JKMU

Journal of
Kerman University of Medical Sciences

https://jkmu.kmu.ac.ir
10.34172/jkmu.2023.08
Vol. 30, No. 1, 2023, 51-54

Original Article

Evaluation of Nuclear Parameters in Relation to Regional Lymph Node Involvement in Oral Squamous Cell Carcinoma: A Cytomorphometric Study

Vahid Karbalaee Khiavi[1], Noushin Jalayer Naderi[2*], Ahad Muhammadnejad[3]

[1]Faculty of Dentistry, Shahed University, Tehran, Iran
[2]Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Shahed University, Tehran, Iran
[3]Cancer Biology Research Center, Cancer Institute of Iran, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Background: Up-regulation of ribosome biogenesis encodes the factors related to carcinogenesis. It has been shown that the nucleus diameter and number of nucleoli increase from normal mucosa to oral squamous cell carcinoma (OSCC). The relationship between nuclear parameters and lymph node involvement in OSCC has not been established, yet. The aim of this study was to evaluate the nuclear parameters comprising nucleoli count and nucleus: nucleoli ratio in relation to regional lymph node involvement in OSCC.

Methods: Thirty-four formalin-fixed, paraffin embedded sections from different histopathologic grades of OSCC were stained with methyl green-pyronin. Mean number of nucleoli and nucleus: nucleoli ratio were calculated in 100 tumor cells from 10 random selected fields and compared based on lymph node involvement.

Results: Nucleoli count in cases with metastasis to regional lymph nodes was not significantly different from that in cases without regional lymph nodes involvement ($P=0.29$). The difference of nucleus: nucleoli ratio in cases with and without lymph nodes involvement were not significant ($P=0.52$). No significant correlation was found between the nucleoli count and lymph node involvement ($r=0.08$, $P=0.78$). The correlation between nucleus: nucleoli ratio and lymph node involvement was significant ($r=0.58$, $P=0.02$).

Conclusion: The nucleus: nucleoli ratio of tumoral cells in OSCC was correlated to lymph node involvement. Based on the results, nucleus: nucleoli ratio can potentially be a useful tool to determine the lymph node involvement in OSCC.

Keywords: Carcinoma, Squamous cell, Cell nucleoli, Oral pathology, Pathology

Citation: Karbalaee Khiavi V, Jalayer Naderi N, Muhammadnejad A. Evaluation of nuclear parameters in relation to regional lymph node involvement in oral squamous cell carcinoma: a cytomorphometric study. *Journal of Kerman University of Medical Sciences*. 2023;30(1):51-54. doi:10.34172/jkmu.2023.08

Received: April 23, 2022, **Accepted**: September 5, 2022, **ePublished**: February 21, 2023

Introduction

Protein synthesis is an essential step in cell growth and proliferation. Recent studies have shown the augmented rate of ribosome biogenesis in malignant cell division and tumor progression. Up-regulation of ribosome biogenesis encodes the oncoproteins, growth factors and cell cycle related regulatory mechanisms. All these mechanisms contributed to carcinogenesis (1-3). Cells need more protein during growth and proliferation and, so they should increase their protein synthesis capacity by increasing the biogenesis of the ribosome. This is completed by increasing the size and number of nuclei in the tumor cells. Response to internal and external stressors, maintaining the genome stability, setting the cell cycle, conserving the chromatin structure and regulating the gene expression are among the functions of nucleoli. When the cell loses these regulations, cancer will initiate and progress (4).

Nearly, 90% of oral cancers are oral squamous cell carcinoma (OSCC). The 5-year survival rate of OSCC is still less than 50%. Tumor size, regional metastasis and clinical stage have been related to poor prognosis of OSCC (5,6). Progressive increases of nucleus diameter and number of nucleoli from normal mucosa to OSCC have been reported (7,8).

Recent studies showed increasing rate of moderately differentiated OSCC in recent decade in Iran. It is important to have a criterion for determining the early prognosis of OSCC (9).

No reliable, definitive diagnostic protocol to predict the prognosis of OSCC has been determined, yet. The morphometric study of nuclear parameters can be a useful tool to predict the outcome of malignant epithelial derived lesions. The relationship between nuclear parameters and lymph nodes involvement is unknown. The aim of this study was to investigate the nuclear parameters in relation to regional lymph node involvement in OSCC.

