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ORIGINAL ARTICLE

Expression and role of p53 in oral lichen planus patients

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Summary

Purpose: *Oral lichen planus (OLP) is an autoimmune skin and mucosal disorder. The range of malignant transformation in OLP varies between 0.1-3%. p53 is a tumor suppressor protein. Defective p53 could allow abnormal cells to proliferate, resulting in cancer. p53 plays an important role in cell cycle control and apoptosis and loss of p53 function has been demonstrated in about half of all human cancers. The purpose of the study was to investigate the malignant potential of OLP on the basis of p53 expression and to correlate p53 expression with clinical and histopathological features of OLP.*

Methods: *40 patients with OLP underwent biopsy. All tissue samples were treated immunohistochemicaly using avidin-biotin peroxidase complex method.*

Results: *In 80% of OLP specimens the nuclei of basal and parabasal keratinocytes were p53-positive, but in low numbers. Low percentage of p53-positive cells in older and medium percentage of p53-positive cells in younger group of OLP patients were noted. Higher intensity of p53 stained keratinocytes, no matter their low number, could represent mutant and more stable form of p53 protein, and at the same time signal for monitoring of disease due to potential malignant transformation. Low percentage and weak intensity of p53-positive cells was detected mostly in OLP specimens with highly expressed civatte bodies (CB). Up-regulation of apoptosis didn't correspond with the expression of CB.*

Conclusion: *We believe that low percentage of p53-positive and well-marked keratinocytes in OLP represent the influence of mutant p53 protein, and that increasing expression of this protein could serve as a valuable diagnostic sign of early carcinogenesis. According to our results intensity of p53 coloration of keratinocytes could help assessing the malignant potential of OLP.*

Key words: *oral lichen planus, p53, premalignant lesion, squamous cell carcinoma*

Introduction

OLP is a chronic inflammatory disease of unknown etiology. Autoimmunity is considered to be the most probable factor of OLP, due to different autoimmune features of disease such as connection of OLP with other autoimmune diseases, depression of immuno-suppressive activity, presence of autocytotoxic T-cell clones on the site of the lesion etc.

Immunohistochemistry is a technique for identifying cellular or tissue constituents (antigens) through antigen-antibody interactions. This technique is often used to characterize the infiltrating cell population (T cells – CD3+, CD4+, CD8+; mast cells and Langerhans cells) [1], describe the expression of molecules regulating apoptosis (Fas, Fas-L, Bcl-2, Bax, p53) [1,2], and clarify the proliferation (mitotic) activity of basal and parabasal cells (Ki-67, p16, cyclins) [3].

Identification of molecules regulating apoptosis could be a significant parameter in the pathogenesis of OLP. Besides, overexpression of these

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molecules and intensive mitotic activity of basal and infiltrating cells are often present in carcinomas. So, on the basis of the results of immunohistochemical examinations it could be predicted the malignant potential of OLP. The most likely range of malignant transformation of OLP described in the literature varies between 0.1-3% [4].

p53 is a tumor suppressor protein. It plays an important role in cell cycle control and apoptosis. p53 protein can trigger arrest in either the G_1 or G_2 phase of the cell cycle. Defective p53 could allow abnormal cells to proliferate, resulting in cancer. In normal cells, the p53 protein level is low. DNA damage, hypoxia, oncogenes and other stress signals may trigger the increase of p53 protein, which has two major functions: growth arrest and apoptosis [5]. The growth arrest stops the progression of the cell cycle, preventing replication of damaged DNA. During the growth arrest, p53 may activate the transcription of proteins involved in DNA repair. Apoptosis is the "last resort" to avoid proliferation of cells containing abnormal DNA. p53 plays an important role in apoptotic cell death in OLP mucosa [6]. This protein is induced in injured keratinocytes and maintains genomic integrity with multiple downstream targets which activate pathways of cell cycle arest, cell repair and apoptotic cell death. p21, one of the downstream target of p53, is a cyclin dependent kinase inhibitor (CDKI), that inhibits cyclin-CDK activity to induce cell cycle arrest. Therefore, cell cycle regulation induced by the p53 and p21 pathway provides an interval for the maintenance of genomic stability [6].

Loss of p53 function has been demonstrated in about half of all human cancers, including oral squamous cell carcinoma (SCC) [7].

Benign versus malignant states may well be balanced between apoptosis and mitosis. Dyskeratotic cells could be considered to represent the apoptotic process, namely the host's reaction for eliminating tumor cells by accelerating keratinization. If the apoptotic process supersedes mitosis, the tumor remains benign [8].

We assumed that expression of p53 in keratinocytes could show progression from healthy oral tissue, through premalignant lesion (OLP) to oral SCC.

