

## Influence of Smoking upon the Ki67 Expressions in Asymptomatic Fully Impacted Lower Third Molar Follicles

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### Abstract

Ki67 expressions are the most widely used markers to show the pathologic proliferation and early-stage tumoral alterations in vital tissues. This study aimed to compare the expressions of Ki67 protein between smokers' and nonsmokers' pericoronal follicles of asymptomatic impacted lower third molars (ILTM), thirty-seven specimens of DFs associated with impacted mandibular third molars fully covered by mucosa or bone were surgically removed from 37 patients. The patients were divided into 2 age groups. 19 of thirty-seven DFs were between (18-24) years (Group 1) and 18 were (< 24) years (Group 2). Ki-67 immunostaining was evaluated in the epithelial component of the all DFs, independent-samples t-test analyses were conducted with SPSS 10.0 with statistical significance set at a P-value equal to 5%. There were statistically significant differences in the mean values of KI-67 (%) expression between the non-smokers group and the smokers group regardless of the patient's age group. The values of KI-67 (%) expression in the non-smokers group were smaller than in the smokers group, in both the (18-24) age group and the (24 >) year age group. Separately in the research sample, this study showed that DFs of smokers have higher Ki67 expressions than nonsmokers follicles.

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### Introduction

Tooth impaction is a situation when tooth fails to erupt into its normal functioning positions within its eruption time<sup>1</sup>. Impacted lower third molars surgery (ILTM) is one of the most common surgical procedures in dentistry<sup>2</sup>. Although there is a real indication for extraction symptomatic third molar, there is no full agreement among surgeons on the requirement for extract asymptomatic impacted lower third molars<sup>3-6</sup>, among totally different populations the impaction incidence of lower third molars varies from (9.5-39) %<sup>7</sup>, Prophylactic extraction of asymptomatic third molars has generated much discussion in dentistry<sup>3</sup>, Asymptomatic means that there is no discomfort or pain from the impacted third molar, but it does not mean free of

risk<sup>8</sup>, (ILTM) is a common problem. It is often associated with pericoronitis, periodontitis, cystic lesions<sup>9</sup>.

Dental follicle (DF) is an ectomesenchymal tissue that surrounds the developing tooth germ<sup>10</sup>. Radiographically, the DFs look like slight semi-circular radiolucencies around impacted third molar. if the DFs are more than 2.5 mm in diameter, they are considered to be a pathological change<sup>11</sup>. Recently immunohistochemical studies have emerged on the importance of the idea of extraction of Asymptomatic Fully Impacted Lower Third Molars<sup>3, 4, 12-14</sup>. The main idea of these studies was to determine which histopathological changes could appear in the (DFs) of the third molars that appear asymptomatic and radiologically appear normal. Differences in the rate of proliferation of epithelial cells or components of the dental epithelium of the dental follicle can play a significant role in the formation of tumors and cysts<sup>12</sup>. It has been agreed that potentially malignant lesions are associated with smoking and drinking alcohol, which are among the most important factors in the transition from a pre-cancerous to a cancerous lesion<sup>15</sup>.

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in dental follicle epithelium Cell proliferation can be the precursor of pathologic changes, such as cystic alterations or squamous metaplasia<sup>14</sup>. Ki-67 is a nuclear antigen who expressed throughout the cell cycle in G1, G2, and S phases in mitosis and is not expressed in resting phase G0 and has been used fundamentally as a cell proliferation marker<sup>16</sup>.

The purpose of this study is to evaluate and compare the variation of cell proliferation activity of DFs surrounding the asymptomatic (ILTM) in smokers and non-smokers using the Ki-67 to determine the effect of smoking on the pathologic potential of the (DFs) depending on the age factor.

