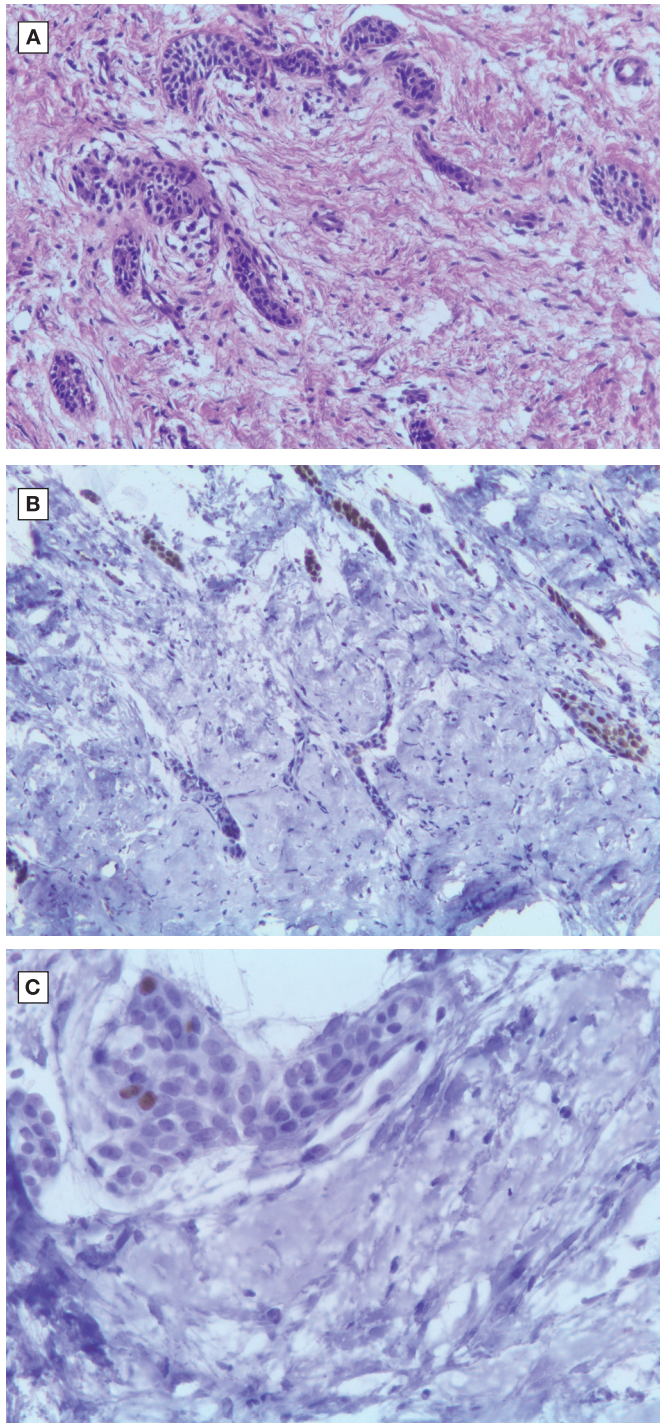


Fig. 1. (A) epithelial component of the dental follicle, HE, x100; (B) Ki67-positive cells in epithelial component of the dental follicle, x100, (C) p53-positive cells in epithelial component of the dental follicle, x200.

in the surrounding bone structures [3,9]. Third molars are the last developing teeth and they become radiographically visible only after the age of 9 years. Final development of the crown and root is attained somewhere around the