The purpose of the study was to investigate the malignant potential of OLP on the basis of p53 expression. It was also examined the correlation of p53 with clinical and histopathological features in OLP.

Methods

The study included 40 patients with diagnosed OLP. Diagnosis of OLP was made on the basis of clinical analysis and histopathological features on the material retrieved from the Laboratory of Pathobiology, University hospital in Nice-France and the Clinic for Oral Medicine and Periodontology in Belgrade, Serbia.

Two control groups were included in this research. The first one consisted of 13 healthy persons and immunohistochemical examination was carried out on the oral mucosa without inflammatory changes. The biopsies of the oral tissue were obtained from patients already indicated for oral surgical treatment. Immunohistochemistry was the same as in the patients with OLP. In the second control group, immunohistochemical examination was carried out on SCC of the oral mucosa obtained by biopsy from 12 patients with highly differentiated SCC. Immunohistochemistry was also the same as in patients with OLP.

Clinical examination

The following clinical parameters were considered in the study: sex, age, clinical type of disease, duration of disease, subjective symptoms, presence of other diseases and drugs used for the therapy of different pathological conditions. Qualitative analysis of oral changes and their localization was determined using clinical examination (size and structure of lesions). Written inform consent was obtained from all study subjects.

Histopathological examination

Histopathological analysis was performed on material obtained by biopsy of oral mucosa of patients with diagnosed OLP. Biopsies were fixed in formalin, embedded in paraffin and routinely sectioned and stained with H&E or PAS.

The following epithelium parameters were analyzed: keratinization – presence and type of keratinization (ortho or parakeratinization), granular layer, hyperplasia, atrophy, acanthosis (ACA), hyperbasal cells (HBZ) – multiplication of basal cells, liquefaction degeneration (LD), civate (eosinophilic) bodies (CB), necrotic cells – keratinocyte cell death, spongiosis – dilatation of intercellular spaces within the basal cell layer of epithelium, exocytosis – penetration of lymphocyte infiltrate from submucosa to the lower layers of epithelium. In the zone of basal membrane and submucosa the following structures were analyzed: Max-Joseph spaces - focal separation of epithelium from connective tissue, thickening of the basal membrane (TBM), cellular infiltration (CI), presence of lymphocytes (LY), presence CB and edema. Semiquntitative examination was performed for all mentioned parameters. Their degrees of expression were also taken into consideration during histopathological analysis, and they were described as mild, moderate and intense.

Immunohistochemical analysis for p53

Immunohistochemistry was carried out using the avidin-biotin-peroxidase complex method. For the immunostaining antigen retrieval, citrate buffer solution (pH 6.0) was used. Tissue sections were transferred to a beaker containing buffer solution and incubated at 95°C in a microwave oven for 17 min to unmask the site of antigen. After taken away from the microwave oven, the

tissue sections were left for 20 min in a beaker at room temperature. Then, they were rinsed with PBS and incubated with 0.3% H_2O_2 for 15 min to block the endogenous peroxidase. Then, the tissue sections were incubated with normal goat serum for p53 staining. They were treated overnight at 4°C with a mouse monoclonal antibody against p53 (DO_7, DAKO A/S Denmark) – dilution 1/50. The samples were incubated with biotinylated animal-matched secondary antibodies (DAKO A/S Denmark) at room temperature, and after rinsing with PBS, they were incubated again with avidin-biotin peroxidase for 45 min. Protein expression was visualized using a kit (DAKO), developed with diaminobenzidine (DAB)-H_2O_2 substrate complex. Each section was left in the DAB solution up to 15 min and counterstained lightly with Mayer's hematoxylin (Hemalun). PBS was used for all washings between the applications of the staining reagents and also as a diluent buffer for the antibodies.

Breast carcinoma (nuclear) was the positive control for the p53 antibody. Staining was considered positive when the nuclear staining of the mucosal epithelium cells of OLP was compatible with that of positive control. For negative control, the same procedure was carried out with normal serum instead of each antibody.

Immunohistochemical measurement parameters included total tissue area, total stained area and intensity of stain. Five hundred keratinocytes or lymphocytes were randomly counted in epithelium (basal and prickle cell layer) and submucosa. Semi-quantitative and semi-qualitative evaluation was performed for p53 staining according to the following criteria: quantitative (0 - negative, grade1 [<1%], grade 2 [1-5%], grade 3 [5-10%], grade 4 [10-25%]); and qualitative (0 - negative, 1 (+) weak, grade 2 (++) moderate, and grade 3 (+++) intense).

Statistics

The data collected were analysed using Fisher exact test for pairs and Wilcoxon rank sum test with continuity correction, to make comparison of differences between the examined groups. Comparisons of multilevel factors between groups were statistically analyzed using Kruskal-Wallis rank sum test.

