Research article/Raziskovalni prispevek

DNA PLOIDY AND NUCLEAR TEXTURE FEATURES OF THE RETICULAR FORM OF ORAL LICHEN PLANUS

DNK PLOIDIJA IN JEDRNE TEKSTURNE ZNAČILKE RETIKULARNE OBLIKE USTNEGA LIHENA

Matjaž Rode,[1] Margareta Strojan-Fležar,[2] Mirela Kogoj-Rode,[1] Marija Us-Krašovec[2]

[1] Ul. bratov Učakar 16, 1000 Ljubljana

[2] Institute of Oncology, Zaloška 2, 1000 Ljubljana

Abstract

Background — *Oral lichen planus (OLP) is a chronic inflammatory disease of the oral mucosa with low risk of malignant transformation. At present there are no reliable tools to predict which OLP will progress to cancer. Abnormal DNA content has been reported to be a valuable diagnostic marker of potential malignant transformation of oral white patches.*

Aims — *To analyze image cytometric DNA ploidy of OLP and assess chromatin changes reflected in nuclear features, when OLP is compared to normal buccal mucosa.*

Materials and methods — *Twenty-eight patients with reticular form of OLP and 50 healthy control subjects were included in the study. No malignant transformation of OLP was observed during a follow-up period of 25 years. Scrapings of buccal mucosa were suspended in transport medium. Monolayer filter cell preparations were Feulgen-Thionin stained. Image cytometric analysis was performed by Cyto-Savant system.*

Results — *All OLP in our study were diploid. Statistically significant differences were found between normal buccal mucosa and OLP in several nuclear texture features.*

Conclusions — *Our results indicate that diploidy predominates in OLP, confirming its benign nature. Minor chromatin changes of OLP were detected by IC, probably of reactive type.*

Key words — *oral lichen planus; image cytometry; DNA ploidy; nuclear texture features*

Izvleček

Izhodišča — *Ustni lihen planus je kronična vnetna bolezen ustne sluznice z nizkim tveganjem prehoda v malignom. Zaenkrat še nimamo zanesljive metode, s katero bi lahko napovedali, kateri ustni lihen planus bo prešel v raka. Nekateri avtorji poročajo, da bi lahko bila spremenjena količina DNK pomemben označevalec morebitne maligne transformacije v presejanju belih madežev v ustni votlini.*

Namen — *S slikovno citometrično metodo oceniti DNK ploidijo pri ustnem lihnu planusu in analizirati spremembe kromatina, ki se kažejo v jedrnih značilkah ter vrednosti primerjati z normalno lično sluznico.*

Metode — *V študijo je bilo vključenih 28 bolnikov z retikularno obliko ustnega lihna planusa in 50 zdravih odraslih brez vidnih sprememb na lični sluznici. Pri nobenem bolniku z ustnim lihnom planusom v 25-letnem obdobju načrtovanih sistematičnih kontrol bolezen ni na-

Correspondence author / Avtor za dopisovanje:

Prof. dr. Matjaž Rode, dr. dent. med., Ulica bratov Učakar 16, 1000 Ljubljana, e-mail: matjaz.rode@siol.net

predovala v malignom. Bris celic ustne sluznice je bil vložen v prenosni medij. Suspenzijo celic smo filtrirali in filtre odtisnili na objektna stekelca. Enoslojne celične preparate smo pobarvali s Feulgen-tioninom. Slikovno citometrična analiza je bila narejena z aparaturo Cyto-Savant.

Rezultati *Vsi celični vzorci pri bolnikih z ustnim lihnom planusom so bili diploidni. Med celicami OLP in celicami normalne lične sluznice smo našli statistično značilne razlike v nekaterih jedrnih teksturnih značilkah.*

Zaključki *Naši rezultati nakazujejo, da pri ustnem lihnu planusu prevladuje diploidnost, ki potrjuje benigno naravo ustnega lihna planusa. S slikovno citometrično analizo smo našli manjše spremembe v strukturi kromatina, ki so verjetno reaktivne narave.*

Ključne besede *ustni lihen planus; slikovna citometrija; DNK ploidija; jedrne teksturne značilke*

Introduction

Oral lichen planus (OLP) is a chronic inflammatory disease of the oral mucous membranes and it affects 0.5 % of population.[1, 2] It could have different clinical presentations: reticular, papular, plaque-like, erosive, ulcerative, bullous and atrophic.[3–5] The reticular form is the most common and is generally asymptomatic while the ulcerative and bullous forms are frequently associated with pain.