### Materials and methods

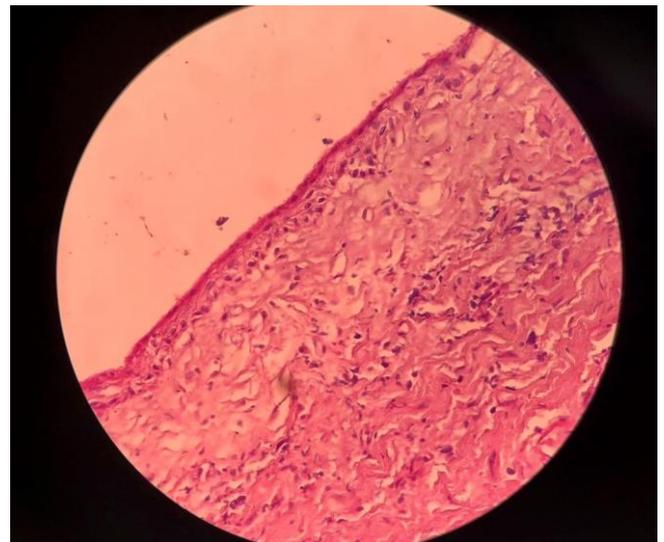
This study was performed at the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Damascus University Thirty-seven of DFs associated with impacted mandibular third molars were surgically removed from 37 patients. The patients were divided into 2 age groups. Group I (included 19 patients aged 18-24 years), group II (18 patients aged 24-47 years). Inclusion Criteria: patient with full impacted asymptomatic mandibular third molar, the patient is in good oral hygiene, between the ages of 18-60 years, Dental follicle measurement is  $\leq 2.5$  mm. Exclusion criteria: Patients with uncontrolled systemic disease or localized lesions at the third molar site, poor oral hygiene, pregnant and lactating women were excluded, and when the dental follicle measured  $> 2.5$  mm. All surgical extraction were carried out under local anesthesia, DFs were carefully removed and the specimens were fixed in 10% buffered formalin for 1 to several days and embedded in paraffin wax. They were sliced into serial 3- $\mu$ m-thick sections and processed routinely and stained by hematoxylin and eosin and monoclonal antibodies to Ki67. Smoking history was quantified. Statistical analyses were conducted with SPSS 10.0, with statistical significance set at a P value equal to .05

The predictor variable of this study is Smoking status. the outcome variables are the Ki67 protein expressions in (ILTM) follicles. The mean Ki67 of the smoking and non-smoking groups were compared by independent-samples t test.

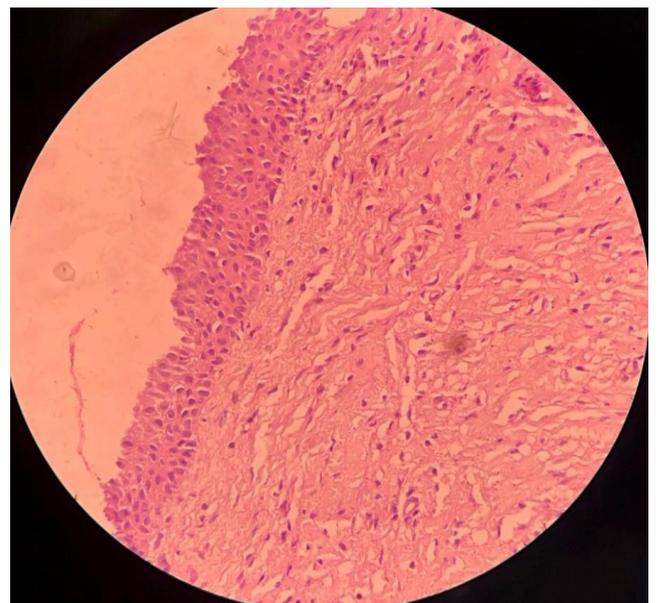
Ki-67 expressions was evaluated in

epithelial component of DFs. Epithelial components of Dental follicle were classified as basal layer of squamous epithelium and suprabasal layer. rests of the odontogenic epithelium Inflammatory cells, and calcification in the mesenchymal components were also noted.