Interdependence between immunohistochemical and clinical or histopathological parameters were considered only in cases where statistical significance was reached or almost reached. A p value<0.05 was considered as statistically significant.

Results

The group of patients with OLP comprised 28 (70%) women and 12 (30%) men with a median age of 58.3 years (range 33-81). There were 4 (10%) patients with systemic lupus erythematosus, 7 (17.5%) with cardiac disease, and one (2.5%) patient with hepatitis C. Fifteen (37.5%) patients were smokers and 11 (27.5%) consumed alcohol. Healthy individuals comprised 7 (53.8%) women and 6 (46.2%) men, while the group of patients with SCC comprised 8 (66.7%) women and 4 (33.3%) men. The erosive type of OLP was found in 35 patients (70%), followed by reticular in 11 patients (22%), plaque-like in 3 patients (7.5%) and bullous in 1 patient (2.5%). The results of the histopathological study are shown in Tables 1 and 2.

Table 1. Histopathological analysis of epithelium in patients with oral lichen planus (n= 40)

Variables		*n*	*%*
Keratosis	No	0	0
Parakeratosis		20	50
Orthokeratosis		20	50
Granulosis	No	12	30
	+	17	42.5
	++	6	15
	+++	5	12.5
Acanthosis	No	22	55
	+	10	25
	++	5	12.5
	+++	3	7.5
Hyperplasia	No	28	70
	+	8	20
	++	3	7.5
	+++	1	2.5
Atrophy	No	32	80
	+	6	15
	++	2	5
	+++	0	0
Hyperbasal cells	No	13	32.5
	+	21	52.5
	++	4	10
	+++	2	5
Liquefaction degeneration	No	0	0
	+	10	25
	++	13	32.5
	+++	17	42.5
Civatte bodies	No	1	2.5
	+	21	52.5
	++	15	37.5
	+++	3	7.5
Necrotic cells	No	1	2.5
	+	16	40
	++	22	55
	+++	1	2.5
Spongiosis	No	29	72.5
	+	9	22.5
	++	2	5
	+++	0	0
Exocytosis	No	1	2.5
	+	30	75
	++	7	17.5
	+++	2	5

Immunohistochemical analysis showed that the nuclei of 80% of basal and parabasal keratinocytes in OLP specimens were positive on p53 staining (Figure 1). Nevertheless, in most cases of OLP (45%) low percentage of cells (level 1) was p53-positive. The number of patients with the highest percentage of keratinocytes stained with p53 (level 3) was higher in healthy controls (23.09%, Figure 2) in comparison to OLP patients (5%). The statistical difference between these two groups was significant (p=0.02). SCC specimens were p53-positive in most cases with highest percentage of stained keratinocytes (75%, Figure 3). They were marked much more with p53 protein than OLP (p<0.001) and healthy specimens (p=0.04) (Table 3).

p53 staining was weak to moderate in OLP (80%), and intense in SCC specimens (83.33%) (Table 4). In healthy controls 53.85% of the specimens were stained weakly to moderate with p53. Comparing all three groups, statistically significant difference in staining intensity of p53 protein was noted between healthy and SCC (p=0.004) and SCC and OLP (p<0.001). Although keratinocytes were stained more intensely in OLP specimens, differences in staining intensity of p53 protein between OLP and healthy cells were non-significant (p=0.19).

Low percentages (level 1) of p53-positive cells were discovered mostly in older groups of patients with OLP (60.71%), while moderate percentages (level 2) were identified more often in younger patients (58.33%). The difference between these two groups was significant (p=0.003) (Table 5). Patients with highest numbers of keratinocytes stained with p53 (level 3) were rare (7.14%).

p53 staining - grade 2+, was identified in most cases in older OLP patients (41.67%), and grade 1 in younger patients (64.29%). The statistical difference between these two groups was almost reached (p=0.06) (Table 6).

Low percentage (level 1) of p53-positive cells in OLP specimens was detected mostly in females

Table 2. Histopathological analyses of basal membrane zone and submucosa in patients with oral lichen planus (n = 40)

Variables		*n*	*%*
Max-Joseph spaces	No	39	97.5
	+	1	2.5
	++	0	0
	+++	0	0
Thickening of basal membrane	No	21	52.5
	+	16	40
	++	2	5
	+++	1	2.5
Cellular infiltration	No	0	0
	+	8	20
	++	11	27.5
	+++	21	52.5
Lymphocytes	No	0	0
	+	7	17.5
	++	33	82.5
Civatte bodies	No	5	12.5
	+	33	82.5
	++	2	5
Edema	No	30	75
	+	8	20
	++	2	5
	+++	0	0