OLP is histopathologically characterized by a cell-mediated immune condition of unknown etiology, in which T-lymphocytes accumulate beneath and in the epithelium of the oral mucosa. Epithelial basal cells are destructed and the basal membrane is thickened or disrupted.[4,5] It has been postulated that OLP is a localized autoimmune disease.[6]

OLP is clinically important because of the possible malignant transformation.[7, 8] A number of mainly retrospective studies from several countries showed, however, only a minimal risk of malignant transformation.[9–12] In our long-term follow-up none of patients with OLP developed malignant transformation of the lesion.[13]

Excision biopsy with histological examination is the golden standard for definitive evaluation of oral lesions and for the assessment of their possible malignant transformation. Since OLP bares minimal risk of malignant potential, it could also be screened by cytopathologic examination of buccal mucosa scrapings or brushings. To obtain cytological sample one needs no special skills, method is easy to perform, needs no anesthesia and is not painful for the patient. Ancillary methods could also be applied to cytologic sample to obtain more information about the lesion.

In this report, we present image cytometric (IC) evaluation of OLP cytologic samples, analyzing DNA ploidy, which was proposed to predict malignant potential in oral white patches. Additionally nuclear texture feature measurements of OLP were performed and compared to normal buccal mucosa.

Materials and methods

Twenty-eight patients with reticular form of OLP and 50 control healthy subjects were included in the study. The data on 28 patients with reticular form of oral lichen were collected from a previous survey by the Department of Clinical Oral Pathology of Medical Center in Ljubljana. The mean age at the initial presentation was 58.3 years (range: 41 to 64 years, SD ± 5.1 yrs.). Majority (80 %) of the patients were women. The oral examinations of all lesions were performed in daylight and using the mouth mirror. Oral reticular lichen was diagnosed and registered on a clinical basis and with histopathological examinations of biopsies that were conducted at the Institute of Pathology, Faculty of Medicine, University of Ljubljana. No patient had characteristic skin lesions.

Cell samples for IC analysis were collected from the sites of OLP in patients and from normal buccal mucosa in control subjects by gentle scraping using wooden spatula. The spatula was then rinsed in transport solution, containing 10 % of alcohol solution.

The cell suspension was filtered through the Costar filter system (Costar Europe Ltd., Shiphol-Rijk, the Netherlands). The filter (5 µm pores, 47 mm diameter of the filter) was gently imprinted onto a special glass slide and fixed in Delaunay solution (ethanol: acetone 1:1 (w/w mixture) with 0.5 ml/l trichloracetic acid) overnight. Slides were stained according to modified Feulgen method using thionin. The cell preparations were fixed in Böhm-Sprenger fixative, then hydrolyzed in 5N HCl for 50 minutes. Afterwards, cells preparations were stained in thionin (Aldrich Chemical Company, Inc., USA) solution for 60 minutes.

The image analysis of cell samples was performed by an automated, high resolution image cytometer (Cyto-Savant, Oncometrics Imaging Corp., Vancouver, British Columbia, Canada). The hardware and its performance are described in detail in the sources listed in the references.[14]

Nuclear images were acquired automatically by random sampling by means of the data acquisition program Acquire, incorporated into the system.

IC DNA histograms were analyzed by two authors. The histograms were considered diploid, DNA index (DI) value of 1, if only one cell population was present, consisting of one G0/G1 peak with corresponding G2/M peak. Histograms with more than one G0/G1 peak would be considered nondiploid.

Over 100 nuclear features were calculated from each of the nuclear images. The nuclear features included common morphometric features (area, diameter, shape features). In addition to nuclear texture features (NTF) evaluating nuclear chromatin structure and organization, descriptive statistics of chromatin distribution (integrated optical density [IOD], variance of optical density [OD], OD skewness and kurtosis), discrete chromatin texture features (area and shape of high, medium, low density chromatin components), fractal texture features, Markovian texture features (entropy, energy), non-Markovian local extreme features (density dark spot) and run length texture features (short run emphasis, long run emphasis, gray level nonuniformity) were analyzed. All nuclear features, their exact formulas and descriptions can be found in the references.[15]

The nuclear features of the G_0G_1 cell population were included in the statistical analyses.