*Corresponding Author: Noushin Jalayer Naderi, Email: jalayer@shahed.ac.ir

Materials and Methods

This retrospective study was completed in Iran National Tumor Bank, Cancer Biology Research Center, Cancer Institute of Iran, Tehran University of Medical Sciences, Tehran, Iran and Oral and Maxillofacial Pathology Department, Faculty of Dentistry, Shahed University during 2016-2018.

Incomplete medical records, previous history of radiotherapy and/or chemotherapy and marginal sections were exclusion criteria. Sufficient tumoral mass, appropriate fixation and tumors without extensive necrosis and hemorrhage were inclusion criteria. After reviewing the hematoxylin-eosin stained sections, best samples with adequate tumoral mass were selected. Considering the power of sample size = 0.9 at 95% confidence level, the minimum number of sample size was determined as 12 cases for each groups. Twenty-eight samples were found and entered the study. Samples were divided into two sets of with and without regional lymph node involvement. Nuclear parameters were evaluated on total of 14 well differentiated OSCC, 8 moderate differentiated OSCC and 6 poorly differentiated. Samples were graded based on Kumar et al description of histopathologic differentiation of SCC (10).

The formalin-fixed, paraffin embedded sections of samples were retrieved from the archive. Four µm sections were prepared from embedded blocks. Sections were deparaffinized and hydrated in distilled water. The slides were incubated in methyl green-pyronin solution (2% aqueous methyl green and 1% aqueous pyronin) (Merck, Germany) for 2-7 minutes, rinsed with distilled water, dipped in absolute alcohol and dried. Then, the sections were cleared in xylene and mounted (11). With methyl green-pyronin staining method, the nuclei and nucleoli were seen green and red, respectively (Figure 1).

Using light microscopy (Olympus BX40) equipped with digital camera (Sony ExwaveHAD, Model No. SSC-DC58AP; Tokyo, Japan), the cells were evaluated at × 1000 (10 × ocular and 100 × objective lenses) magnification. A total of 100 tumoral cells in 10 fields were selected randomly (11). For each sample, the number of nucleoli in 10 high-power field (HPF) were counted. The nucleus and nucleoli diameters were measured and the nucleus: nucleoli ratio were calculated (12).

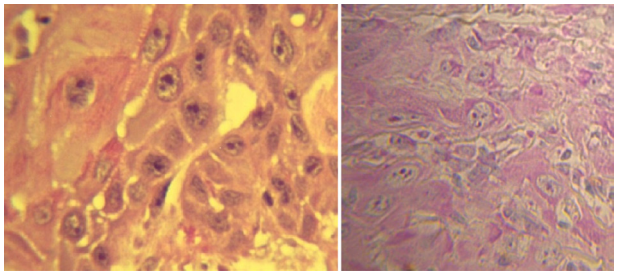

Figure 1. Methyl green-pyronine stained section of oral squamous cells carcinoma (magnification × 1000)

The nuclear parameters were assessed and scored as follows (13):

(A) Number of nucleoli: Score 0 (Normal): 0-4 nucleoli in 10 HPF, Score 1: 4-6 nucleoli in 10 HPF and Score 2: more than 6 in 10 HPF. (B) Nucleus: nucleoli ratio: Score 0 (Normal): the nucleus: nucleoli ratio < 1: 5, Score 1: the nucleus: nucleoli ratio 1:3 and Score 2: the nucleus: nucleoli ratio > 1: 3

The number of involved lymph nodes were scored as follows:

N0: No regional lymph node involvement, N1: 1-5 involved regional lymph nodes, N2: 5-10 involved regional lymph nodes and N3: more than 10 involved regional lymph nodes.

Statistical analysis

The frequency of nucleoli count and nucleus: nucleoli ratio were presented as number (%). To compare the nucleoli count and nucleus: nucleoli ratio with lymph node involvement and to compare the nuclear parameters in different histopathologic grades, Mann-Whitney U test were used at $P \leq 0.05$ probability level. The association of nucleoli count and nucleus: nucleoli ratio with lymph node involvement were tested using the Spearman's correlation coefficient test at $P \leq 0.05$ probability level. The SPSS statistical software package (Version 22; IBM Company, Chicago, IL, USA) was employed.