Table II. Correlation between age, sex and immunohistochemical markers

Age	Ki67	p-0.0551
	p53	p-0.4330
Sex	Ki67	p-0.09
	p53	p-0.34

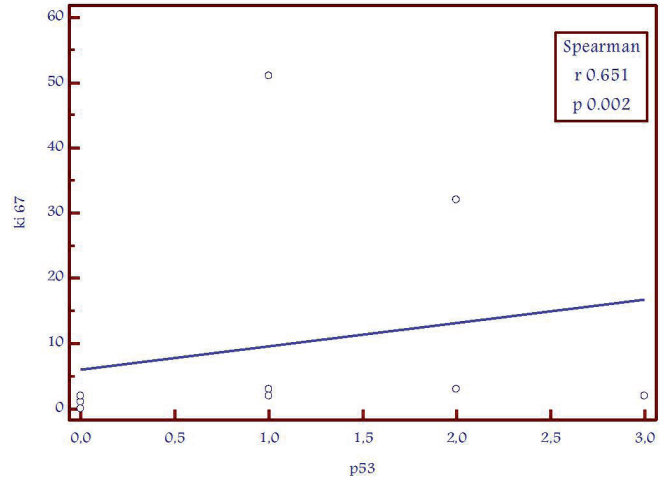


Fig. 2. Correlation between ki67 and p53 for the follicles of canines

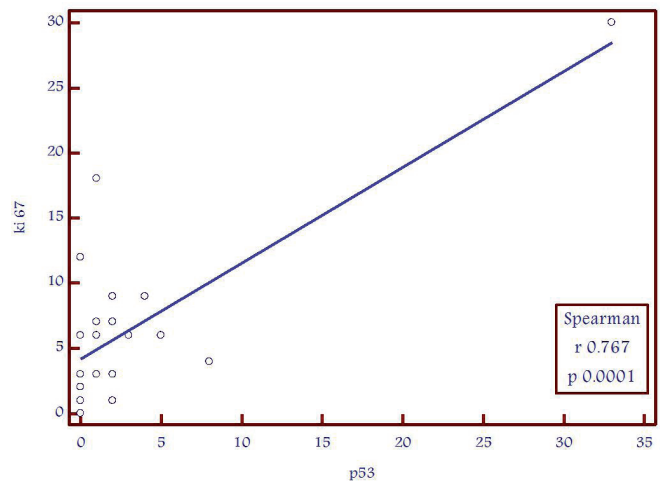


Fig.3. Correlation between ki67 and p53 for the follicles of molars

age of 14-18 years, and the eruption occurs around the age of 20 years. Canines develop earlier, around the age of 9-10 years, and they also erupt around this period.

The present study reveals that the impaction of wisdom teeth is more common than that of canines. Regarding the age of patients, the youngest patient was 14 years old and the oldest subject with impacted canine was 57 years of age.

The age difference between the cases with canine impaction and those with molar retention can be explained by the fact that adult patients are more concerned with their aesthetics, particularly as canines are located in the frontal segment of the dental arch. The lower average age calculated for the cases with impacted third molars may be explained by the fact that younger patients are frequently subjected to orthodontic treatment, which often implies surgical removal of impacted third molars in order to gain more space on the dental arch.

There are many studies in the literature related to dental impaction that also assess the gender of patients with this diagnosis. Most of them, including the papers from the national literature, demonstrate that canine impaction is more common in women compared to males [10,11]. For wisdom teeth the studies performed in Singapore and Iran

indicate the same predominance of cases with dental impaction in women and this finding is consistent with the result of the present study [12,13].

There are few studies related to the assessment of proliferative potential of the odontogenic epithelium and the epithelial islands that can be found in the dental follicles surrounding impacted teeth with the use of proliferation markers. The proliferative capacity of odontogenic epithelium is considered to be very important as the imbalance between epithelial cell proliferation and apoptotic process may result in the development of benign and malignant odontogenic lesions in the periodontal tissues of impacted teeth. Also, there is no consensus regarding the management of impacted teeth in asymptomatic patients. Some authors advocate the routine extraction of impacted molars [5], while others sustain that their removal is only recommended when they either associate with pathology or symptoms or during orthodontic treatment aimed to create additional space. Other authors recommend regular monitoring only in cases with impacted third molars [14]. A method proposed for assessing the progress towards a cystic lesion of a follicle presumes tracking the size of pericoronal space around impacted teeth. However the results of studies related to this topic are controversial due to the small number of cases studied and also because the maximum size of this space is not fully established.

