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Original Paper

CHARACTERISTICS OF THE DUAL NATURE OF THE P27 PROTEIN IN ORAL LEUKOPLAKIAS AND CANCER

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Dysregulation of the cell cycle is an important factor in a potentially malignant oral disorder. There have not been many studies on the role of the cell cycle regulator p27 in oral non-homogenous leukoplakia. The aim of our study was to characterise the p27 protein in homogenous and non-homogenous oral leukoplakias (OL), in comparison with healthy mucosa and squamous cell carcinoma tissues. The current study included 25 patients with OL, 15 cases with oral squamous cell carcinoma, and 15 samples of healthy oral mucosa, both as comparison groups. Immunohistochemical p27 antigen expression was determined by a standard EnView imaging system. The expression level of p27 in nodular and verrucous leukoplakia was lower than in homogenous OL but higher than in erythroleukoplakia. There was a statistically significant difference ($p < 0.05$) between the expression of p27 in healthy mucosa and homogenous OL. There was a significantly lower amount of p27 positive cells in oral cancer than in OL ($p < 0.0001$); however, its intracytoplasmic presence was diagnosed. Our study proved the instability of p27 protein and its dual nature in non-homogenous OL and OSCC, and therefore, it can be used as a predictive marker for the clinical course of these conditions.

Keywords: *oral precancers, oral carcinoma, CDKN1B, tumour suppressor gene p27, oncogene p27.*

INTRODUCTION

Oral leukoplakia is described as the most common oral potentially malignant disorder (OPMD), although not all leukoplakia lesions necessarily transform to malignancies (Kuribayashi *et al*., 2015; Iocca and Solecito, 2020). Currently, to characterise the course and prognosis of leukoplakia, the degree of epithelial dysplasia is currently used. The histopathological classification of oral epithelial dysplasia has been revised several times between 1978 and 2017, with the view to optimise the assessment of histopathological criteria. Three degrees of dysplasia grade were accepted by the WHO in 2017: mild, moderate, and severe, with severe dysplasia and carcinoma *in situ* considered to be synonyms (El-Naggar *et al*., 2017; Müller, 2017; Ranganathan *et al*., 2019). However, this classification does not account for early specific changes at the molecular level. Antigen detection in leukoplakia tissues can show unpredictability of OL and reflect the mechanisms or steps of malignant transformation. Even mild dysplasia has a potentially high rate of recurrence and progression to malignancy (Arnaoutakis *et al*., 2013). This means that, in case of leukoplakia, where changes in cell differentiation and growth already exist, it is important to examine the cell cycle process, since down-regulation of the cell cycle plays an important role in carcinogenesis. To prevent malignancies, the cell cycle is regulated by controlled mechanisms (Rath *et al*., 2016; Abbastabar *et al*., 2018; Thambiah *et al*., 2018).

The cell cycle process is influenced by cyclins, cyclin-dependent kinases (CDKs) and their inhibitors. During the

cell cycle, cyclins activate CDKs, and cyclin-CDK complexes induce cell cycle progression, but the activity of CDK is down-regulated by binding inhibitors. The main cell cycle inhibitors are the CIP/kip family and the INK-4/ARF family. The Cip/kip family of cyclin-dependent kinase inhibitors include p21, p27 and p53, but the most significant members of the INK-4/ARF family are p16 and p14 (Queiroz *et al*., 2010; DE Almeida *et al*., 2015; Thambiah *et al*., 2018). Other studies reported the role of p-proteins like p63, p73, p75 etc. in cell cycle regulation and tumorigenesis (Ramasubramanian *et al*., 2013; Sinha *et al*., 2015).

The cyclin-dependent kinase inhibitor 1B (CDKN1B), also known as p27, is a gene encoding a protein that binds to and inhibits the activation of cyclin E/CDK2 or cyclin D/CDK4 complexes in order to regulate cell cycle progression through the G1 and S phases. Action of p27 protein leads to cell cycle arrest in the G1 phase (Vallonthaiel *et al*., 2016; Thambiah *et al*., 2018). The location of the p27 gene is on chromosome 12p13 (Abbastabar *et al*., 2018). The crystallised structure of the p27 domain contains 69 amino acids (Sgambato *et al*., 2000; Rath *et al*., 2016). Other functions of p27 described in the biological literature are cell differentiation and apoptosis (Abbastabar *et al*., 2018). It has been suggested that cell differentiation is reached during cell growth arrest induced by CDK inhibitors like p21 and p27 (Visioli *et al*., 2012). Current findings show that p27 has a switching function — it changes the cell function from proliferation to differentiation (Shiozawa *et al*., 2001). p27 protein in healthy mucosa is present in quiescent cells in intermediate and superficial layers (Queiroz *et al*., 2010). The expression of p27 antigen in the superficial layers of normal oral mucosa can also be explained by maturation of squamous epithelia (Shintani *et al*., 2002).