Results

The results of nucleoli count showed that, out of 14 cases with metastatic involvement of lymph nodes, 8 (57.14%) cases were score 1 and 6 (42.86%) ones were score 2. Out of 14 cases without lymph node involvement 10 (71.43%) cases were score 1 and 4 (28.57%) ones were score 2. The results of nucleus: nucleoli ratio showed that, in both cases with and without lymph node involvement, 7 (50%) cases were score 1 and 7 (50%) ones were score 2 (Table 1).

Mann-Whitney U test revealed that the number of nucleoli count and nucleus: nucleoli ratio in cases with metastasis to regional lymph nodes were not significantly different from cases without lymph node involvement ($P = 0.29$ and $P = 0.52$, respectively).

In cases with lymph node involvement, Spearman's correlation coefficient test revealed no significant correlation between the nucleoli count and lymph node involvement ($r = 0.08$, $P = 0.78$). The correlation between nucleus: nucleoli ratio and lymph node involvement was significant ($r = 0.58$, $P = 0.02$). In cases with lymph node involvement, the correlation of nucleoli count and nucleus: nucleoli ratio with histo-pathologic grade were not significant ($r = 0.12$, $P = 0.68$ and $r = 0.12$, $P = 0.68$, respectively). Nucleoli count and nucleus: nucleoli ratio showed no significant correlation with histopathologic grade in cases without lymph node involvement ($r = 0.12$, $P = 0.66$ and $r = 0.30$, $P = 0.28$, respectively).

Table 1. Distribution of nuclear parameters in cases with lymph node involvement and without lymph node involvement

	Nucleoli count			Nucleus: Nucleoli ratio		
	Score 0	Score 1	Score 2	Score 0	Score 1	Score 2
Cases with lymph node involvement (n=14)	0	8 (57.14%)	6 (42.86%)	0	7 (50%)	7 (50%)
Cases without lymph node involvement (n=14)	0	10 (71.43%)	4 (28.57%)	0	7 (50%)	7 (50%)

Discussion

The nucleoli count and nucleus: nucleoli ratio were not significantly different in cases with and without regional lymph node involvement. The nucleus: nucleoli ratio was significantly correlated to lymph node involvement.

Accumulation of distinct genetic events is a causal factor in initiation and progression of oral cancer which is ultimately terminated to an invasive tumor. Increased cellular proliferation and reduced apoptosis are the first steps of cancer progression. In a normal situation, proto-oncogenes and tumor suppressor genes code a number of proteins to control cell cycle, apoptosis and angiogenesis. The loss of nucleoli function in maintaining the stability of the genome can cause the development of cancer. Currently, the attention of researchers has been focused on cellular nucleoli as a potential therapeutic pathway (4). Previous studies have been shown a progressive increase of nucleus diameter and number of nucleoli from normal mucosa toward the higher degree of histopathologic grades in OSCC (7).

In the present study, the nucleus: nucleoli ratio was significantly correlated to lymph node involvement. This is compatible with previous studies which showed a higher ribosome biogenesis in OSCC (4,7,14). This is in the line with previous reports that showed higher aggressive function of malignant tumors with discrepancies of ribosome biogenesis. This is in harmonious with poor outcome of disease.

The nucleoli count and nucleus: nucleoli ratio in cases with metastasis to regional lymph nodes were not significantly different from cases without lymph node involvement. It seems that increased activity of nucleoli is associated with increased cell proliferation and is not correlated to metastatic behavior. This is compatible with higher protein synthesis in cells during proliferation (1).

The correlation of nucleoli count and nucleus: nucleoli ratio with histopathologic grade were not significant in cases with and without lymph node involvement. This was not consistent with the findings of Mohtasham et al who showed that the mean number of nucleoli were significantly associated with higher histopathologic grades of OSCC (7). Since Mohtasham et al have been focused on diameters and mean number of nucleoli in different grades of OSCC, the difference may be due to different sampling methods and study design.

In the present study, the nucleoli count was not significantly related to regional lymph nodes involvement. No similar study was found to compare the findings.

