Expression of P63 in Oral Mucosa Covering Impacted Teeth: An Immunohistochemical Study

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Abstract

Objective: The purpose of the study was to examine the expression of p63 in asymptomatic enclosed lower third molar covering oral mucosa.
Design: : 50 samples of normal oral mucosa taken from 50 patients who underwent third molar surgery were included into the study. The samples were subdivided into two groups. One group was made of oral mucosa covering completely impacted teeth, while other group was made of oral mucosa covering partially impacted teeth.
Only nuclear staining of the cells was evaluated by percentage of positive cells.
Results: : Immunoexpression of p63 was almost equal in submucosa cells of both groups, showing a faint to intermediate positivity. Basal layer of oral mucosa covering completely enclosed teeth have shown a stronger expression of p63 than it was the case with the other group.
Conclusion: Expression of p63 is noticed in basal and suprabasal layers of oral mucosa covering impacted teeth. Weak expression of p63 in basal layer of oral mucosa covering partially impacted teeth, migh be associated with teeth eruption, lesser number of stem cells and thinner mucosa patch than it is a case with oral mucosa covering completely impacted teeth.

KEYWORDS: p63, immunohistochemistry, oral mucosa, impacted teeth, stem cells.

Introduction

P63 gene is the member of p53 gene family, found by Young in 1998 (1). It was found that the gene is located in 3q27-29 chromosome and according to presence or absence of a transcriptional activation domain, has a six isoformes, named α, β, γ subdivided into a two groups; TA p63 and ΔNp 63, characterized by different transactivation potentials and biologic properties (1-8).

It was expected, that due the belongs to the familly, p63 gene behaves resembling the p53 as the tumor supressor gene. However, further investigations have shown that only one group of the TAp63 isoformes demonstrate similarity to p53 (1-8).
TAp63 induces apoptosis and mediate cell cycle control by transactivation of p53 target gene, while ΔNp63 behaves dominantly negative, blocking both the activity of TAp63 and p53, inducing a cell proliferation (1-3,5,9). By this way ΔNp63 behaves as an oncogene, which has been confirmed by various studies, where different kinds of squamous cell carcinomas and oral precancerous lesions have shown an overexpression of the ΔNp63 isoformes (2,4,7-16).

Except carcinogenesis, p63 isoformes play more important roles in ectodermal differentiation during embryogenesis, stem cell biology, proliferation of craniofacial structures and epithelial development (2,5,9,11,17-22).

Transcriptionally the most active p63 isoformes are the γ isoformes, while α isoformes contain protein-protein interaction domain known as a sterile alpha motif (SAM), which is involved in developmental processes (5,6,17). It is found that in some congenital syndromes such are ankyloblepharon ectodermal dysplasia and clefting syndrome (AEC or Hay-Wells syndrome) and limb-mammary syndrome (LMS) the SAM domain is mutated (5,17).

Under normal conditions, p63 is expressed in select epithelial cells of the tissues such as embryonic ectoderm, skin, breast myoepithelium, oral epithelium, prostat and urethra (2,5,11,13,18). It is also shown that p63 is involved in maintance of epidermal stem cells and stratification of the epidermis (18-22).

In last decade, the p63 gene was a subject of different immunohistochemical studies, which included normal oral mucosa and pathological changes associated with it (4,7-16).

As we know third molar surgery is one of the most performed procedures in dentistry, characterized by extraction of impacted tooth with curettage of dental follicular and pericoronary tissues. Due the results of our previous study in which, we have investigated pathological changes and immunoexpressivity of p63 in dental follicles of asymptomatic partially and completely impacted lower third molars (22), the aim of this study is to analyze the immunoexpressivity of p63 in the oral mucosa covering these teeth.

MATERIALS AND METHODS

The study was approved by the Istanbul University Ethics Committe for Scientific Research in humans.

Cases selection

Fifty patients (25 male, 25 female), age range from 18 to 35 years, with partially or completelly impacted asymptomatic lower third molars were included into the study. All of the patients were medically healthy non smokers and were not taking any drugs at least for two months before surgery.

