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Evaluation of pathogenicity of the tissues surrounding partially and fully impacted teeth in smokers and non-smokers

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ABSTRACT

The aim of this study was to investigate the presence of cell cycle transcription factors p53 and Ki67 in dental follicle cells of asymptomatic impacted teeth to determine the propensity for precancerous lesions. One hundred impacted third molar teeth follicle tissues from 100 patients were used in the research. Following formaldehyde fixation, expression of p53 and Ki67 were determined by immunohistochemical staining, and pathologic evaluation was carried out in hematoxylin and eosin (H&E) stained specimens. The results were correlated according to the age, gender, smoking habits, and degree of tooth impaction in the patients. Histopathological observation revealed the presence of inflammation, granulation tissue, and pseudoepitheliomatous hyperplasia. Dysplasia and neoplastic changes were not detected. A positive correlation was present between Ki67 and p53 ($P \leq 0.01$). A Significant increase of p53 was detected in follicular tissues of teeth partially exposed to the oral environment. The immunohistochemical expression of p53 was positively associated with the state of the tooth impaction; however, the impact of the smoking habits of the patients on the cell cycle transcription factor expression was somehow inconclusive within the limitations of this research.

Keywords: *Dental follicles, cell cycle transcription factors, P53, Ki67, smoking*

INTRODUCTION

Located in the lower and upper jaws, impacted third molar teeth are the most commonly extracted teeth (1). While they can remain in the mouth for years without symptoms, sometimes they can lead to resorption, pain, infections, and formations such as cysts and tumors in the adjacent teeth (2). Cysts can form from and tumoral changes can occur in the pericoronal tissues and tooth follicles of fully impacted third molar teeth (3,4). While genetic influence has been emphasized in explaining pathological changes, it has been a matter of discussion on the other hand that environmental factors (such as smoking, food, etc.) and some viruses may be possible causes (5).

Because the Ki67 protein can only be detected in proliferating cells, it indicates the growth fraction of a tumor and is thus regarded as evidence of tumor proliferation (6). It is used quite often for early diagnosis in cancer cases and in clinical studies as a possible indicator of genetic anomalies that accompany premalignant and malignant lesions of the oral cavity (7).

p53, a nuclear phosphoprotein, is a tumor suppressor gene known to often undergo changes in head and neck and other types of cancer including, in particular, oral squamous cell carcinoma among oral cancers (8). It binds directly to DNA as a result of cell injury and recognizes the damage. Because it is an essential key in the apoptosis mechanism, the presence of the p53 protein is important for the elimination of genetically damaged cells (9). p53 protein is found in very small amounts in normal tissues and because it has a very short half-life and is subject to enzymatic degradation, it cannot be detected by immunohistochemical methods. However, because the p53 gene undergoes mutation in cancerous tissues, its half-life gets prolonged and becomes easily stained in immunohistochemical staining. If there is a risk of a pathological formation, its values rise (10). A high rate of p53 has been shown to indicate a bad portrait in terms of prognosis and differentiation in cancers (11).

Several studies exist, which show the positive relationship between Ki67 and p53 (12,13). The proteins p53 and Ki67 are usually used together in studies in order to identify malignant changes at an early stage and to identify apoptosis and cell proliferation activity. An increase in the distribution and density of Ki67 and p53 has been shown to take place in proliferative changes in the oral mucosa (7).

A correlation has been found between smoking and Ki67 staining (14). Cigarettes contain several carcinogens, and these can lead to cell proliferation and tumoral changes (14,15).

A scanning of the literature revealed that there is no assessment at all of the tissue surrounding partially impacted (in a position that is exposed to the oral environment) teeth in research on pathological changes in impacted third molar teeth. In this study, the effect of impacted teeth and their surrounding tissues being exposed/unexposed to the oral environment on pathological predisposition is assessed.

The aim of this study is to assess the effect of impacted teeth being exposed/unexposed to the oral environment in smoker and non-smoker patient groups on Ki67 and p53 expression densities and distributions.

METHODS

Samples were taken from 300 patients aged between 18-60 years, whose impacted teeth had received an indication for extraction. 100 patients whose file numbers ended with an even number were randomly selected from these samples. This study was been approved by the Ethics Committee of the Van Yuzuncu Yil University Faculty of Medicine (13032014/07) and carried out by providing conditions all stages of the Declaration of Helsinki. The participants gave their written consent at the beginning of the study.

Inclusion criteria: having a third molar tooth with an indication for extraction, being ASA1 healthy volunteers, and not having any condition that poses an obstacle to surgical extraction. The patient's age, sex, systemic state, smoking status, and the relationship of the extracted teeth to the oral environment (being fully or partially impacted) were recorded.

Routine impacted tooth extraction techniques were applied to extract the teeth. Care was taken to remove the tooth and surrounding tissues (pericoronal follicle, the epithelium in the distal part of the tooth) in one piece and without damage. The samples were placed in a 10% neutral formalin solution and were sent for histopathological and immunohistochemical examination.