Large number of samples were missed due to the lack of clinical information, insufficient tissue of paraffin embedded sections and absence of archived blocks. It was an important limitation of this study.

Specific histochemical staining methods provide better interpretation of nuclear materials of cells. Toluidine Blue, Schiff reagents and Feulgen are specific DNA detectors, whereas AgNOR is specific for displaying RNA (12). Feulgen is used for deoxyribonucleoprotein staining. With this staining method nucleoli is negative and nuclear DNA is positive (15). AgNORs are chromosomal segments that encode the ribosomal ribonucleic acid situated within the nucleolus. Increased number of AgNORs marks which is visualized as black dots have been correlated to increased proliferation rate of cells (16). Counting the black dots is difficult and time-consuming, especially for a no calibrated, experienced person. Methyl green-pyronin is a reliable and simple technique in the assessment of RNA and DNA simultaneously. Higher grade of polymerized nucleic acids in DNA is stained with methyl green and lower grade of polymers from RNA is stained with pyronin (17). With methyl green-pyronin, the nuclei and nucleoli were seen green and red, respectively. Evaluation of nucleus and nucleoli parameters in oral dysplastic lesions (8), OSCC (7) and oral sub-mucous fibrosis (12) showed the usefulness of methyl green-pyronin staining in demonstrating the malignant transformations. Accurate distinction of nuclei and nucleoli with methyl green-pyronin staining method and its accuracy in demonstrating the malignant transformation are important advantages of methyl green-pyronin staining method. To omit the effect of staining method on results, methyl green-pyronin stain was used. It was an important strength of study to reach a more reliable result.

Based on our knowledge, the present study was the first research on evaluation of nuclear parameters in relation to regional lymph node involvement in OSCC. More efforts are required to assess the nuclear parameters in relation to prognostic outcome of OSCC.

A significant correlation was found between nucleus: nucleoli ratio and lymph nodes involvement in OSCC. Comparing the clinic-pathologic outcome of different histopathologic grades is recommended in future studies.

Conclusion

The nucleus: nucleoli ratio was correlated to lymph nodes involvement. Based on results, nucleus: nucleoli ratio can potentially be a useful tool to determine lymph node involvement in OSCC.

Acknowledgements
The authors thank Dr. Amir Nader Emami Razavi and team of Iran National Tumor Bank, Cancer Biology Research Center, Cancer Institute of Iran, Tehran University of Medical Sciences, for kindly assistance in archive retrieval and histopathologic staining.

Author Contributions
Conceptualization: Noushin Jalayer Naderi, Ahad Muhammadnejad.
Data curation: Vahid Karbalaee Khiavi, Ahad Muhammadnejad.
Formal Analysis: Noushin Jalayer Naderi.
Investigation: Vahid Karbalaee khiavi, Noushin Jalayer Naderi, Ahad Mohammadnejad.
Methodology: Noushin Jalayer Naderi.
Project administration: Noushin Jalayer Naderi.
Supervision: Noushin Jalayer Naderi.
Validation: Noushin Jalayer Naderi, Ahad Muhammadnejad.
Visualization: Vahid Karbalaee khiavi, Noushin Jalayer Naderi, Ahad Mohammadnejad.
Writing – original draft: Vahid Karbalaee khiavi, Noushin Jalayer Naderi.
Writing – review & editing: Vahid Karbalaee khiavi, Noushin Jalayer Naderi, Ahad Mohammadnejad.

Conflict of Interests
The authors declare that have no conflict of interests.

Ethical Approval
The study was approved by the ethical committee of Shahed University under registration number IR.Shahed.REC.1395.157.

Funding
The study financially was supported by Shahed University. Biological materials were provided by the Iran National Tumor Bank which is funded by Cancer Institute of Tehran University of Medical Sciences, for Cancer Research.