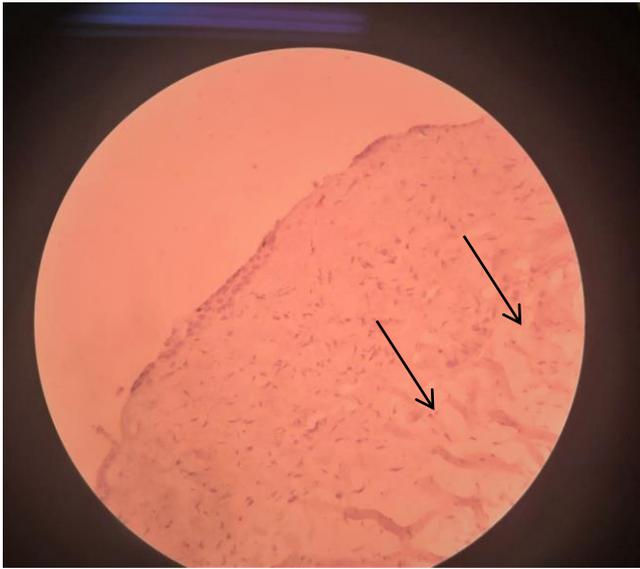
The positivity of Ki-67 was evaluated by counting the number of positive cells per 1000 cells in both basal and upper part of the epithelium of DFs. Ki-67 percentage was calculated for each sample.



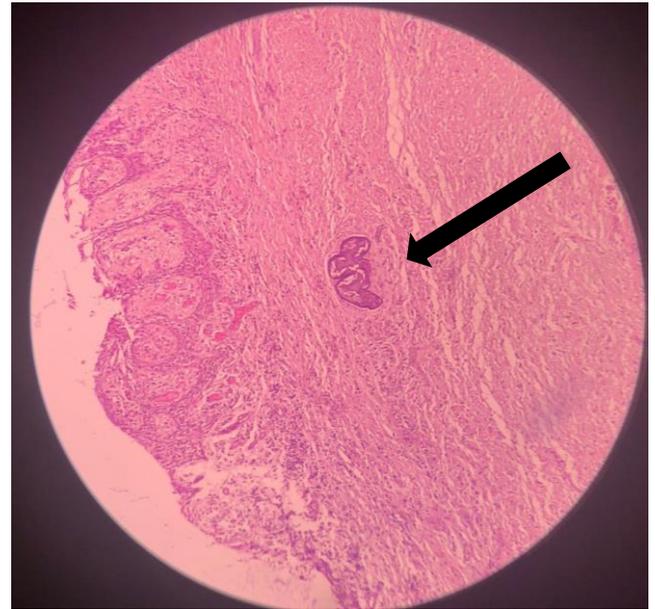
**Figure 1.** shows one of the first group samples after the traditional hematoxylin and eosin staining (x400).



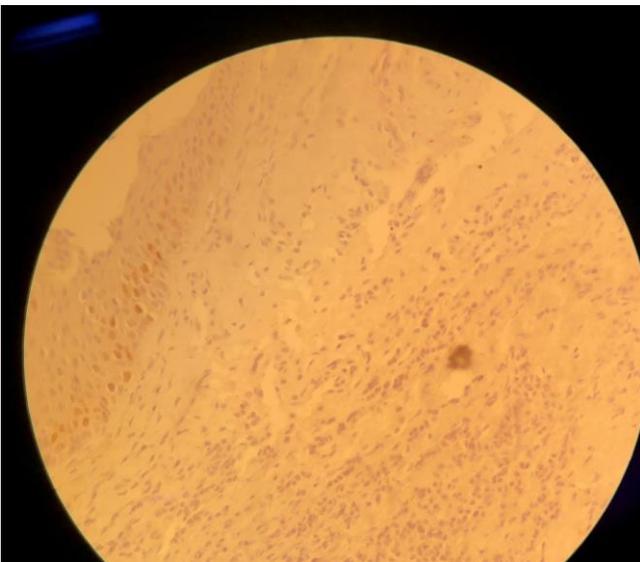
**Figure 2.** shows one of the second group samples after the traditional hematoxylin and eosin staining, showing Squamous proliferation deemed histologically cystic (x400).