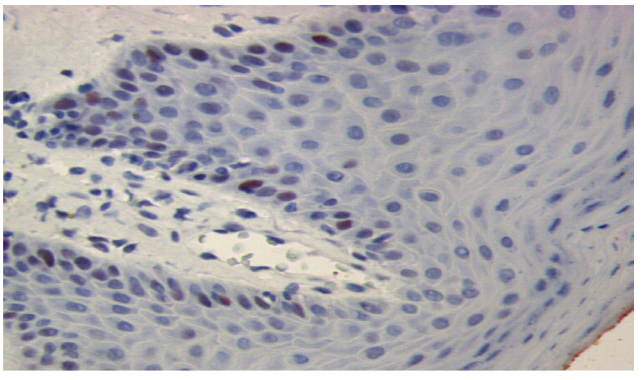

Figure 2. Staining of keratinocytes with p53 protein (healthy controls, H&E x40).

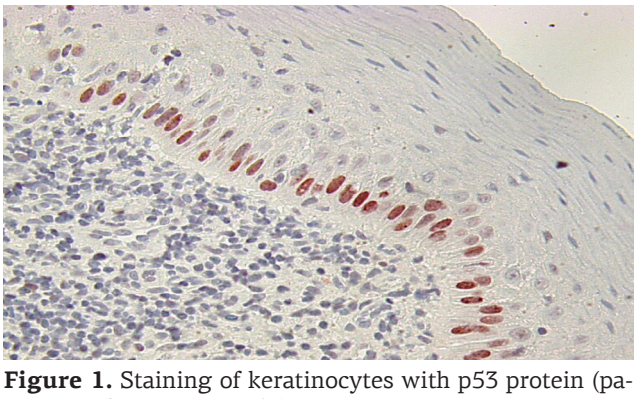

Figure 1. Staining of keratinocytes with p53 protein (patients with OLP, H&E x40).

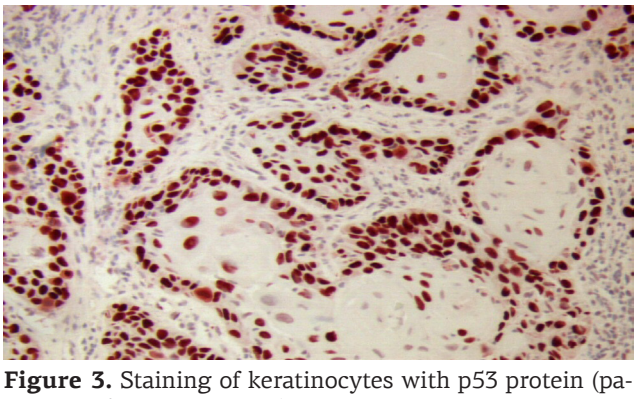

Figure 3. Staining of keratinocytes with p53 protein (patients with SCC, H&E x20).

(60.71%), while moderate percentage (level 2) of these cells was identified more often in males (58.33%). The difference between these two groups was significant (p=0.04) (Table 5).

Low percentage (level 1) of p53-positive cells in OLP specimens was noticed mostly in 2nd+ grade of LD (56.67%) (Figure 4), and moderate percentage (level 2) in 1st grade of LD (50%) (Figure 5). The statistical difference between the examined groups was significant (p=0.006) (Table 5).

Weak intensity of p53 staining (grade 1) was identified more often in OLP specimens with 2nd+ grade of LD (66.67%). Moderate to high intensity of p53 staining (grade 2+) was detected mostly in OLP specimens with 1st grade of LD (70%) (Table 6). The statistical difference between these two parameters was significant (p=0.0007).

Low percentage (level 1) of p53-positive cells in OLP specimens was registered mostly in 2nd+ grade of CB (50%), and moderate percentage (level 2) in 1st grade of CB (47.62%). The statistical difference between the examined groups was significant (p=0.009) (Table 5).

Weak intensity of p53 staining (grade 1) was identified almost equally in OLP specimens with 1st (52.38%) and 2nd+ (50%) grade of CB. Moderate to high intensity of p53 staining (grade 2+) was detected more in OLP specimens with 1st grade of CB (42.86%), with statistical significance (p=0.019) (Table 6).

TBM grade 2+ was p53-negative in 50%, while TBM grade 1 was p53-negative in 68.75% of the examined OLP patients. Correlation between moderate to high staining intensity of p53 protein (grade 2+) and degree of the TBM was negative and without statistical significance (p=0.23) (Table 6).