The tissues were prepared for histopathological and immunohistochemical examination by a pathologist and transferred to slides. One of the resulting 9 slides was stained with hematoxylin-eosin in the automated slide staining device. The remaining 8 slides were stained using antibodies developed against the p53 (NCL-L-p53-DO7) and Ki-67 (NCL-L-Ki67-MM1) proteins and positive external controls in the automated immunohistochemical staining device (Leica Bond-Max, Leica Biosystems, United Kingdom). The hematoxylin-eosin stained slides were examined for histopathological findings such as benign and malignant neoplastic lesions and dysplasia in particular and the slides that were immunohistochemically stained were assessed by a pathologist under a light microscope.

Assessment of Immunohistochemical Staining

Nuclear stains in the dental follicle epithelium were assessed as "positive" for the p53 and Ki67 antibodies. Cases with no staining were regarded as "negative" for p53. The staining intensities of Ki67 and p53 in the dental follicle were evaluated; the extent of staining was determined by identifying "hot points" and the place of the cell showing the highest level of positivity in the epithelium from the basal layer upward was assessed.

Accordingly: The most densely stained areas were identified with x40 lens [1 high power field] on a light microscope to assess the extent of staining of Ki67 and p53 in the dental follicle epithelium and these foci were considered as "hot points". p53 positive cells were counted in three hot points and their arithmetic mean was calculated. The resulting value was scored as follows for p53:

- Score 1: 1-20 cells
- Score 2: 21-50 cells
- Score 3: 51-100 cells
- Score 4: 101-150 cells
- Score 5: 151 cells and higher

The value obtained was scored as follows for Ki67:

- Score 1: 1-20 cells
- Score 2: 21-40 cells
- Score 3: 41 cells and higher

Statistical Method

While descriptive statistics were expressed as mean, standard deviation, and minimum and maximum values for continuous variables in the study, they were expressed as numbers and percentages for categorical variables. Student's t-test was used to compare the group means. Pearson correlation was

used to examine the relationships between variables. The level of statistical significance was taken as 5% in calculations and data analyses were performed using the IBM SPSS Statistics for Windows (IBM Corp., Armonk, NY, USA, version 20).

RESULTS

The tissues surrounding the asymptomatic impacted/partially impacted teeth of 100 patients consisting of 45 females and 55 males aged between 18-56 years with a mean age of 24.71 years were examined in the study.

28% of the patients were smokers and 72% were non-smokers. 10% of the tooth follicles investigated were on the upper jaw and 90% were lower jaw tooth follicles. While 26 of the teeth were exposed to the oral environment, 74 were completely unexposed.

The average of the Ki67 positive cell average was found to be 26.64 and the average of the p53 positive cell average was found to be 40.54. There is a significant relationship between the Ki67 and p53 positive cell averages. As Ki67 positivity increases, p53 positivity also increases (p=0.00) (Figure 1).

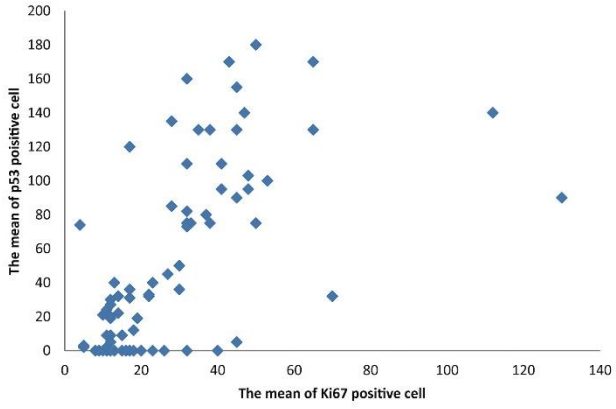

Figure 1. The relationship between mean p53- and Ki67-positive cell numbers

The p53 positive cell average was seen to be significantly high in a total of 43 patients. When we looked at the relationship between sex and active and chronic inflammation variables, we determined that sex and these variables are independent of each other. No significant relationship was found between chronic inflammation and the Ki67 and p53 positive cell average scores (p>0.05). However, as the score increased in both tumor markers, the rate of chronic inflammation also increased (Figure 2).

No dysplasia or neoplasia was revealed in histopathological examinations in the research. All markers were assessed according to smoking status and the relationship of the impacted tooth to the oral environment. No significant relationship was found between the Ki67 positive cell average and the relationship of the impacted tooth to the oral environment (P=0.960) and smoking status (P=0.838). The p53 positive cell average was found to be significantly high in patients with an impacted tooth exposed to the oral environment (P=0.039). There was a significant correlation between smoking and the p53 positive cell average. (Figure 3).