Twenty-five teeth were fully covered by mucosa and bone (completely impacted), while 25 teeth were partially covered with bone and mucosa (partially impacted). All surgeries were performed by the same surgeon and under local anesthesia, in the normal course of practice and similar conditions. During the surgery, approximately 2x3mm of covering mucosa tissue sample, taken from the horizontal portion of the incision was excised, immediately fixed in 10% paraformaldehide and after that embedded in parafin, which was cut into 5 μm section by a rotary microtome. From every specimen 2 preparations were made (one preparation was stained with hematoxylin-eosin, while the other preparation was prepared for immunostaining for p63).

Immunohistochemical analysis

Immunohistochemical (IHC) staining of the sections were carried out by streptavidin-biotin peroxidase method using p63 antibody (Gene Tex, Inc, San Antonio, USA). Briefly, 5 micrometer sections were deparaffinized and hydrated through graded alcohols to water. The slides were immersed in citrate buffer in a microwave oven for antigen retrieval for 20 min (5min.x4). After cooling for 20 min and washing in PBS (phosphate buffer saline) for 5 min, endogen peroxidase was blocked with %3 hydrogen peroxidase and then washing in PBS for 15 min. the slides were incubated with block solution for 15 min. Afterward the sildes were incubated with primary antibodies for 30 min., and then washed and incubated with linking reagent for 25 min, the slides were incubated streptavidin-biotin solution for 25 min. Staining was visualized using AEC chromogen. Mayer hematoxylin was used as a counterstain.

IHC staining was evaluated as percentage of positive stained cells and the intensity of the stain according to following criteria: negative nuclear staining (0), when the stained cells were comprised from 0 to < 10%; faint positivity (1+), when the stained cells were comprised from 10 to < 30%; intermediate positivity (2+), when the stained cells were comprised from 30 to < 60%; and strong positivity (3+), when the stained cells were comprised from > 60%.

Data were analysed using SPSS INC; Chicago USA version 16. and significant differences (95% or $P < 0.05$), between groups were determined using Kruskal-Wallis test.

RESULTS

Fifty samples subdivided into two equal groups, of masticatory mucosa covering completely and partially impacted lower third molars, were immunohistochemically examined for expression to p63. Positive nuclear reaction was noticed in basal and suprabasal layers of both groups.

Analyzing the expression of p63 in basal layer of mucosa, in the group of completely impacted teeth; 1 (4%) specimen showed negative nuclear staining, 9 (36%) specimens faint, 9 (36%) intermediate and 6 (24%) specimens showed strong nuclear positivity. (Figure 1. and Figure 2.) The results of another group showed 16 (64%) samples with faint and 9 (36%) samples with intermediate nuclear positivity.

(Figure 3) No sample from this group showed a negative or strong positivity.

P63 immunoexpressivity was almost the same in suprabasal layers of mucosa from the both groups . In the group with completely impacted teeth 2 (8%) samples did not showed nuclear staining for p63 and a number of samples with the same results from another group was 5 (20%). Faint positivity was noticed in 21 (84%) of mucosa samples from the group of completely impacted and 19 (76%) of partially impacted teeth, whereas intermediate positivity for p63 was present in 2 (8%) mucosa samples taken from the group of completely impacted teeth and 1 (4%) from partially