Statistical significance of any differences in the values of nuclear features between the cells from OLP and normal buccal mucosa were tested with ANOVA. The differences were considered statistically significant at $p < 0.05$.

Results

Histopathologic findings: All tissue biopsies demonstrated histological characteristics of OLP. No atypia or dysplasia was found.

IC DNA ploidy: Cytologic samples of all patients with OLP (28) and of all control subjects (50) contained only diploid cell populations.

Nuclear features: After interactive removal of artifacts and double or multiple nuclei, there were 18939 nuclei of diploid cells (corresponding to nuclei of intermediate squamous cells) available for analysis in OLP group and 41813 nuclei in control group. The pycnotic nuclei (3228 in OLP group and 5649 in control group) were analyzed separately.

No statistically significant differences were found in the group of morphometric features between the nuclei from OLPs and controls (Figure 1).

In the group of discrete NTF, we found small but statistically significant differences in several NTF including »hiDNAarea«, which defines the size of the area of

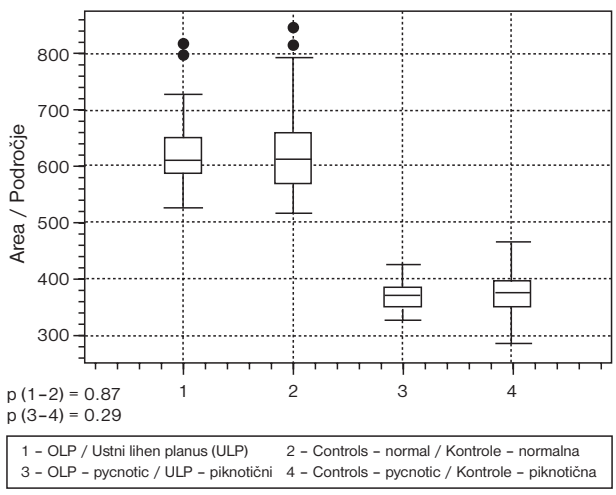

Figure 1. *Nuclear area of OLP nuclei compared to nuclei of cells of normal buccal mucosa; pycnotic nuclei were analyzed separately.*

Sl. 1. *Primerjava jedrne površine celic ULP s površino celic normalne bukalne sluznice; jedra piknotičnih celic obeh skupin smo analizirali ločeno.*

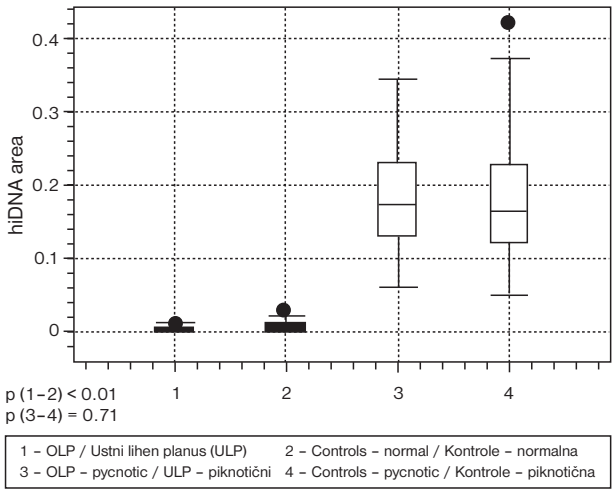

Figure 2. *Nuclear texture feature «high DNA area« of OLP nuclei compared to nuclei of normal buccal mucosa cells; pycnotic nuclei were analyzed separately.*

Sl. 2. *Primerjava jedrne teksturne značilke »high DNA area« celic ULP s celicami normalne bukalne sluznice; jedra piknotičnih celic obeh skupin smo analizirali ločeno.*