Table 3. Percentage of keratinocytes stained with p53 in patients with squamous cell carcinoma, oral lichen planus and healthy controls

Variables	*SCC*		*OLP*		*H*	
	n	%	*n*	%	*n*	%
p53-negative	2	16.67	8	20	6	46.15
p53≤1%	1	8.33	18	45	2	15.38
p53-(1-5%)	0	0	12	30	2	15.38
+ p53-(5-10%)	9	75	2	5	3	23.09
Total	12	100	40	100	13	100

SCC: squamous cell carcinoma, OLP: oral lichen planus, H: healthy controls, SD: standard deviation
Fisher's test, p < 0.001; Fisher's Exact Test for pairs:SCC vs. OLP, p < 0.001; SCC vs. H, p = 0.04; OLP vs. healthy controls, p = 0.02

Table 4. Staining intensity of keratinocytes stained with p53 in patients with squamous cell carcinoma, oral lichen planus and healthy controls

Variables	*SCC*		*OLP*		*H*	
	n	%	*n*	%	*n*	%
p53-negative	2	16.67	8	20	6	46.15
p53 grade 1	0	0	21	52.50	4	30.77
p53 grade 2+	10	83.33	11	27.50	3	23.08
Total	12	100	40	100	13	100

SCC: squamous cell carcinoma, OLP: oral lichen planus, H: healthy controls, SD: standard deviation
Fisher's test, p < 0.0005; Fisher's Exact Test for pairs:SCC vs. OLP, p < 0.001; SCC vs. H, p = 0.004;OLP vs. healthy controls, p = 0.19

Table 5. Percentage of keratinocytes stained with p53 in OLP patients according to age, gender,different degrees of liquefaction degeneration (LD) and different degrees of expression of civate bodies (CB)

Variables	*Gender (%)*		*Age (%)*		*LD*		*CB*	
	Male	*Female*	*<55y*	*>55y*	*Grade 1*	*Grade 2*	*Grade 1*	*Grade 2*
p53-negative	8.33	25	33.33	14.29	20	20	4.76	38.89
p53≤1%	25	53.87	8.33	60.71	10	56.67	38.10	50
p53 1-5%	58.33	17.56	58.34	17.86	50	23.33	47.62	11.11
+ p53-(5-10%)	8.34	3.57	0	7.14	20	0	9.52	0
Fisher test	p = 0.04		p = 0.003		p = 0.006		p = 0.009	
Total	15	35	18	32	10	30	21	19

Table 6. Staining intensity of p53 in OLP patients according to age, different degrees of liquefaction degeneration (LD), different degrees of expression of civate bodies (CB) and different degrees of the basal membrane thickening (TBM)

Variables	*Age (%)*		*LD*		*CB*		*CB*		
	<55y	*>55y*	*Grade 1*	*Grade 2+*	*Grade 1*	*Grade 2+*	*ØTBM*	*Grade 1*	*Grade 2*
p53-negative	33.33	14.29	20	20	4.76	38.89	12.5	16.67	50
p53 grade 1	25	64.29	10	66.67	52.38	50	68.5	44.44	33.33
p53 grade 2+	41.67	21.42	70	13.33	42.86	11.11	18.75	38.89	16.67
Fisher test	p = 0.06		p = 0.0007		p = 0.019			p = 0.23	
Total	18	32	10	30	21	19	16	18	6

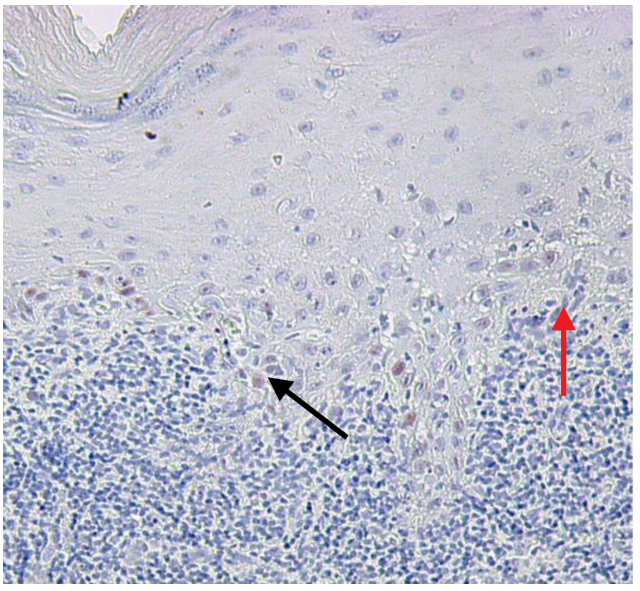

Figure 4. Low number and weak coloration of keratinocytes (black arrow) with p53 marker, in OLP patients with high expression of LD (red arrow) (H&E x20).

