Original Article

Epidermal Growth Factor Receptor (EGFR) Expression and Incidence of Cystic Changes in Asymptomatic Dental Follicles in Smoking Patients

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ABSTRACT

Background: Smoking is a well-known predisposing factor for pathological changes in oral tissues. Epithelial proliferation due to smoking is caused by the increased expression and activation of epidermal growth factor receptor (EGFR), which is a protooncogen.

Aims: The objective of this study was to evaluate Epidermal Growth Factor Receptor (EGFR) expression and intensity and the incidence of histologically diagnosed cystic changes in asymptomatic impacted lower third molar (ILTM) follicles of smoking and non-smoking patients.

Materials and Methods: A hundred dental follicles obtained from asymptomatic ILTMs were examined histopathologically and immunohistochemically using antibody against EGFR. After initial microscopic analyses, 18 specimens were excluded from the study because of lack of epithelium. The final EGFR expression intensity score of samples was determined by multiplying the percentage of positively stained cells by the staining intensity.

Results: Cystic changes were detected with microscopic evaluation. In the smoking group, the relation between EGFR scores and cystic changes were found statistically significant ($p<0.05$). The relation of age and EGFR scores that showed cystic changes in the smoking group was also found statistically significant ($p<0.05$).

Conclusion: Asymptomatic ILTMs of the smoker patients may be suspected for the higher possibility of cystic changes compared with the nonsmokers. Smoking may be considered as a factor for the decision of removal of an asymptomatic ILTM.

Key Words*:* EGF Receptor, cystic changes, dental follicles, smoking

INTRODUCTION

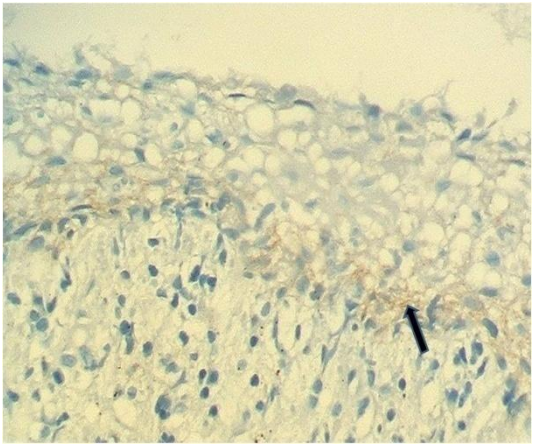
There are well-established indications for the removal of impacted teeth. However, prophylactic removal of asymptomatic impacted teeth is still controversial. Several histopathological studies have demonstrated that the incidence of pathosis in follicular tissues is higher than perceived when assessed by radiographical examination alone [1-6]. Pericoronal follicles occasionally remain in the tissue adjacent to the crown of impacted teeth, which may lead to the development of cysts or tumors that arise from odontogenic epithelial rests [2, 3]. Smoking is a well-known predisposing factor for pathological changes in oral tissues [7]. Epithelial proliferation due to smoking is caused by the increased expression and activation of epidermal growth factor receptor (EGFR), which is a protooncogen [8]. Metabolites of cigarette smoke induce expression and activation of EGFR [8]. Studies investigating odontogenic cysts have shown that EGFR expression increases due to the proliferative activity of the lesions [9]. At the same time, different levels of EGFR expression in odontogenic cysts have been reported, with the strongest expression observed in odontogenic keratocysts [9-12].

The aim of this study was to examine the effects of smoking on the incidence of cystic changes in pericoronal follicles, located around asymptomatic impacted lower third molars (ILTM) of smoking and non-smoking patients, by comparing EGFR expression intensity. We also investigated whether smoking may be considered an exogenous predisposing factor when considering the removal of an asymptomatic ILTM.

METHOD AND MATERIALS

Patients referred to our clinic for removal of asymptomatic, fully impacted lower third molars were enrolled in this study. Clinical and radiographical examinations were performed to determine both whether the teeth were fully covered by the mucosa and partially or completely covered by bone. Smoking and non-smoking patients were selected randomly. Inclusion criteria were: healthy patients, without serious medical alterations or blood dyscrasias. Exclusion criteria included a history or signs of infection, enlarged tissue surrounding the impacted third molars, and history of smoking for non-smoking group. Follicular spaces were measured from panoramic radiographs by each author independently, without regard to the magnification factor reported by the manufacturer. Follicular spaces greater than 2.5 mm were excluded from the study [6]. Reasons for removal of the ILTM were orthodontics, destruction of adjacent tooth, and prophylactic removal in the presence of medical or surgical conditions. Approval for the study was obtained from the local ethics committee and informed consent was obtained from all the participating patients (Ethical Committee of Süleyman Demirel University Faculty of Medicine, Decision Number: 202-4826). All operations were carried out under local anesthesia by conventional third molar surgery. One hundred follicles were obtained from 100 patients, and were sent to the pathology department for histopathological and immunohistochemical analyses. All histopathological and immunohistochemical analyses were performed by the same pathologist in a blind fashion.