In carcinogenesis, p27 can act dually: in the nucleus as a tumour suppressor and in the cytoplasm as an oncoprotein (Abbastabar *et al*., 2018). The sequestered p27 in the cytoplasm cannot provide its nuclear function as a cyclin-dependent kinase inhibitor; it induces functions of the cancer cells and inhibits apoptosis of the cells (Vallonthaiel *et al*., 2016; Abbastabar *et al*., 2018). In the cytoplasm, it can be more easily degraded through the ubiquitin-proteasome pathway. Decreased p27 protein expression has been reported in several human tumours — oesophageal (Shibata *et al*., 2001), breast (Alkarain *et al*., 2004), pancreatic (Jeannot *et al*., 2015), cervical cancer (Shiozawa *et al*., 2001) and others. It was observed that CDKN1B is altered in 0.98% of head and neck carcinoma patients (AACR Project GENIE Consortium, 2017). This indicates that p27 protein may be used as a predictive factor in prognosis of squamous cell carcinomas, and the interest in the role of this gene in tumorigenesis has re-emerged in the last decade (Bencivenga *et al*., 2017). More recent studies noted there is a need to analyse individually nuclear and cytoplasmic p27 protein, not only in cancers, but also in premalignant lesions of the oral cavity specifically (Vallonthaiel *et al*., 2016; Teng *et al*., 2020).

As information on the role of the p27 antigen in different types of OL is still scant and contradictory, we consider that it is important to analyse the expression and localisation of p27 protein in different clinical types of oral potentially malignant lesion, and to compare the distribution of the p27 antigen by immunohistochemistry in oral squamous cell cancer of various TNM and clinical stages by analysing also grade (G) values.

The aim of our study was to characterise p27 protein in homogenous and non-homogenous oral leucoplakias, in comparison with healthy mucosa and squamous cell carcinoma tissues, and to determine the role of the p27 antigen in the premalignant process of the oral cavity.

MATERIALS AND METHODS

We analysed 25 cases of oral leucoplakias and compared them with 15 samples containing oral squamous cell carcinoma and 15 samples of normal oral mucosa (control group). The patients were treated in the Centre of Oral Medicine of the Institute of Stomatology of Rīga Stradiņš University and in the Centre of Maxillo-Facial Surgery of Pauls Stradiņš Clinical University Hospital. The limited number of leukoplakia used in our study arose because we selected only those leukoplakia that could be evaluated clinically, surgically excised and then evaluated morphologically and immunohistochemically. We analysed the age and sex of the patients, clinical features like localisation, size, and colour of the lesion. Oral leukoplakias were classified as homogenous and non-homogenous, and in the latter group we included nodular, verrucous subtypes and erythroleukoplakias. In oral leukoplakia, three degrees of dysplasia were recognised: mild, moderate, and severe. Oral squamous cell carcinoma (OSCC) was described accord to the WHO classification, taking into consideration TMN values. "T "stage plus number describes the size of the tumour and its depth of invasion into nearby tissue of oral cavity (El-Naggar *et al*., 2017). Clinical stages of OSCC from I till IV-b were evaluated.

Biopsies (usually 1–2 samples) and surgical material from the oral cavity were fixed in 10% neutral (pH = 7) buffered formalin solution and processed in an automatic tissue processor. All samples of oral mucosa were stained with haematoxylin and eosin, and three layers of the non-keratinised oral mucosa were evaluated: basal, intermediate, and superficial. Routine morphologic examination of healthy oral mucosa, leukoplakia and carcinoma was done by two independent pathologists.

Our study was designed and carried out in compliance with laws and regulations, and **the ethical principles** set out in the Helsinki Declaration. The study protocol was approved by the Committee of Ethics of the Rīga Stradiņš University (Decision of the Ethics Committee No. 3.18.08.2016).

Immunohistochemistry was used to detect the expression of the cell cycle protein p27 (Dako Denmark; Clone – DCS-

60.2, dilution 1:50). Anti-p27 Kip1/CDKN1B antibody (F-8) is recommended for detection of p27 Kip1 of mouse, rat, and human origin. It labels nuclei of epithelium and stromal cells and in case of the pathological lesions also cytoplasm. A standard polymer-based visualisation system (EnVision method by Dako/Agilen, Denmark/USA) was used to determine antigen expression. The slides were stained using the manufacturers' protocols. The expression of p27 protein in the nucleus and the cytoplasm of epithelium was considered positive if more than 10% of the cells were stained with a strongly positive reaction in brown colour and negative if less than 10% of the cells were stained. Only cells with intense nuclear staining were scored. The sections were examined in randomly selected microscopic fields. The expression of the p27 antigen was counted in epithelial cells in three fields of view using original magnification of 200× and calculated per one field of view. Detailed microscopic examination of cell structure was done at 400× magnification. All sections were examined and photographed with a Leitz microscope (Leitz, Wetzlar, Germany) with a DFC 450C digital camera.