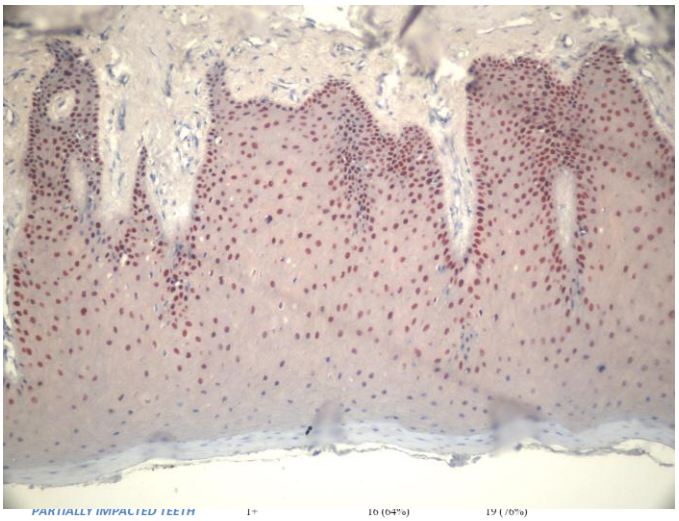

Figure -1 Immunohistochemical stain of oral mucosa covering completely impacted teeth with intermediate (2+) nuclear positivity (p63 x 200).

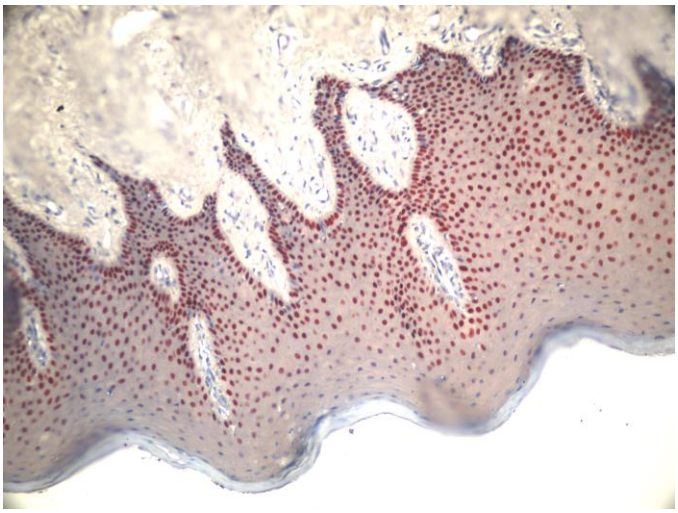

Figure -2 Immunohistochemical stain of oral mucosa covering completely impacted teeth with strong (3+) nuclear positivity (p63 x 200).

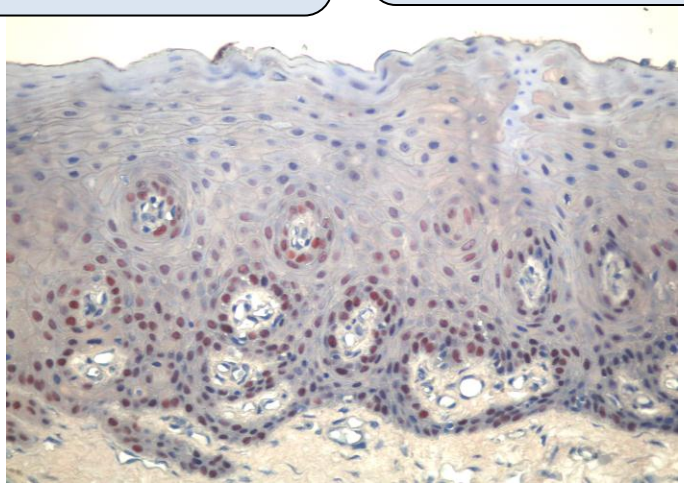

Figure -3 Immunohistochemical stain of oral mucosa covering partially impacted teeth with faint (1+) nuclear positivity (p63 x 400).

	P63 EXPRESSION	BASAL LAYER	SUPRABASAL LAYER
COMPLETELY IMPACTED TEETH	0	1 (4%)	2 (8%)
	1+	9 (36%)	21(84%)
	2+	9 (36%)	2 (8%)
	3+	6 (24%)	0 (0%)
PARTIALLY IMPACTED TEETH	0	0 (0%)	5 (20%)
	1+	16 (64%)	19 (76%)
	2+	9 (36%)	1 (4%)
	3+	0 (0%)	0 (0%)

Table -1 Statistical analysis of p63 expression in oral mucosa covering completely and partially impacted teeth.

impacted teeth. No sample from the both groups did show a strong nuclear positivity. (Table 1)
Comparing the results from the both groups, it is noticed that immunoexpressivity of p63 is almost the same in suprabasal layers (P=1). Although basal layers from the group of completelly impacted teeth show a stronger immunoexpressivity to p63, the results are not statistically significant (P= 0.89).