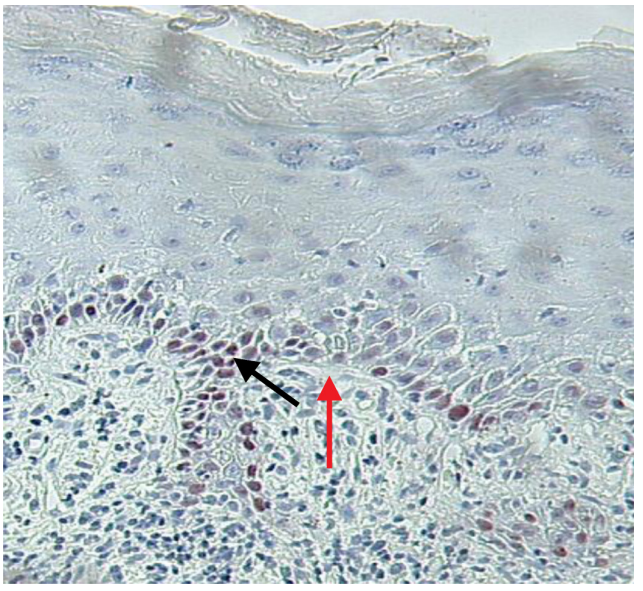

Figure 5. Medium percentage and weak coloration of keratinocytes (black arrow) with p53 marker, in OLP patiens with moderate expression of LD (red arrow) (H&E x20).

Discussion

The balance between cell proliferation and cell death is essential for the maintenance of normal tissues and organs. The maintenance of balance, partially dependent on apoptosis, seems to be disturbed in cancer cells. Apoptosis is a prominent morphologic feature of OLP. Programmed cell death is mediated by the action/interaction of many proteins (gene products) such as p53, c-myc and bcl-2 [9]. These proteins can act as inducers or inhibitors of apoptosis.

p53 gene has earned the name "guardian of the genome", because of its role in apoptosis [10]. It is a tumor suppressor that maintains genomic stability either by inducing cell cycle arrest or apoptosis. Activation of p53 after DNA damage is an important protective mechanism, which facilitate DNA repair and stimulates apoptosis of the abnormal cells. Overexpression of p53 may result from p53 gene mutation, exposure to genotoxic stress, or binding with other cellular or viral oncoproteins [11]. Functional loss and altered expression of p53 are the most frequent genetic changes in human cancers. The fact that overexpression of p53 often occurs not only in oral SCC but also in dysplastic lesions, suggests that p53 overexpression plays a role in the early stage of oral carcinogenesis [12].

In 80% of OLP specimens from this study nuclei of basal and parabasal keratinocytes were positive in p53 staining. Similar findings have also been reported in several earlier studies [2,13,14]. However, in most cases of OLP in our research, only a low number of keratinocytes was p53-positive. The number of keratinocytes stained with p53 was higher in p53-positive specimens of healthy controls than in OLP, which is a little bit surprising. Nevertheless, the staining intensity was higher in OLP in comparison to controls. Rosa et al. also found in their study weak expression of p53 in OLP areas, suggesting that even in the presence of typical histological appearance, OLP can undergo malignant transformation. These findings emphasize the importance of diagnosis and continuous monitoring of OLP lesions [15].

Taking into consideration the fact that wild type of p53 has a short half-life (6-20 min) [16]. and that this is even shorter in the cases with intense inflammation, the wild type of p53 could not easily be detected in the lesions with chronic inflammation. Indeed, the majority of patients from this survey had erosive type of OLP with high degree of inflammation. On the other hand, the mutated protein has a much longer half-life of up to 6 hrs and, accordingly, it is accumulated and may be detected. So, the discovered p53 in OLP specimens could represent mainly mutated p53, what is in accordance with the results of Ögmundsdóttir et al. [17] and de Sousa et al. [18], but opposite from the results of many other researchers [19,20]. In their opinion, overexpression of p53 in OLP occurs unlikely due to gene mutation, but may be a physiologic response to the proliferative state, a protective mechanism that would arrest the cell cycle to allow repair of damaged DNA or trigger apoptosis [19]. Gudkov and Komareva have reported that increased level of p53 protein in OLP represents a protective response to increased levels of DNA damage resulting from chronic inflammation, in a manner similar to other situations of chronic inflammation [20]. With this protective role of p53 they try to explain the low incidence of carcinoma associated with OLP. The inflammatory infiltrate and its secretions can cause DNA damage [21,22], with a consequent higher expression of p53, as demonstrated in immunohistochemical studies [23]. Gonzalez-Moles et al. concluded that the system promoting the repair of DNA damage acts on the injury caused by the inflammatory infiltrate and its secretions, and cases of malignant transformation correspond to the few occasions in which this system is inactivated [24]. The cases of cancerization could correspond to situations where mutations or other inactivating mechanisms prevent the p53 system from acting correctly, and this in turn possibly constitutes a key element for initiation of the malignant transforma¬tion process. A number of authors estimate that p53 overexpression constitutes a form of cell response to the hyperprolifer¬ative state frequently seen in OLP. It is possible that p53 mutations may constitute an im¬portant oncogenic event in malignantly transformed OLP. Thus, the low frequency of p53 mutations could explain the low cancerization rate of OLP [25]. Safadi et al. underline the significance of inflammatory infiltrate with a wide array of cytokines that may stabilize p21 and p53 proteins in OLP. They believe that the effect of these cytokines may contribute to malignant predisposition of OLP, as is the case in other chronic inflammatory diseases [26]. We believe that the low percentage of p53-positive and well-marked keratinocytes represent mutant p53, and that increasing expression of this protein could serve as a valuable diagnostic sign of early carcinogenesis.