Statistical analysis and plotting were performed using GraphPad Prism v.9.0.2 software for Mac (USA, San Diego, CA). The normality of the quantitative data was determined by the D'Agostino and Pearson, and Shapiro–Wilk tests. Between-group comparison was performed by non-parametric Kruskal–Wallis variance analysis followed by the two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli as a post-test. The relationship of variables was assessed using Spearman's rank correlation analysis. The mean levels were expressed as the median with the interquartile region (IQR) as the dispersion characteristic. Statistical significance was set at $p < 0.05$.

RESULTS

Clinical and morphological characteristics. Surgical material and biopsies from twenty-five cases of oral leukoplakia were analysed, including 11 cases of homogenous and 14 cases of non-homogenous ones. In the last group, four cases of nodular leukoplakia, six of verrucous leukoplakia and four of erythroleukoplakia (speckled OL) were included. The mean age of the patients with OL was 59.1 ± 14.9 (ranging from 27 to 82) years. The ratio between male and female patients was 2.1:1. Oral leukoplakia was localised on buccal mucosa in 52% of the cases, on lingual mucosa in 36% and on the mouth floor in 12%. Dysplastic changes of the 1st, 2nd and 3rd grade were found in 9% of all oral leukoplakia cases.

The data of oral leukoplakia and healthy mucosa were compared with 15 surgical material cases of oral squamous cell cancers. The carcinomas represented the following clinical stages: stage I and III – 13.3% for each; II stage – 46.7%; stage IV with metastases in the neck lymph nodes – 26.7%, however without distant spread to other organs (Mo). The 1st and 2nd level of OSCC was observed in 40% of cases for each, and grade 3 was found in 20% of carcinoma cases. The mean age of patients with OSCC was 58 ± 10.2 (ranging from 44 to 71) years. The ratio between female and male patients was 1.1:1. 60% of cases of oral cancer were localised on the mouth floor, 33% on buccal mucosa and 6% on lingual mucosa.

Immunohistochemical p27 antigen characteristics. The p27 antigen in healthy oral mucosa was expressed only in epithelial cell nuclei. p27 protein was found almost in all cells of intermediate and superficial layers, but not in the basal cell layer. The average number of p27 positive cells in the control group was 76 (63–99) in one field of view.

The average number of the p27 labelled cells in all types of leukoplakia in one field of view was 111 (79–160). In oral homogenous leukoplakia, the p27 antigen was observed only in the nuclei of epithelium, and it was present in intermediate and superficial layers of OL. Expression of p27 protein had a homogenous pattern, and the intensity of nuclear staining in the whole sample was similar throughout (Fig. 1). The expression of p27 protein in non–homogenous oral leukoplakia occurred in intermediate and superficial layers and in some cells of the basal and parabasal layers (Fig. 2). Lack of p27 expression in the epithelium with keratohyalin granules was observed. In verrucous and nodular leukoplakia, fewer p27 positive epithelial cells (average 105 (75–163)) were observed than in homogenous leukoplakia (average 120 (99–187)), however more than in erythroleukoplakia 75 (67–84). There was a statistically significant difference ($p = 0.0229$) between the expression of p27 in healthy mucosa and homogenous oral leukoplakia. Regarding clinical types of OL, a significant difference ($p = 0.0229$) between homogenous leukoplakia and erythroleukoplakia (Fig. 3) was observed. The number of p27-positive cells was found to decrease with increasing severity of its clinical type — moderate negative correlation of $r_S = -0.352$ (Spearman's rank correlation coefficient). In

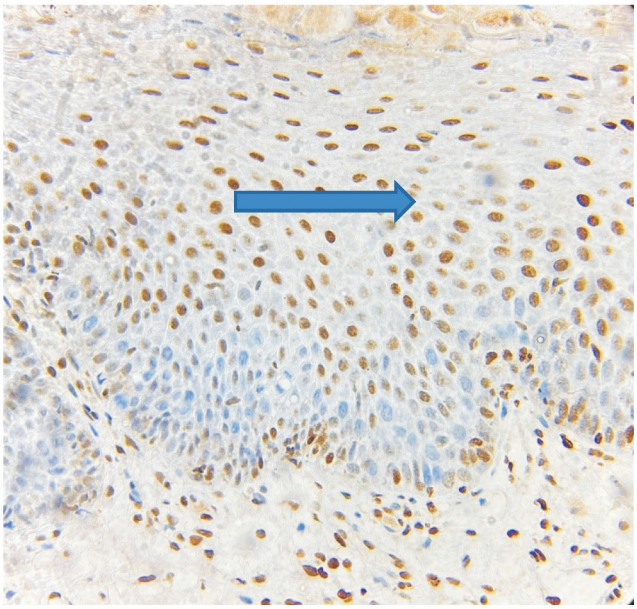

Fig. 1. Nuclear overexpression of p27 protein in the nuclei of non-keratinised epithelium, mainly in upper part of homogenous leukoplakia (arrow). Immunoperoxidase, anti-p27, 200×.