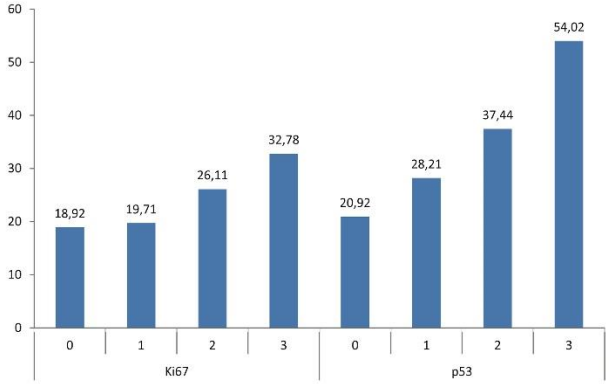

Figure 2. Chronic inflammation ratios in relation to mean p53- and Ki67-positive cell numbers in dental follicles

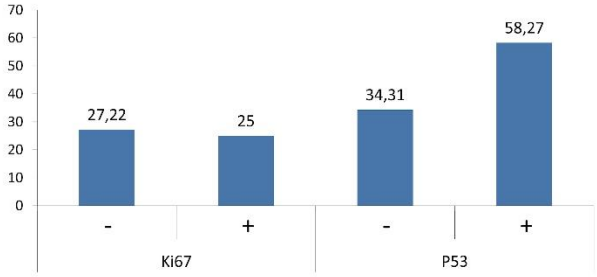

Figure 3. Average Ki67- and p53-positive cell numbers in relation to the status of the impacted teeth included in this study

DISCUSSION

Our hypothesis in this study was that smoking could lead to pathological changes in the tissues surrounding the tooth and speed up the process compared to non-smokers and likewise with impacted teeth being unexposed to the oral environment compared to being exposed.

The Ki67 and p53 proteins and their densities have been reported to increase in the case of lichenoid lesions, cysts, and proliferative changes in impacted tooth follicles and the oral mucosa and tumoral formations (7,16-19). At the same time, it is also being said that p53 and Ki67 expressions do not constitute certain evidence for malignancy and that

decreased p53 even indicates poor prognosis in pathologies (15-19). In this respect, it is still not possible to exactly talk about a certainty for p53 and Ki67, other than the risk of a pathological formation. Although p53 and Ki67 are detected at high levels in the case of premalignant mucosal changes, the significance of these proteins has not gone further than being "suspicious" (20). In this study, there was a statistically significant correlation when Ki67 and p53 were evaluated together and in samples with a high Ki67 positive cell average, the p53 positive cell average was also found to be high. However, no identifiable dysplasia/neoplasia was observed in the tissues. Due to there being no systemic problem in the individuals from whom the samples were collected, their immune systems may not have allowed for proliferative changes in the tissue. On the other hand, the positive cell average rate of these two tumor markers may have been affected by cellular activity in patients in whom chronic inflammation was observed. This is because although no significant correlation was revealed between chronic inflammation and the Ki67 and p53 positive cell average score, the rate of chronic inflammation was observed to increase as the score increased in both tumor markers. Ki67 and p53 can also be detected in tissues with chronic inflammation in which no proliferative/neoplastic change was observed.

Smoking is a well-known exogenous factor in oral tumorigenesis (21,22). Studies show that smoking causes pathological and tumoral changes by stimulating epithelial cell proliferation (23). Özarslan et al (24). report that the epidermal growth factor in the tissue surrounding the impacted third molar teeth in smokers gets stained more compared to non-smokers.

Despite those arguing that smoking is influential in p53 and Ki67 expressions being high, no significant relationship was found between Ki67 and p53 and smoking status in our study results. In other studies, it is reported that environmental factors such as smoking and alcohol activate HPV and trigger the development of cancer (25-27). In the study that Toptaş et al.(28) conducted in order to clarify under which conditions smoking became a predisposing factor, no statistically significant difference was detected between smokers and non-smokers in terms of Ki67 and p53 expressions in individuals younger than 22 years old, while a difference was seen to occur in individuals older than 22 years old. This means that as the duration of smoking increases, its possibility of causing pathogenicity also increases. The fact that smokers and non-smokers were not equally distributed in the results we obtained may have had an impact on the conclusion that we reached. In addition, the effect of the daily amount of smoking on smokers and for how long they have been smoking on these expressions can also be separately evaluated.

In this research, whether or not there is a relationship between maxillary and mandibular impacted third molar teeth being exposed or unexposed to the oral environment and the presence of p53 and Ki67 was also investigated. In the literature, expression studies only include data for fully impacted teeth, and no study on the pathogenicity potential of partially impacted teeth was found. While a statistically significant relationship was found as a result of the study when the p53 positive cell average and the state of being fully/partially impacted were evaluated, no such relationship was found with Ki67. p53 was detected at higher levels in teeth that were exposed to the oral environment. It should also be kept in mind that partially erupted teeth can carry the same possibility of potential pathogenicity as fully impacted teeth.

One of the conditions that limited the research was keeping the patient population limited due to its cost. If a histopathological and immunohistochemical assessment of all 300 patients could be done, different results could have been obtained. We are of the opinion that it would be good for these types of studies to be reevaluated by increasing the number of samples and reinforcing them with advanced testing methods.

In conclusion, it has been revealed within the limits of this study that the amount of smoking can be an important factor in pathogenicity and that partially impacted teeth can carry the same potential for pathogenicity as fully impacted teeth. Clinically, it may be better to subject not only follicles but also adjacent soft tissues that are in direct contact with the tooth to histopathological assessment after all kinds of impacted and partially impacted tooth extractions, considering that the follicle and oral epithelium that is in contact with the tooth may serve as a reservoir for pathogenic factors.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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