As expected, the highest number of p53-positive cells with most expressed staining intensity in this research was detected in the group of patients with SCC.

Our results have shown low percentage with moderate to high intensity of p53-positive cells in older and moderate percentage with weak intensity of p53 protein in younger patients with OLP. Ogmundsdottir et al. also established in their research lower expression of p53 protein in OLP specimens of older patients [19]. Generally, more progressed disease was developed in the older group of OLP patients according to the examined clinical and histological parameters in the present study. It could be accidental, but we cannot exclude the influence of age on disease progression. Higher intensity of p53-stained keratinocytes, no matter their low number, could represent mutant and more stable form of p53 protein, and at the same time signal for disease monitoring due to potential malignant transformation.

In OLP specimens from this survey, keratinocytes were stained in higher percentage and intensity with p53 protein in males than in females. In most other researches no correlation between genders in p53 staining of keratinocytes was detected, and probably the established difference from this study was accidental.

In this research assessed was the correlation between some histological parameters and staining of keratinocytes with p53 protein. Low percentage and weak intensity of p53-positive cells in OLP specimens was noticed mostly in moderate to high grade of LD, while moderate percentage and moderate to high intensity of p53-positive cells was noticed mostly in low grade of LD. With destruction of basal keratinocytes in OLP, the nucleus is also been destroyed. Considering the fact that p53 is a nuclear-binding protein, higher prevalence of this protein is expectable in circumstances with preserved cells with nuclear material. So, with highly expressed LD, many basal cells are destroyed and consequently p53-positive keratinocytes are sparsely present. Intensity of staining of p53-positive cells in the spinal cell layer of the patients with OLP is lower in comparison to the cells from the basal cell layer. In developed LD, basal cells of OLP specimens were almost completely destroyed. That could be the reason why keratinocytes are less stained with p53 protein by higher degree of LD.

Similar relationships were discovered between p53-stained keratinocytes and CB. Low percentage

and weak intensity of p53-positive cells in OLP specimens were detected mostly in high degree of CB, and moderate percentage and moderate to high intensity of p53-stained keratinocytes in low grade of CB. Regarding the fact that p53 is a pro-apoptotic protein, and that CB are symbols of apoptosis, positive correlation between these parameters would be expected. However, upregulation of apoptosis doesn't correlate with the expression of CB. Apoptosis is a brief process, and many CB, as apoptosis is getting more serious, drop into the papillary dermis. So, in more developed apoptosis with higher expression of p53, CB are not strongly expressed.

Conclusion

p53 protein could be a useful marker in the diagnosis of OLP patients with higher risk of SCC development. The p53-positive OLP patients with intense expression of p53 protein (regardless of percentage of p53-positive cells) need to be followed up more frequently, in order to prevent potentially malignant transformation.

Conflict of interests

The authors declare no confict of interests.