DISCUSSION

From the last decade, in oral and maxillofacial surgery, the p63 gene is one of the most investigated subjects. Studies by different authors have deal with a role of p63 from the stem cell biology and developemental processes of teeth formations to carcinogenesis (4,5,7-16,20-22).
In one of our previous studies we were examinated pathological changes and immunoexpressivity of p63 in dental follicles of asymptomatic impacted lower third molars (22). We found out that the immunoexpressivity for p63 was stronger in the dental follicles of the group with completely impacted teeth, than it was a case of dental follicles of the group with partially impacted teeth, and concluded that these results might be associated with the follicular stem cells (22). From the same patients, we were in this study analized the expression of p63 in the mucosa samples, where immunoexpression for p63 was noticed in basal and suprabasal layer of both group of samples.
Studies made by the other authors have also shown the expression of p63 in basal and suprabasal layers of normal oral epithelium (4,8-10,12,15). A results from a study of Lo Muzio et al.(4) have shown a high p63 expression in the cells of basal and parabasal layers of oral epithelium, comparing the results with oral squamous cell carcininoma samples. The author concluded that the p63 overexpression in oral carcinogenesis is one of indicators of the ability of oral cancer cells to proliferate and stay undifferentiated (4). With the similar results Nylander et al.(9) and De Oliviera et al.(10) analyzing the expression of p63 in different tissues including oral mucosa and squamous cell carcinomas were disposed . A results from Bortoluzzi et al(8). have shown the presence of p63 immunoexpressivity in basal, parabasal and suprabasal layers of healthy oral mucosa, as same as the strong expression in oral squamous cell carcinomas and oral dysplasia. Chen et al.(12,15) in two different studies, have shown that TAp63 and ΔNp63 isoformes are present in basal and suprabasal layers of buccal mucosa, also with predominant expression of ΔNp63 isoforme in primary well-differentiated buccal carcinoma and oral epithelial dysplasia, confirming once more an oncogenetic role of p63 isoformes. However, ΔNp63 isoformes are not overexpressed only in cancer cells. Basal cells of normal human epithelium show a strong expression of ΔNp (especially α isoforme), which overexpression blocks keratinocyte differentiation. Soon as the cell withdraw from the stem cell section and become differentiated, the ΔNpα expression is lost (13,14,17,18). Some authors suggests that for these reasons, p63 can be a marker for keratinocyte stem cells and its expression may be more related to stem cells maintenance and cell differentiation, than tumorogenesis (8,18,21). Findings from Hatakeyama et al.(20) about expression of p63 in the oral gingival epithelium, resulted with conclusion that basal layer of oral gingival epithelium contain stem cells, which may play an essential role in the supply of gingival epithelial cells.

In our study using for immunohistochemistry a primary antibody which recognize all isoformes of p63, the expression was stronger in basal layer of mucosa covering completely impacted teeth, than it was a case with basal layer of mucosa covering partially impacted teeth. However, the results from Table 1. suggests that the larger number of oral mucosa samples from both groups, would give more statistically significant results, than we have observed (P= 0.89). In mukosa suprabasal layers from the both groups, expression for p63 was almost the same (P=1).
In the English literature we did not found similar studies to our, because of we could not discuss about potential influence of tooth eruption to the stem cells of the covering mucosa and expression of the p63 associated with it. We are in opinion that although statistically not significant, expression of p63 in basal layers of the mucosa covering partially impacted teeth might be associated with teeth eruption, lesser number of keratinocyt stem cells and thinner mucosa patch, than it is a case with oral mucosa covering completely impacted teeth.

Also we hope, that other researchers with their studies will supplement our investigations.

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