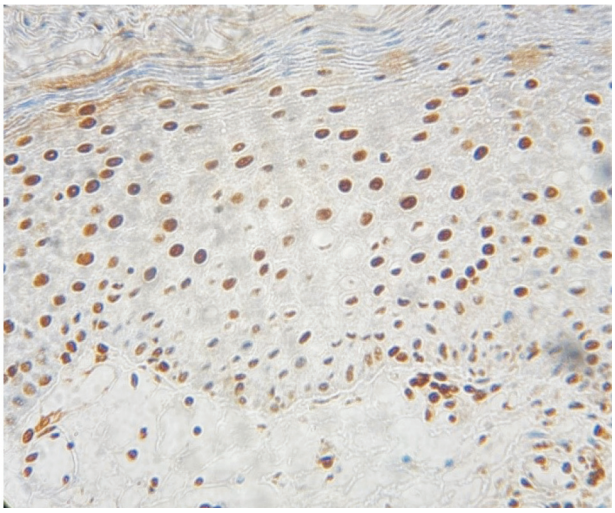

Fig. 2. Nuclear expression of p27 in all layers of oral non-homogenous leukoplakia with moderate dysplasia. Immunoperoxidase, anti-p27, 200×. *J. Oral. Pathol. Med.,* **45**.

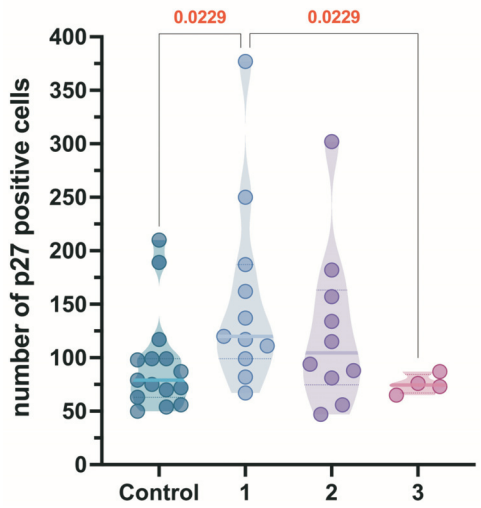

Fig. 3. p27 expression levels in normal mucosa (control) and in different types of leukoplakia (1-homogenous, 2-verrucose and nodular, 3-erythroleukoplakia); significant differences between groups are shown as *p* values highlighted in red.

erythroleukoplakias, patchy nuclear expression of the p27 antigen was also diagnosed in basal layer of the mucous membrane, and intracytoplasmic protein expression occurred in small groups of epithelia. A moderate positive correlation (r_S = 0.340) was found between the number of the p27 positive cells and gender (female, male) – the median number of the p27 positive cells in females was 94 (75–114) and 127 (83–186) for males.

In squamous cell carcinoma, the nuclear expression of p27 decreased markedly and even disappeared, but cytoplasmic expression increased (Fig. 4). The average number of p27 positive cells in carcinoma 16 (10–36) was significantly lower in comparison to the control and leukoplakia — 79 (63–99) (p = 0.007) and 111 (79–160) ($p < 0.0001$), respectively (Fig. 5). In advanced oral cancer, the pattern of p27 protein expression had a mosaic-like pattern, and the intensity of brown colour was from pale to dark (Fig. 6).

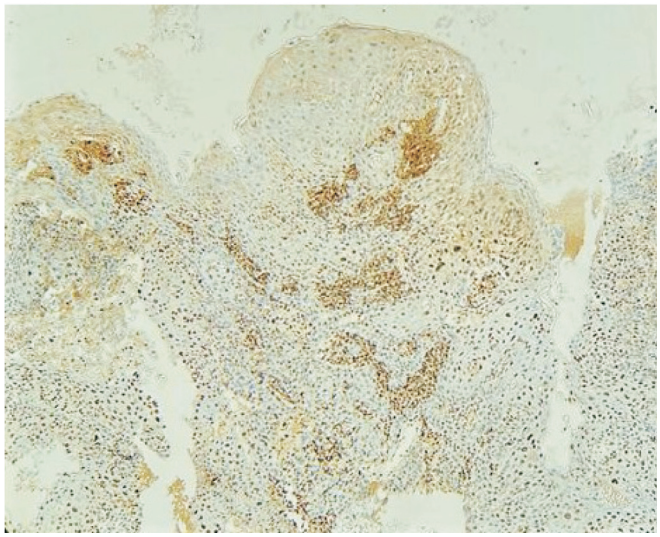

Fig. 4. Intranuclear and intra-cytoplasmatic expression of p27 antigen in oral squamous cell cancer. Immunoperoxidase, anti-p27, 100×. *J. Oral. Pathol. Med.,* **45**.