References

1. Roopashree MR, Gondhalekar RV, Shashikanth MC, George J, Thippeswamy SH, Shukla A. Pathogenesis of oral lichen planus--a review. J Oral Pathol Med 2010;39:729-34.
2. Sagari S, Sanadhya S, Doddamani M, Rajput R. Molecular markers in oral lichen planus: A systematic review. J Oral Maxillofac Pathol 2016;20:115-21.
3. Hadzi-Mihailovic M, Cakic S, Jankovic S, Raybaud H, Nedeljkovic N, Jankovic L. Ki-67 expression in oral lichen planus. J BUON 2012;17:132-7.
4. Irani S, Esfahani AM, Ghorbani A. Dysplastic change rate in cases of oral lichen planus: A retrospective study of 112 cases in an Iranian population. J Oral Maxillofac Pathol 2016;20:395-99.
5. Oliveira Alves M, Balducci I, Rodarte Carvalho Y, Cabral L, Nunes F, Almeida J. Evaluation of the expression of p53, MDM2, and SUMO-1 in oral lichen planus. Oral Dis 2013;19:775-80.
6. Vousden KH. p53:death star. Cell 2000;103:691-4.
7. de Oliveira MG, Ramalho LM, Gaião L, Pozza DH, de Mello RA. Retinoblastoma and p53 protein expression in pre-malignant oral lesions and oral squamous cell carcinoma. Mol Med Rep 2012;6:163-6.
8. Sankari SL, Babu NA, Rajesh E, Kasthuri M. Apoptosis in immune-mediated diseases. J Pharm Bioallied Sci 2015;7(Suppl 1):S200-2.
9. Hadzi-Mihailovic M, Raybaud H, Monteil R, Cakic S, Djuric M, Jankovic L. Bcl-2 expression and its possible influence on malignant transformation of oral lichen planus. J BUON 2010;15:362-8.
10. Lin T, Lin Y. p53 switches off pluripotency on differentiation. Stem Cell Res Ther 2017;28:8.
11. Lambrus BG, Holland AJ. A New Mode of Mitotic Surveillance. Trends Cell Biol 2017;7:S0962-8924(17)30014-4.
12. Raybaud-Diogene H, Tetu B, Morency R, Fortin A, Monteil RA. p53 overexpression in head and neck squamous cell carcinoma: review of the literature. Eur J Cancer B Oral Oncol 1996; 32:143-9.
13. Bowen AR, Burt L, Boucher K, Tristani-Firouzi P, Florell SR. Use of proliferation rate, p53 staining and perforating elastic fibers in distinguishing keratoacanthoma from hypertrophic lichen planus: a pilot study. J Cutan Pathol 2012;39:243-50.
14. Sousa FA, Paradella TC, Carvalho YR, Rosa LE. Immunohistochemical expression of PCNA, p53, bax and bcl-2 in oral lichen planus and epithelial dysplasia. J Oral Sci 2009;51:117-21.
15. Rosa EA, Lia EN, Macebo SB, Amorim RF. In situ carcinoma developed over oral lichen planus; a case report with analysis of BUB3, p16, Ki-67 and SOX4 expression. J Appl Oral Sci 2015;23:442-7.
16. Perri F, Pisconti S, Della Vittoria Scarpati G. p53 mutations and cancer: a tight linkage. Ann Transl Med 2016;4:522.
17. Ogmundsdottir HM, Hilmarsdottir H et al. Oral lichen planus has a high rate of TP53 mutations. A study of oral mucosa in Iceland. Eur J Oral Sci 2002;110: 192-3.
18. Sousa FACG, Paradella TC, Ccarvalho YR, Rosa LEB. Comparative analysis of the expression of proliferating cell nuclear antigen, p53, bax, and bcl-2 in oral lichen planus and oral squamous cell carcinoma. Ann Diagn Path 2009;13:308-12.
19. Ogmundsdóttir HM, Hilmarsdóttir H, Björnsson J, Holbrook WP. Longitudinal study of TP53 mutations in eight patients with potentially malignant oral mucosal disorders. J Oral Pathol Med 2009;38:716-21.
20. Gudkov AV, Komarova EA. P53 and the Carcinogenicity of Chronic Inflammation. Cold Spring Harb Perspect Med 2016;1:6.
21. Mignogna MD, Fedele S, Lo Russo L, Lo Muzio L, Bucci E. Immune activation and chronic inflammation as the cause of malignancy in oral lichen planus: is there any evidence? Oral Oncol 2004;40:120-30.
22. Ebrahimi M, Boldrup L, Coates PJ, Wahlin YB, Bourdon JC, Nylander K. Expression of novel p53 isoforms in oral lichen planus. Oral Oncol 2008;44:156-1.

23. Taghavi N, Yazdi I. Prognostic factors of survival rate in oral squamous cell carcinoma: clinical, histologic, genetic and molecular concepts. Arch Iran Med 2015;18:314-9.

24. Gonzalez-Moles MA, Gil-Montoya JA, Ruiz-Avila I, Esteban F, Bascones-Martinez A. Differences in the expression of p53 protein in oral lichen planus based on the use of monoclonal antibodies DO_7 and pAb 240. Oral Oncol 2008;44:496-503.

25. Leyva-Huerta ER, Ledesma-Montes C, Rojo-Botello RE, Vega-Memije E. p53 and bcl-2 immunoexpression in patients with oral lichen planus and oral squamous cell carcinoma. Med Oral Pathol Oral Cir Bucal 2012;17:e745-50.

26. Safadi RA, Al Jaber SZ, Hammad HM, Hamasha AA. Oral lichen planus shows higher expressions of tumor suppressor gene products of p53 and p21 compared to oral mucositis. An immunohistochemical study. Arch Oral Biol 2010;55:454-61.