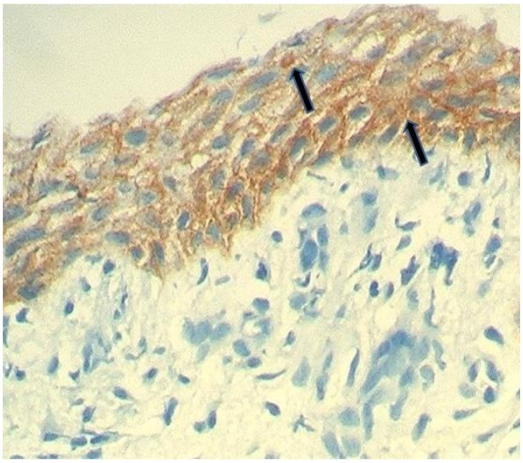
Fig 1: Low intensity EGFR staining in basal layer of the squamous epithelium (EGFR x 400)



Specimens were fixed in 10% neutral buffered formalin, and fixed tissue samples were processed routinely. One section from each follicle was stained with hematoxylin-eosin (Hematoxylin, Bio-Optica, Milano S.p.A., Italy; Eosin, Merck&Co., Inc., NY, USA) for histopathologic examination, and the other section was stained with anti-EGFR monoclonal antibody (Gene Tex, Inc., CA, USA) for immunohistochemical evaluation.

Following initial microscopic analyses, 18 specimens were excluded from the study owing to a lack of epithelium. The proportion of stained cells and staining intensity were combined to assess the immunohistochemical stain. The final EGFR expression intensity score of the samples was determined by multiplying the percentage of

Fig 2: High intensity EGFR staining in all layers of the squamous epithelium (EGFR x 400)



positively stained cells by the staining intensity. The proportion of EGFR staining was graded and scored as: 0 points for negative staining, 1 point for <10% positive cells, 2 points for 10–50% positive cells, and 3 points for >50% positivity. According to the intensity of the positive reaction of EGFR, the staining intensity was graded and scored as: 1 point for weak staining (+), 2 points for moderate staining (++), and 3 points for strong staining (+++). To achieve the overall evaluation of EGFR expression in positive specimens, the overall score for each test specimen was obtained by multiplying the extent of the immunoreactivity score by the intensity score. In this study, an overall score of ≥4 was defined as strong expression or over-expression, and <4 as weak expression of EGFR. Scores were grouped as either low-intensity or high-intensity EGFR. The staining intensity in the low-intensity group varied from 0–3, whereas that for the high-intensity group varied from 4–6 [13] (Fig 1 and 2). Statistical analyses were conducted using SPSS 16.0 software (SPSS Inc., Chicago, USA), with statistical significance set at $p = 0.05$.

RESULTS

Pericoronal follicles of clinically and radiographically asymptomatic ILTMs were

obtained from 82 patients enrolled in the study: 41 in the smoker group and 41 in the non-smoker group. Patient ages ranged from 17 to 43 years (mean age = 22.1 years). The mean age was 23.6 ± 5.1 years in the smoker group, and 20.7 ± 2.4 years in the non-smoker group. In the smoker group, 51% were female, and 49% were male; in the non-smoker group, 71% were female, and 29% were male.

Table 1: The relation between the EGFR scores of the smoking and non-smoking patients

	EGFR Scores		
	Low	**High**	**Total**
Non-smoking (f) (%)	22 (54%)	19 (46%)	41 (100%)
Smoking (f) (%)	11 (27%)	30 (73%)	41 (100%)
Total (f) (%)	33 (40%)	49 (60%)	82 (100%)

p<0.05, Significant

High-intensity EGFR scores were observed in 73% of the smoker group and 54% of the non-smoker group. According to the distribution of these EGFR scores, chi-square analyses showed a statistically significant relationship between smoking and EGFR expression ($p < 0.05$) (Table 1).

Table 2: The relation of cystic changes in smoking and non-smoking groups

	Cystic Changes		
	+	_	Total
Non-smoking (f) (%)	18 (44%)	23 (56%)	41 (100%)
Smoking (f) (%)	12 (29%)	29 (71%)	41 (100%)
Total (f) (%)	30 (38%)	52 (84%)	82 (100%)

p>0.05, Non significant

During microscopic evaluation of the tissues, any soft tissue specimen with the presence of a dense, fibrous connective wall, lined by a few layers of stratified squamous epithelium, was defined as cystic changes.[5] On the basis of this definition, 30 specimens showed cystic changes (36.55%); cystic changes were observed in 18 (44%) specimens in the non-smoking group and 12 specimens (29%) in the smoking group. The relationship between smoking and the incidence of cystic changes was not statistically significant ($p > 0.05$, NS) (Table 2). The presence of cystic changes and EGFR scores were analyzed in both the smoking and non-smoking groups separately. EGFR score was not significantly related to the presence of cystic changes in the non-smoking group ($p > 0.05$, NS). However, the relationship between EGFR scores and cystic changes was statistically significant ($p < 0.05$) in the smoking group (Table 3); all specimens displaying cystic changes were accompanied by high EGFR scores.