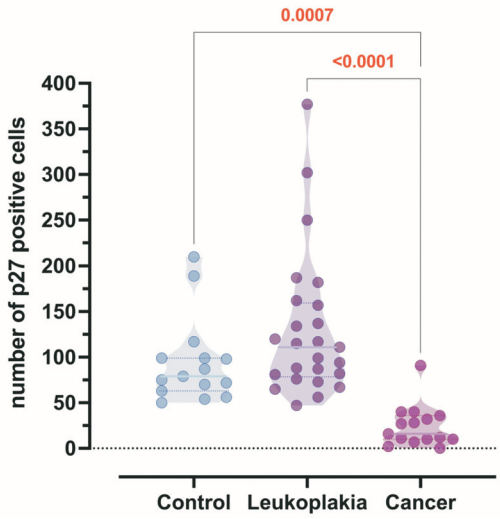

Fig. 5. p27 expression levels in normal mucosa (control), leukoplakia and cancer; significant differences between groups are shown as *p* values highlighted in red.

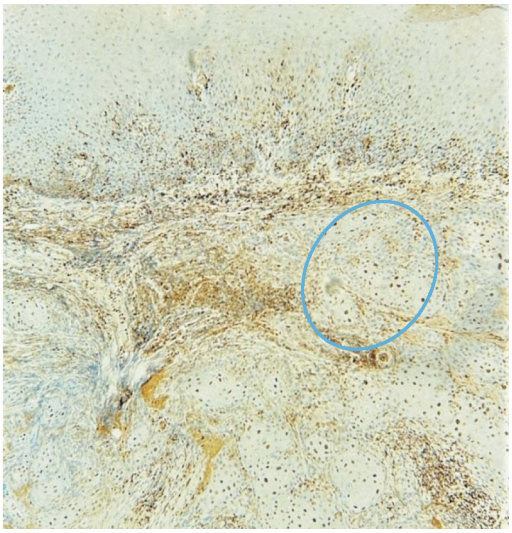

Fig. 6. Invasion of oral squamous cell cancer into lamina propria with very low expression of p27 protein (oval). Immunoperoxidase, anti-p27, 100×. *J. Oral. Pathol. Med.,* **45**.

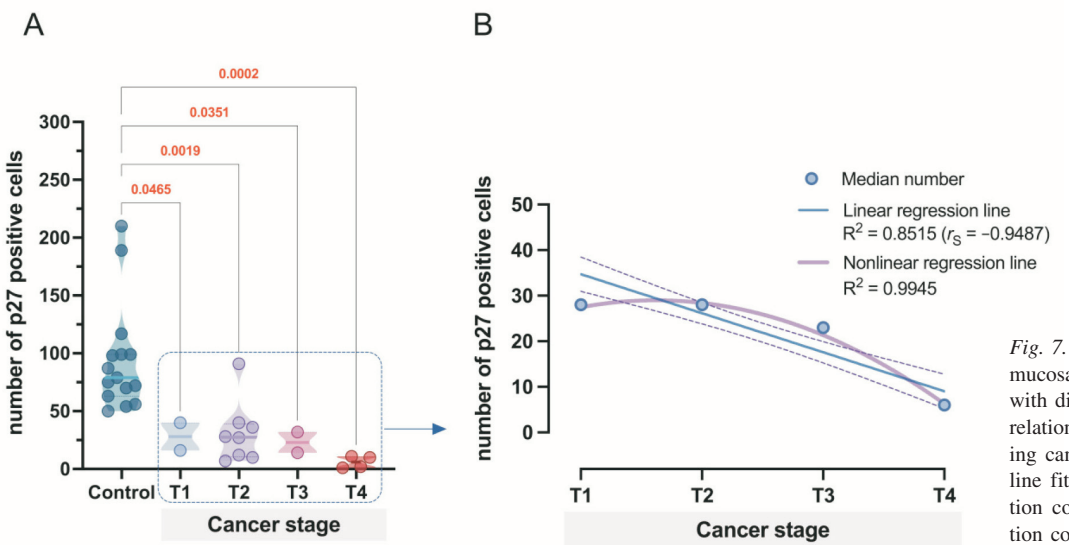

Fig. 7. **A**, Expression of p27 in normal mucosa (control) and in tissue samples with different stages of cancer; **B**, correlation of p27 expression level regarding cancer stages, linear and nonlinear line fit, r_S – Spearman's rank correlation coefficient, R^2 – squared correlation coefficient.