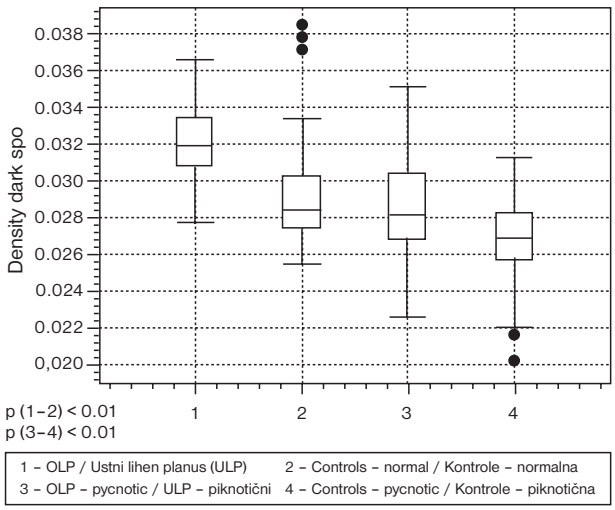

Figure 3. *Nuclear texture feature »density dark spot« of OLP nuclei compared to normal buccal mucosa cells; pycnotic nuclei were analyzed separately.*

Sl. 3. *Primerjava jedrne teksturne značilke »density dark spot« celic ULP s celicami normalne bukalne sluznice; jedra piknotičnih celic obeh skupin smo analizirali ločeno.*

high density chromatin in the nucleus (Figure 2). The difference was found between the normal nuclei of intermediate cells and also between the pycnotic nuclei of the two groups.

We also found statistically significant differences in »fractal dimension«, belonging to fractal texture feature group. The »fractal dimension« yields high values to the nuclear images with spatially frequent, high contrast variations in chromatin condensation. The differences were detected between the nuclei of the intermediate cells and also between the pycnotic nuclei of the two studied groups.

Another group of features that differed in their values in the intermediate and in the pycnotic nuclei between the two studied groups were non-Markovian texture features, represented by NTF »density dark spot« (Figure 3).

In the run length feature group, there were no significant differences either for the intermediate or the pycnotic nuclei for »average gray level«, the feature that gives maximal values to the nuclei with large areas of condensed chromatin. On the other hand, the differences, although small, were statistically significant for »average short runs«. This feature gives maximal values to nuclei with small areas of condensed chromatin.

Discussion

OLP is considered a lesion with a low risk of malignant transformation to squamous cell carcinoma of the oral cavity, which rates among the ten most common cancers in the world.[16] The five year survival rate of oral cancer is still being less than 50 %, however, oral cancer can be cured, if detected and treated early enough. Therefore, one should emphasize the importance of an early diagnosis of potentially malignant oral lesions, including OLP.[17]

A useful screening procedure of OLP could be oral scrapings or brushings with subsequent cytologic examination. The clinical advantages of cytologic examination are evident: it is non-invasive, painless and fast regarding the acquisition of material.[18] However, cytological examination has its limitations, since mostly upper layers of the lesion can be sampled. False negative diagnoses were reported in cytologic samples of keratinizing well-differentiated squamous cell carcinoma.[19–21]

Ancillary methods could improve diagnostic accuracy of cytologic evaluation of OLP. Image cytometric analysis of cell samples offers analysis of DNA ploidy and objective and reproducible assessment of chromatin texture, measuring over 100 NTF.[16] Foremost, an abnormal DNA content was found a valuable diagnostic marker of malignant potential in non-dysplastic oral white patches.[17] In our study, IC diploid pattern was found in all the cases, which is in concordance with no malignant transformation of OLP in our study group with the 25-years of follow-up. However, one has to bear in mind that diploid pattern of the lesion does not exclude malignant potential reliably.

More information about OLP could be obtained by analyzing NTF. Therefore, we also compared NTF of OLP to normal buccal mucosa. We found no differences in morphometric nuclear features, including area, which is in concordance with other quantitative studies of oral mucosa. They found that the change in area of oral squames was detected only, when dysplasia was found on histological examination of the lesion.[20]

We found statistically significant differences in some groups of NTF, which evaluate chromatin structure and organization, but the differences observed between OLP and normal buccal mucosa were small. Besides, we observed that the pycnotic cells, that originate from the upper parakeratotic layer, characteristic for this lesion, did not increase the differences found between the two study groups.

In our previous study, we demonstrated differences in NTF values between buccal mucosa of smokers and non-smokers and between males and females.[18] Similar differences were found between OLP and normal mucosa in the present study. However, these small differences, although statistically significant, were not useful for recognition of smokers among subjects with unknown smoking history when applied to a classifier in our previous study. Since the differences in the present study were in the same range, we assume, that small chromatin changes in OLP are not connected to neoplasia, but rather reflect the reactive changes of epithelium as a consequence of chronic inflammation.