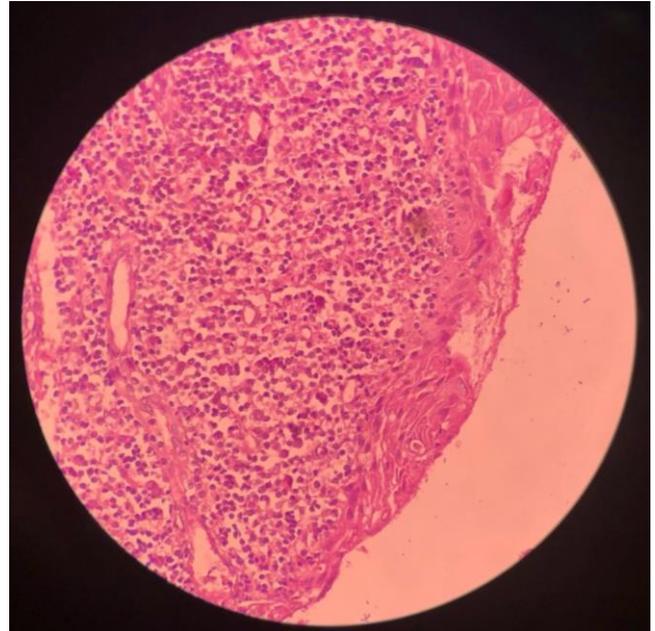
**Figure 3.** shows the distribution of Ki-67-positive cells in Group 1. Ki-67-positive cells. The positive cells were rarely observed on supra-basal layer (x400).



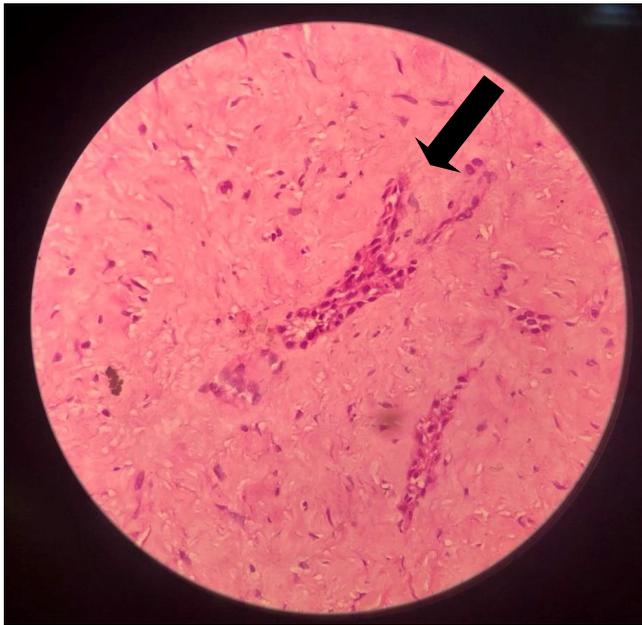
**Figure 5.** shows one of the second group samples after the traditional hematoxylin and eosin staining, showing calcification in the Connective tissue (x100).



**Figure 4.** shows the distribution of Ki-67-positive cells in Group 2. Ki-67-positive nuclei were confined to both basal and supra-basal portions of the epithelium and were more than those in group 1 (x400).



**Figure 6.** shows one of the second group samples after the traditional hematoxylin and eosin staining, showing inflammatory cells in the Connective tissue (x400).



**Figure 7.** shows one of the first group samples after the traditional hematoxylin and eosin staining, showing Remnant of reduced enamel in the Connective tissue (x400).