Examination of larger pieces of surgical material of oral OSSC showed that in the areas adjacent to carcinoma, overexpression of p27 protein in both stem and serrated cells of basal layer was found, but in the cancer invasion area immunopositivity gradually disappeared. A significant decrease in the amount of p27 positive cells, partially depending on the T-stage of carcinoma, was detected in cancer patients compared to the control (Fig. 7A). Correlation analysis with linear and non-linear regression tools revealed a strong negative correlation (r_S = –0.9487) for cancer stages (T), and this relationship was found to be best fit by a non-linear quadratic polynomial model — R^2 = 0.9945. (Fig 7B). Figures 3, 5, and 7 show the levels of p27 expression in normal oral epithelium, oral leukoplakia and cancer.

DISCUSSION

The last major systematic review and meta-analysis of the malignant transformation rate of oral leukoplakia covered the period of 2015–2020, and included 24 studies from Italy, Japan, Spain, Brazil, China, India, Iran, Taiwan, Sri Lanka, South Korea, USA, Australia, Romania, and Sweden, with a total of 16 604 patients (Mello *et al*., 2018; Iocca and Solecito, 2020; Aguirre-Urizar *et al*., 2021). This study estimated that the malignant transformation proportion was in the range between 1.1% and 40.8% and that determining criteria were female sex, non-homogeneous clinical type, and presence of epithelial dysplasia. In relation to the latter criteria, it should be noted that in our study, the non-homogenous type of leukoplakia and presence of dysplasia also dominated. Despite the fact that in our study leukoplakia was two times more common in men than in women, the statistically significant drop in expression of the p27 antigen indicates a more unfavourable course of oral leukoplakia in females. The mean age of our patients with OL was 59.1 ± 14.9, which largely coincides with estimates from other studies, which showed that malignant transformation occurs in OL at age > 50 years (Wu *et al*., 2018; Wang *et al*., 2019; Li *et al*., 2020; Sundberg *et al*., 2020). In our study, OL was mainly localised on the buccal mucosa, followed by the lateral border of the tongue. In a study covering the period of 1960–2013, the most common reported site for occurrence of OL was the buccal mucosa (Warnakulasuriya and Ariyawardana, 2016), while other studies showed that OL was mainly localised on the tongue, followed by the buccal mucosa (Pinto *et al*., 2020; Aguirre-Urizar *et al*., 2021).

For better understanding the outcomes of oral leukoplakia, biopsy and/or its surgery must be done with morphological examination of mucosa and underlying tissue. Clinical classification of oral leukoplakias into homogenous and non-homogenous is important for oral surgeons, dentists, and hygienists. Medical staff need to know when to follow up their patients or do immediate surgery, or to take only a biopsy from white or speckled lesions. In 9% of our analysed patients with OL, dysplasia of different degrees was proved. In European and Eastern patients, the incidence of dysplasia varies from 19 to 46% (Brouns *et al*., 2014; Müller, 2017). Real premalignant changes on the cellular level are observed in severe dysplasia only. Researchers worldwide consider that transformation of dysplasia into cancer occurs approximately within a period of four to seven years (Brouns *et al*., 2014; Alsalem, 2019; Tovaru *et al*., 2022). Therefore, it is necessary to study not only on nuclear changes, nuclear-cytoplasmic ratio, and architectural disarrangement in dysplasia, but also the subcellular processes of oral epithelium, particularly the molecular components in nucleus and cytoplasm. There are plenty of proteins in cells: histones, transcription factors, and ribosomal proteins taking part in gene transcription, protein translation, and cell signalling (Drescher *et al*., 2018). p-proteins are only a small part of cell cycle proteins associated with DNA in the nucleus. Proteins like p12, p14, p16, p21, p27, p53, p63, and p73 occur in oral mucosa. For instance, p16 is involved in protein-protein interactions and is a tumour suppressor protein (Queiroz *et al*., 2010; Thambiah *et al*., 2018), p21 can function as a sensor and as an effector of multiple antiproliferative signals (Visioli *et al*., 2012), and p63/p73 proteins attach to DNA and control specific genes (Ramasubramanian *et al*., 2013).

p27 antigen was of interest in this study. Our results showed that this marker is present in a rather wide spectrum of epithelium: in healthy oral mucosa, in areas of hyperplasia, dysplasia and oral squamous cell cancer. In the present study, we characterised the p27 protein in homogenous and non-homogenous oral leukoplakia, in comparison with the respective amount in carcinoma. There are few studies about p27 protein expression in different clinical types of OL with precise calculation of its immunopositivity of nuclei and the analysis of p27 antigen appearance in the cytoplasm of epithelium (Kövesi and Szende, 2006: Queiroz *et al*, 2009; Vallonthaiel *et al*., 2016). The cases of non-homogenous OL as the main potentially malignant oral disorders were of special interest, and detailed statistical analyses was conducted regarding the amount of p27 protein.