Some authors recommended that the maximum value for this space to be 2 to 2.5 mm, and those that exceed 3-4 mm to be already considered cystic lesions. However the results of a 12-year study that monitored cases with a pericoronal size of 3-4 mm has not revealed any association between the mentioned larger dimensions and the possibility of pathological changes [15,16]. Most studies analyze the immunoexpression of proliferation markers such as Ki67, p53, PCNA, MCM2, BCL2 only in cases with impacted molars, or with cystic lesions and/or tumors. Only one study evaluated the immunoexpression of two markers, Ki67 and BCL2, in the dental follicles around impacted molars, canines and supernumerary teeth, but did not assess the correlation between immunohistochemical parameters and the type of the impacted tooth [17].

Immunoexpression of Ki67 nuclear protein is associated with cell proliferation and is expressed in all phases of the cell cycle, except the G0 phase. In interphase the protein expression is exclusively nuclear, but during mitosis it is present also in the cytoplasm as it relates to the surface of the chromosomes. In many studies, ki67 is used as a biomarker to assess the proliferative activity and progression from normal tissue to dysplasia and malignant lesions.

The p53 protein is expressed in the G1 phase of the cell cycle and has an important role in DNA repair and in the induction of apoptosis. In the literature there are few studies that assess the immunoexpression of this protein in the dental follicles of impacted teeth. In most cases the expression of p53 is assessed in cases with benign and malignant lesions and the results are still controversial.

Regarding the immunohistochemical Ki67 labeling the present study revealed a positive reaction in the residual epithelium and in the odontogenic epithelium of follicles in 80% of cases with impacted canines and in 95.23% of cases with impacted third molars. For p53 there were fewer cases with positive reactions, 60% and 66.67%, respectively. Our results are similar to those from previous studies [18]. Investigations performed on dental germs collected from human fetuses revealed the immunoexpression of both proliferation markers and the over-expression of Ki67 for developing molars, followed by growing canines [19].

In the present study, although the number of Ki67-positive cases was greater than the number of cases found positive for p53, both in canines and wisdom teeth, no statistically significant difference was found between the two markers to evaluate the proliferative potential of these epithelial residues. However, a positive correlation was identified between the two markers for the two types of follicles and a statistically significant value for Ki67 in terms of median values.

Some studies show a correlation between the advanced age and the development of cystic lesions around impacted teeth, over half of them developing after the age of 20 years, with peak incidence between 20 and 25 years. Regarding the cystic lesions the literature also reveals an increased immunoexpression of the two markers in cases with radicular cyst and dentigerous cyst where cell proliferation is enhanced by the associated inflammation [20]. In keratocyst the immunoexpression is more pronounced, but it is due to the proliferative pattern of the cells located in this type of lesions, compared to the neoplastic cells of an ameloblastoma [15, 21, 22, 23].

Considering that there was no correlation between age and sex of the patients and the immunoexpression of the two markers in the dental follicles of the impacted canines and third molars, we believe that the proliferative potential of epithelial residues and odontogenic epithelium located in the dental follicles is preserved mainly in the molar sites.

## Conclusions

Dental inclusion is found more frequently in women and at older ages for canines. The residual epithelium and the odontogenic epithelium derived from wisdom teeth retain their proliferative capacity better than those located in the follicles of the impacted canines. This explains the higher frequency of cyst development at the site of impacted third molars compared to those located in impacted canines.

Ki 67 can be used as an independent marker of proliferation for the detection of epithelial residues associated with the dental follicles of both impacted third molars and canines.

p53, although expressed in a less number of cases, correlates positively with immunoexpression of Ki67, proves to be a useful marker of proliferation with that used in the panel.

Detection of these two markers in the follicles of impacted teeth confirms the proliferative capacity of this type of tissue around the impacted tooth and also its ability to convert into cyst at any age. For this reason we support the routine removal of impacted third molars.

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