**Results**

The patients were divided into 2 age groups. nineteen cases out of 37 DF were between the age group of (18 -24) (Group 1) and eighteen were (< 24) years (Group 2). all of the Dental follicles were lined with squamous epithelium. Fibrous connective tissues [Figure 1].

The histologic examination of DF specimens showed 21% and 44% squamous proliferation [Figure 2], 10% and 22% inflammatory cells [Figure 6], 0% and 5.6% calcification [Figure 5], 5.3% and 5.6% reduced enamel in Group 1 and Group 2 cases respectively [Table 2].

Patients in each group were divided into smoker and non-smoker as in the [Table 1], [Table 3] shows the mean, standard deviation, standard error, minimum and maximum of Ki-67 expression in the research sample according to smoking habit and age group of the patient. the distribution of Ki-67 Expressions in Group 1 was mainly confined to the basal location. The positive cells were rarely observed in the supra-basal layer [Figure 3] but in Group 2 positive cells were confined to both basal and supra-basal portions of the epithelium and more than those in group 1 [Figure 4].

	Patients number			Percentage		
	Non-smoker	smoker	Total	Non-smoker	smoker	Total
Group 1	13	6	19	68.4	31.6	100
Group 2	7	11	18	38.9	61.1	100

**Table 1.** The distribution of the research sample according to smoking habit and age group of the patient.

	Group 1 (%)	Group 2 (%)
squamous proliferation	21	44
inflammatory cells	10	22
calcification	0	5.6
reduced enamel	5.3	5.6

**Table 2.** Shows Histologic data of some components

KI-67 Expression (%)	Smoking	Dfs number	mean	standard deviation	standard error	Minimum (%)	Maximum (%)
			Group 1	No	13	0.85	0.83
Group 2	Yes	6	3.97	1.98	0.81	1.2	6.6
	No	7	2.06	0.96	0.36	1	4
Group 2	Yes	11	7.03	3.89	1.17	3.6	17

**Table 3.** Shows the mean, standard deviation, standard error, minimum and maximum of KI-67 expression in the research sample according to smoking habit and age group of the patient.

		T	mean	P value
KI-67 Expression (%)	Group 1	-4.937	-3.12	<b>0.000</b>
	Group 2	-3.280	-4.97	<b>0.005</b>

**Table 4.** Independent samples T-test was performed to study the significance of differences in the average expression rate of KI-67 (%) between non-smokers and smokers in the research sample according to the patient's age group.

two variables studied = expression distribution of KI-67 × patient age group			
DF's number	X <sup>2</sup>	P value	result
34	14.454	0.000	there is statistically significant differences

**Table 5.** Chi-Square Test was performed to study the significance of differences in the frequency of The distribution of Ki67 among epithelial layers between group1 and the group2 of the research sample as follows.

The above table indicates that the value of the significance level is much smaller than the value 0.05, ie that at 95% confidence level there are statistically significant differences in the frequency of expression distribution of protein KI-67 between the group of the research sample.

## Discussion

Cell proliferation is a vital biological process due to the role it plays in the growth and maintenance of tissue balance and is confined to the basal layer of normal epidermis. Therefore, the presence of cell proliferation activity in the rest of the layers is considered abnormal proliferation, and the process of cell proliferation helps in the classification of tumors based on Histology, where several studies have reported that abnormal cell proliferation may be an indicator of tumor formation<sup>17</sup>. Histopathological studies have shown that the incidence of pathological changes in the lower third molar follicles can be as high as 50%<sup>18</sup>.