The largest amount of p27 protein was at cell cycle stage G1, to facilitate the entrance into the "S" phase, where there is realised replication of DNA and intensive protein synthesis. We can argue that in the epithelium of homogeneous OL, the cell cycle is intact as there are plenty of non-proliferating cells. Thus, while there is p27 protein in the nucleus, we know that oral epithelium will grow and divide in a controlled manner and there will be turnover of it in time, which is typical for each site of the mouth. There was a statistically significant relationship between the increase of number of labelled cells and p27 expression in healthy mucosa and homogenous OL. In our opinion, this is due to the fact that in homogenous OL, hyperplastic processes dominate and the p27 protein still works as a tumour suppressor. However, we cannot agree with some authors who conclude that p27 is expressed not in all but only in a part of OL cases and show results in percentages. For instance, it was estimated that p27 antigen expression varies from 40% to 78% of the cases (Kudo *et al*., 2000; Tsuzuki *et al*., 2003; Queiroz *et al*., 2009). We confirmed that p27 protein is present in all samples of healthy mucosa and OL. The only question is regarding the amount of this protein, its stability, and its localisation in the cell?

The results of our study showed that the number of cells expressing p27 antigen tended to decrease from homogenous OL to non-homogenous OL. However, a statistically significant difference in terms of expression of p27 was observed only between homogenous leukoplakia and erythroleukoplakia, but not with other types of non-homogenous OL. It is important to note that there were similar numbers of cells expressing p27 in normal mucosa and erythroleukoplakia. In this case, it is not necessary to compare the numbers. However, we want to emphasise the localisation pattern of p27 expression in erythroleukoplakia: immunopositivity appears in the basal layer of mucosa and in the cytoplasm of dysplastic cells and therefore the number of expressing cells become similar in both of these cases. It should be noted that the keratinised area superficially also disappears. The simultaneous decrease of p27 in the cell nuclei and the appearance of mild expression of this protein in the cytoplasm indicated that already in this potentially malignant lesion, p27 translocation from the nucleus to the cytoplasm had begun, which was very pronounced in our analysed cases of carcinomas. Weak expression of the p27 antigen in the cytoplasm of epithelial cells of erythroleukoplakia can be explained by the migration of macromolecules and ions between nucleus and cytoplasm through nuclear pores (Ibarra and Hetzer, 2015). However, proteins are transported to ribosomes, endoplasmatic reticulum and Golgi complex where active protein metabolism occurs (Zheng and Jiang, 2021).

Since we evaluated the p27 expression in oral leukoplakias and carcinomas at three points, the results of our study and those of other scientists reflect the expression of this antigen in one histological slide, but not in the whole volume of the pathological lesion, in which the total quantitative amount of p27 antigen is many times greater than in some fields of view. This should be considered in the future when developing target tumour therapy in cell cultures, animal models and human tissues with any antigen. Therefore, it is important that the stage of the carcinoma (T 1, 2, 3, 4) correlated very strongly in descending order with expression of p27 ($R^2 = 0.8515$), Fig. 7B. However, T values do not reflect the volume of malignancy, which is important to consider in the case of individual tumour target therapy. In molecular pathology and oral medicine, experimental and clinical studies on cell cycle proteins should also be accurately distinguished, since results from cell cultures and animal models studies may be ambiguous (Vairaktaris *et al*., 2007; Satoh *et al*., 2016).

We analysed the expression of p27 not only in oral leukoplakia of various clinical variants but also compared the results with a group of oral cancers. Squamous cell cancer of oral cavity was diagnosed in 40% of patients in the advanced stages (III and IV-a) with its spread to the lymph nodes. The information about p27 in oral cancer is controversial, as in last two decades totally opposite results appeared: some observed an increased amount of p27 protein (Mineta *et al*., 1999; Hasmi *et al*., 2019) whereas others found decreased expression of this antigen (Kudo *et al*., 1998; Shintani *et al*., 2002). The opposite results can be explained by different methods used for the calculation of the p27 expression. even though the same antibody was used.

In our study, the amount of the p27 antigen labelled cells in oral cancer was lower by 4.7 times in comparison with erythroleukoplakia, seven times with homogenous OL and 4.8 times in comparison with healthy mucosa. A statistically significant difference in the p27 amount was observed between oral cancer and healthy mucosa, as well as between OSCC and all leukoplakias. In our cases of oral carcinoma, intracytoplasmic immunopositivity for p27 was expressed much more than in erythroleukoplakia. More and more pathologists evaluate the location of p27 in cytoplasm in malignancies as an oncogene, but some researchers do not take this fact into consideration (Ramasubramanian *et al*., 2013). We do not agree with this, since expression of p27 protein is proven by standard immunohistochemistry as a protein with a molecular weight of 16.5 kDa, it consists of 156

amino acids and has a consistent function. It is common knowledge that in the cell there are 20 different amino acids that can undergo denaturation and lose the normal amino acid sequence. Disturbances of an amino acid sequence is responsible for its altered function in the nucleus and cytoplasm.