In our study group with a 25-year follow-up, we found no malignant transformation of OLP,[13] so the values of NTF obtained in these samples could be considered as a baseline reference values for oral white patches with no malignant potential. However, this hypothesis should be tested in prospective studies including other pathological lesions of oral cavity, premalignant and malignant, to assess possibility of IC assessment of oral mucosa as a screening tool to detect early oral cancer.

References

1. Brown RS, Bottomley WK, Puente E, Lavigne GL. A retrospective evaluation of 193 patients with oral lichen planus. J Oral Pathol Med 1993; 22: 69–72.
2. Lodi G, Scully C, Carrozzo M, Griffiths M, Sugerman PB, Thongprasom K. Current controversies in oral lichen planus. Part 2. Clinical management and malignant transformation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005; 100: 164–78.
3. Altman J, Perry HO. Variations and course of lichen planus. Arch Dermatol 1961; 84: 179–91.
4. Andreasen JO. Oral lichen planus. A clinical evaluation of 115 cases. Oral Surg Oral Med Oral Pathol 1968; 25: 31–42.
5. Cooke BD. Keratinizing lesions affecting the oral mucosa. Proc Roy Soc Med 1967; 60: 819–22.
6. Epstein JB, Wan LS, Gorsky M, Zhang L. Oral lichen planus: progress in understanding its malignant potential and the implications for clinical management. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 96: 32–7.
7. Kovesi G, Banoczy J. Follow-up studies in oral lichen planus. Int J Oral Surg 1973; 2: 13–22.
8. Walsh LJ, Savage NW, Ishi T, Seymour GJ. Immunopathogenesis of oral lichen planus. J Oral Pathol Med 1990; 19: 389–96.

9. Barnard NA, Scully C, Everson JW, Cunningham S, Porter SR. Oral cancer development in patients with oral lichen planus. J Oral Pathol Med 1993; 22: 421–4.
10. Holmstrup P, Thorn PP, Rindum J, Pindborg JJ. Malignant development of lichen planus affected oral mucosa. J Oral Pathol 1988; 17: 219–25.
11. Holmstrup P. The controversy of a premalignant potential of oral lichen planus is over. Oral Surg Oral Med Oral Pathol 1992; 73: 704–6.
12. Scully C, El-Kom M. Lichen planus: review and update on pathogenesis. J Oral Pathol 1985; 14: 431–58.
13. Rode M, Kogoj-Rode M. Malignant potential of the reticular form of oral lichen planus over a 25-year observation period in 55 patients from Slovenia. J Oral Science 2002; 44: 109.
14. Jaggi B, Poon S, Pontifex B, Fengler J, Palcic B. A quantitative microscope for image cytometry. J SPIE 1991; 1448: 89–97.
15. Doudkine A, MacAulay C, Poulin N, Palcic B. Nuclear texture measurements in image cytometry. Pathologica 1995; 87: 286–99.
16. Cuffari L, de Siquiera JT, Nemr K, Rapaport A. Pain complaint as the first symptom of oral cancer: a descriptive study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 102: 56–61.
17. Massano J, Regateiro FS, Januario G, Ferreira A. Oral squamous cell carcinoma: Rewiew of prognostic and predictive factors. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 102: 67–76.
18. Lavrenčak J, Fležar M, Žganec M, Us-Krašovec M. Image cytometry of normal buccal mucosa smears: influence of smoking and sex-related differences. Radiol Oncol 1998; 32: 71–5.
19. Cowpe JG, Longmore RB, Green MW. Quantitative exfoliative cytology of abnormal oral mucosal smears. J Roy Soc Med 1988; 81: 501–13.
20. Sudbø J, Kildal W, Risberg B, Koppang HS, Danielsen HE, Reith A. DNA content as a prognostic marker in patients with oral leukoplakia. N Engl J Med 2001; 344: 1270–8.
21. Tucker JH, Cowpe JG, Ogden GR. Nuclear DNA content of normal, premalignant and malignant oral smears. Anal Cell Pathol 1994; 6: 117–28.

Arrived 2007-01-03, accepted 2007-09-27