References
1. van Riggelen J, Yetil A, Felsher DW. MYC as a regulator of ribosome biogenesis and protein synthesis. Nat Rev Cancer. 2010;10(4):301-9. doi: 10.1038/nrc2819.
2. Montanaro L, Treré D, Derenzini M. Nucleolus, ribosomes, and cancer. Am J Pathol. 2008;173(2):301-10. doi: 10.2353/ajpath.2008.070752.
3. Ruggero D. Translational control in cancer etiology. Cold Spring Harb Perspect Biol. 2013;5(2):a012336. doi: 10.1101/cshperspect.a012336.
4. Orsolic I, Jurada D, Pullen N, Oren M, Eliopoulos AG, Volarevic S. The relationship between the nucleolus and cancer: current evidence and emerging paradigms. Semin Cancer Biol. 2016;37-38:36-50. doi: 10.1016/j.semcancer.2015.12.004.
5. Seoane-Romero JM, Vázquez-Mahía I, Seoane J, Varela-Centelles P, Tomás I, López-Cedrún JL. Factors related to late stage diagnosis of oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal. 2012;17(1):e35-40. doi: 10.4317/medoral.17399.
6. Oliveira ML, Wagner VP, Sant'ana Filho M, Carrard VC, Hugo FN, Martins MD. A 10-year analysis of the oral squamous cell carcinoma profile in patients from public health centers in Uruguay. Braz Oral Res. 2015;29. doi: 10.1590/1807-3107BOR-2015.vol29.0075.
7. Mohtasham N, Mahdavi-Shahri N, Salehinejad J, Ejtehadi H, Torabi-Parizi M, Ghazi N. Detection of nucleoproteins in squamous cell carcinoma, and dysplastic and normal mucosa in the oral cavity by methyl green-pyronin staining. J Oral Sci. 2010;52(2):239-43. doi: 10.2334/josnusd.52.239.
8. Metgud R, Gupta K, Chandra U. Simultaneous quantification of nucleoproteins and comparison of methyl green-pyronin Y and Feulgen staining in sections of oral squamous cell carcinoma, dysplastic lesions and normal mucosa. Biotech Histochem. 2014;89(4):267-72. doi: 10.3109/10520295.2013.839826.
9. Ghafari R, Jalayer Naderi N, Emami Razavi A. A retrospective institutional study of histopathologic pattern of oral squamous cell carcinoma (OSCC) in Tehran, Iran during 2006-2015. J Res Med Sci. 2019;24:53. doi: 10.4103/jrms.JRMS_882_18.
10. Kumar V, Abbas A, Aster J. Robbins & Cotran Pathologic Basis of Disease. 10th ed. Canada: Elsevier; 2021.
11. Metgud R, Gupta K. Expression of cell cycle and apoptosis-related proteins in ameloblastoma and keratocystic odontogenic tumor. Ann Diagn Pathol. 2013;17(6):518-21. doi: 10.1016/j.anndiagpath.2013.06.006.
12. Metgud R, Gupta K, Prasad U, Gupta J. Cytomorphometric analysis of oral submucous fibrosis and leukoplakia using methyl green-pyronin Y, Feulgen staining and exfoliative brush cytology. Biotech Histochem. 2015;90(1):8-13. doi: 10.3109/10520295.2014.919025.
13. Phukan JP, Sinha A, Deka JP. Cytological grading of breast carcinoma on fine needle aspirates and its relation with histological grading. South Asian J Cancer. 2015;4(1):32-4. doi: 10.4103/2278-330x.149948.
14. Moradzadeh Khiavi M, Vosoughhosseini S, Halimi M, Mahmoudi SM, Yarahmadi A. Nucleolar organizer regions in oral squamous cell carcinoma. J Dent Res Dent Clin Dent Prospects. 2012;6(1):17-20. doi: 10.5681/joddd.2012.004.
15. Dehghannezhad M, Jalayer Naderi N, Semyari H. Micronucleus assay of buccal mucosa cells in waterpipe (hookah) smokers: a cytologic study. Iran J Pathol. 2020;15(2):75-80. doi: 10.30699/ijp.2020.101701.2010.
16. Aggarwal T, Sawke G, Sawke N. Application of AgNOR (argyrophilic nucleolar organizer regions) staining in distinction of non neoplastic and neoplastic endometrial lesions. People's Journal of Scientific Research. 2015;8(1):23-7.
17. Singh E. Comparative analysis of nucleomorphometric parameters in methyl green-pyronin-stained sections of oral epithelial dysplasia, oral submucous fibrosis and oral squamous cell carcinoma. J Oral Maxillofac Pathol. 2020;24(2):401. doi: 10.4103/jomfp.JOMFP_360_19.