The cyclin-dependent kinase inhibitor p27 is a tumour suppressor, however, it may also function as an oncogene within the cytoplasm (Sharma and Pledger, 2016; Vallonthaiel *et al*., 2016). Our study proves that the amount of p27 in oral carcinoma gradually disappears. In our opinion, the gradual disappearance of p27 from the nucleus affects the activity of centrosomes and distorts the proper division of epithelial cells. Thus, p27 protein is a tumour promoter, but rather unstable oncogene. The variable expression of the p27 protein at the area adjacent to the tumour and its centre indicates the dual nature of this antigen in tissues located even at a distance of a few millimetres.

On the other hand, based on data from other researchers, we see that instead of the presence of p27 in malignancies, p57, p63, p73 and other proteins have been diagnosed. For example, the latter has a molecular weight of 77 kDa with 636 amino acids. Thus, it is possible that amino acids regroup and form other proteins. Researchers have given the name of "intrinsically unstructured protein" or "enigmatic protein" for the p27 antigen, and mostly due to advances in genomic analyses, reappraisal of the *CDKN1B* $p27^{Kip1}$ encoding gene role in tumorigenesis was conducted (Bencivenga, 2017). Apparently, the biochemical structure of the p27 protein is fragile, and it is possible that these proteins transform into each other, as evidenced by numerous publications on variations in the expression of p14, p16, p21, p27, p53, p73 and other antigens in the squamous epithelium of premalignant and malignant processes of oral cavity (Choi *et al*., 2003; Kresty *et al*., 2008; Visioli *et al*., 2012; Sridharan *et al*., 2016).

CONCLUSIONS

Based on the current results, we conclude that protein metabolism of oral epithelial cells is very important in the malign transformation process of oral leukoplakia. However, it is impossible to prove only by immunohistochemistry at which of five protein metabolism steps of p27 (amino acid synthesis, transcription, translation, post translational modifications or protein folding), the biochemical and/or genetic defect appears. Our study proved the instability of p27 protein and its dual nature in non-homogenous OL and OSCC. This cell cycle protein has demonstrated function as a tumour suppressor and an oncogene in both lesions of oral mucosa. Further studies are required with a larger number of patients to investigate the dynamics of the p27 protein restructuring from nucleus to cytoplasm and its subsequent disappearance in the oral cancer cells. Our study group considers that electron microscopic evaluation of nucleus and its centrosome can explain the restructuring of the p27 protein, which is extremely important in the formation of atypical cells in a premalignant lesion, including different clinical types of oral leukoplakias.

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P27 PROTEĪNA DUĀLĀS DABAS RAKSTUROJUMS MUTES DOBUMA GĻOTĀDAS LEIKOPLAKIJĀS UN KARCINOMĀ

Šūnas cikla regulācijas traucējumi ir svarīgs faktors mutes gļotādas priekšvēža stāvokļa attīstībā. Tikai dažos pētījumos tiek raksturota šūnas cikla regulatora p27 ekspresijas nozīme mutes nehomogēnajās leikoplakijās. Mūsu pētījuma mērķis bija raksturot p27 proteīna ekspresiju homogēnās un nehomogēnās mutes leikoplakijās (ML) un salīdzināt ar normāliem un karcinomas audiem. Pētījumā iekļāvām 25 gadījumus ar ML, bet salīdzinošajā grupā — 15 normālas gļotādas un 15 plakanšūnu karcinomas audu paraugus. Imūnhistoķīmisku p27 antigēna ekspresiju noteica ar standarta vizualizācijas sistēmu EnVision. p27 ekspresija nodulārā un verukozā leikoplakijā bija mazāka nekā homogēnā leikoplakijā, bet lielāka nekā eritroleikoplakijā. Homogēnu leikoplakiju gadījumos tika pierādīta statistiski ticama atšķirība, salīdzinot ar normālu gļotādu ($p < 0.05$). Mutes dobuma plakanšūnu vēzī bija ievērojami mazāk ar p27 marķētu šūnu kodolu nekā homogēnā un nehomogēnā leikoplakijā ($p < 0.0001$), bet tika diagnosticēta papildus šī proteīna ekspresija citoplazmā. Mūsu pētījumā tika pierādīta p27 proteīna nestabilitāte un tā duālā daba nehomogēnās OL un mutes gļotādas plakanšūnu vēžos, un tādēļ marķieris var tikt izmantots kā abu šo veidojumu klīniskās gaitas prognostisks marķieris.