The results of this study showed that Ki67 expressions in the follicular epithelium of asymptomatic ILTMs was statistically higher in the smoker group than in the nonsmoker group. Cabbar et al investigated the pathologic proliferation potential of 59 asymptomatic third molars and found that Ki67 intensity in asymptomatic third molar follicles was statistically higher than Ki67 intensity in healthy gingival tissues. They suggested the prophylactic removal of asymptomatic third molars<sup>14</sup>. Macluskey et al compared Ki67 expressions in normal oral mucosa, dysplastic mucosa, and squamous cell carcinoma. They found statistically higher expressions in squamous cell carcinoma and dysplasia than in normal oral mucosa. A comparison of Ki67 scores of dysplasia and squamous cell carcinoma showed no major difference. They claimed that Ki67 expression is an indicator of pathologic changes in tissues, but not a good indicator of neoplastic transformations<sup>19</sup>.

The reasons that makes Ki67 a reliable and widely used cell proliferation marker is its Expression during all active phases of the cell cycle (G1, S, G2 and mitosis) except G0<sup>20</sup>. For these reasons, the authors In many studies, used Ki67 protein as a proliferation biomarker to recognize the pathologic alterations in ILTM follicles, Therefore, we preferred Ki-67 proliferation marker in this study. Iamaroon et al compared the expression of Ki67 in normal epithelium, dysplasia, hyperkeratosis, and squamous cell carcinoma. Dysplasia exhibited a considerably higher Ki67 expression than normal epithelium<sup>21</sup>. Some studies have shown that expressions of Ki67 in odontogenic keratocysts

are higher than in radicular and dentigerous cysts<sup>22, 23</sup>. Studies have showed that smoking induces pathologic and tumoral changes by stimulating epithelial cell proliferation<sup>24, 25</sup>, and Smoking is known as exogenous factor in oral tumorigenesis<sup>26, 27</sup>. Our study concurs with Toptaş et al where they found that the mean expression ratios of KI-67 in asymptomatic lower third molar follicles in smokers were higher than in non-smokers, where they considered that smoking could be one of the external factors causing pathological changes in asymptomatic molar follicles. Therefore Preventive extractions, due to the increased risk of pathological changes, should be considered in smokers<sup>28</sup>. Ozarslan et al showed that mean epidermal growth factor receptor staining scores of ILTM follicles in smokers is higher than in nonsmokers and suggested the extraction of asymptomatic ILTMs in smokers<sup>29</sup>. According to the present results, mean Ki67 expression scores in the pericoronal follicles in smokers were statistically higher than in nonsmokers. The present study has shown that smoking can cause pathologic proliferation and alteration in asymptomatic ILTM follicles as an exogenous factor. Therefore, prophylactic removal of ILTMs, because of the greater pathologic alteration risk of follicles, can be considered in smokers.

Many studies have showed that age is One of the most important criteria of whether to prophylactically remove asymptomatic ILTMs. These Studies have claimed that The pathologies and pathologic risks related to the dental follicle increase as age advances<sup>3 18, 30, 31</sup>.

Boehme et al and Brown et al found different distribution of Ki67 among epithelial layers<sup>32, 33</sup>. Detection of Ki67 expressions in suprabasal layers can be an indicator of the pathologic alteration potential of tissue<sup>34</sup>. In the present study, Ki67 expressions in epithelial layers were statistically different between smokers and nonsmokers (Table 5). But in Toptas, there was no statistically different between smokers and nonsmokers<sup>28</sup>.

Thomson et al reported that a 20% suprabasal layer expression of Ki67 in healthy oral mucosa and an expression higher than 30% in dysplasia and carcinomas<sup>35</sup>. In the present study, 32.4% of all follicles showed Ki67 expression in the suprabasal layers. Asymptomatic ILTM follicles might have a strong potential for premalignant alteration risk

independent from smoking, according to the present findings.

## Conclusions

According to the epithelial layer staining in the present samples Asymptomatic impacted third molars follicles have the potential for pathologic changes. Smokers, especially those older than 24 years, have a higher pathologic modulation risk than non-smokers. These factors should be considered when deciding whether to remove ILTMs. According to the present study, we suggest the extraction of asymptomatic ILTMs, especially in smoker patients.

## Declaration of Interest

The authors report no conflict of interest.

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