Table 3: The relation of cystic changes and EGFR scores in the smoking group

	EGFR Scores		
Cystic Changes	Low	High	Total
+ (f) (%)	00 (00%)	12 (100%)	112 (100%)
- (f) (%)	11 (38%)	18 (71%)	29 (100%)
Total (f) (%)	11 (27%)	30 (73%)	841(100%)

p<0.05, Significant

The relationship between patient age and EGFR scores in samples that showed cystic changes in the smoking group was also found to be statistically significant. The EGFR scores in patients ≥22 years old were higher than the scores of patients <22 years old in the smoking group ($p < 0.05$). Moreover, 15 of 17 EGFR scores were high in patients ≥22 years old and 6 of 13 EGFR scores were high in patients <22 years old.

DISCUSSION

Although pathology associated with impacted third molars is a clear indication for removal, there is no universally accepted treatment for asymptomatic impacted third molars [2,14,15]. Results of radiological and histopathological studies suggest that the incidence of pathological changes associated with asymptomatic ILTMs is much higher than reported in radiographic studies alone [2,4,5]. It is unknown whether an exogenous predisposing factor such as smoking may contribute to pathological changes observed in asymptomatic ILTMs. In this study, we found that EGFR scores of the smoking group were higher than the non-smoking group. Therefore, the risk of pathological changes in asymptomatic ILTMs may be higher in smokers than the non-smokers owing to the overexpression of EGFR in pericoronal follicles caused by smoking.

Several studies have shown that impacted third molar follicles may be related to infection, odontogenic tumors, and cysts [3,6,16-18]. Of the potential complications, cystic changes are the most commonly reported pathosis in the literature [2,4,6,19]. Cystic changes around asymptomatic ILTMs have been reported in 23–50% of patients [2,4-6,19,20]. In this study, 36.35% of the specimens analyzed showed cystic changes. The incidence of cystic changes was 44% in the non-smoking group and 29% in the smoking group. According to these histopathological results, no relationship between smoking and cystic changes

in asymptomatic ILTMs was observed. After calculating EGFR scores immunohistochemically in both groups, we found a statistically significant relationship between EGFR scores and cystic changes in the smoking group; all specimens displaying cystic changes were accompanied by high EGFR scores. In the non-smoking group, high EGFR scores were observed in 50% of the analyzed specimens. Although no relationship was observed between smoking and cystic changes by histopathological examination, higher EGFR scores in specimens that showed cystic changes in the smoking group demonstrated an increased expression and activation of EGFR. Indeed, this increased expression and activation of EGFR is a known cause of epithelial proliferation due to smoking [8]. In response to tobacco smoke, epithelial cells activate EGFR through various mechanisms [8]. At the same time, increased EGFR expression has been reported in odontogenic cysts and tumors in several studies [21].Therefore, although the possibility of the development of a clinically relevant dentigerous cyst needs further investigation, it can be speculated that pericoronal follicles of smoking patients carry a higher risk of cystic changes due to increased epithelial proliferation and activation related to tobacco smoke.

Adelsperger, et al. found non-keratinizing squamous epithelium of variable thickness in 34% of the samples, indicating the presence of dentigerous cysts in the pericoronal follicles of impacted third molars without radiolucency [2]. They reported increased cellular activity and epithelial proliferation, evidenced by the presence of proliferating cell nuclear antigen in the majority of the cystic tissues and its absence in the healthy follicular tissues [2]. Li, et al. concluded that members of the EGF family are important, and play a differential role in the initiation and growth of odontogenic cysts [9,10]. Furthermore, von Oijen, et al. demonstrated an increased number of proliferating cells in the upper digestive tract epithelium of smokers [22]. Taken together, these results suggest that smoking may be an exogenous predisposing factor that may increase the risk of cystic changes in asymptomatic impacted lower third molars in smokers.

In the decision to remove impacted lower third molars, age is reported as an important criterion because of increased post-operative complications and pathological progression risk observed in older patients [2,4,5,19,20,23,24]. It has been reported that the patients over the age of 20 demonstrate higher indices of pathological change [2,4,5,25]. In our previous data concerning the incidence of cystic changes associated with asymptomatic ILTMs, 56% of patients were older than 20 years [6]. Yıldırım, et al. reported cystic changes in 89% of the patients ≥20 years old [20]. In this study, there was a statistically significant relationship between age and the EGFR scores that showed cystic changes in the smoking group. The EGFR scores of patients >22 years old were higher than the EGFR scores of patients ≤22 years old. Increasing age among smokers may also correlate with an increased the amount of smoke exposure, which may explain the higher EGFR scores of the smoking patients with cystic changes.

CONCLUSION

Asymptomatic ILTMs of smokers may increase the possibility of high EGFR expression, especially in patients above 20 years of age, compared with non-smokers. This increased expression and activation of EGFR can be cause of epithelial proliferation due to smoking. Therefore, smoking or a history of smoking may be considered as a factor in the decision to remove an asymptomatic ILTM. More studies are necessary to prove smoking and EGFR association.

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