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SIZE AND NUMBER OF SILVER-STAINED NUCLEOLAR ORGANIZER REGIONS AND SURVIVAL RATE IN PATIENTS WITH INTRAORAL CARCINOMAS

SUMMARY

Determination of future clinical behavior of carcinomas still present the subject of numerous investigations in which different clinical and histopathological findings have been analyzed. The value of argyrophilic nucleolar organizers (AgNOR) is one of them, and the association between their number and size and clinical findings have been analyzed in numerous investigations, on different tumors and different locations in the human body.

The aim of this investigation was to determinate the value of number and size of AgNOR in predicting the clinical behavior of intraoral carcinomas throughout survival rate of patients.

The AgNOR size and number were analyzed in 50 patients with intraoral squamous cell carcinomas with different survival rates. By regression analysis, the relationship between AgNOR size and number and survival rate was not found.

According to our results, it can be said that the values of AgNOR size and number in patients with intraoral carcinomas are not in correlation with survival rate.

Key words: carcinomas, intraoral, AgNOR

INTRODUCTION

Intraorally located squamous cell carcinomas are the ones with the shortest clinical development and lowest survival rate. Unfortinately, in the United States, every year 19.500 newly diagnosed cases of intraoral carcinomas are recorded. Because of asymptomatic beginning, most of newly diagnosed carcinomas are in the third and fourth tumor stage (1). The incidence is not equally distributed in different countries, and varies from 5–8 % of all carcinomas in the United States to 50 % in India and South East Asia. The incidence is closely associated with consuming of tobacco and its products (1). Survival time after therapy is variable, but it can be said that it equals 28% with two-year survival period for men and 42 % for women, and 50% for men and 59% for women in five-year period (2).

It is well-known that tumors with the same clinical and histopathological presentation can have different clinical behavior. Determination of future clinical behavior of carcinomas still presents the subject of numerous investigations in which different clinical and histopathological findings have been analyzed. Based on clinical findings, tumors can be subdivided in two main groups, "aggressive "and " non-agressive". Difficulties in precise determination of these terms forced numerous authors to find methods for easier and objective estimation of future bilogical behavior of neoplasms.

Numerous attempts have been performed to establish the technique which preoperatively can

provide information about future behavior of intaroral carcinomas, and can guide surgical therapy to avoide the procedures which unnecessarily destroy healthy structures. Two basic groups of parameters have been investigated for determining the factors associated with clinical development: (a) parameters connected with clinical presentation of tumour, and (b) parameters associated with histopathological presentations (3).

Argyrophilic nucleolar organizers (AgNOR) are the segments of DNA which are coded for ribosomal RNA. Ribosomal RNA genes manage ribosomes and protein synthesis. It is considered that the number of AgNOR maintains the degree of nuclear and cellular activity. The increased number of AgNOR is the result of more active cellular proliferation, distorted nucleolar association, increased ploidy or greater transcription activities. The argyrophilic staining of interphase nucleolar organizer regions (AgNOR technique) has been demonstrated to be useful in distinguishing some benign lesions from their malignant counterparts, on the basis of greater quantity of AgNOR proteins. The level of AgNOR and AgNOR-associated proteins (AgNOR proteins) varies with cell activity, including ribosomal biogenesis occurring in proliferating cells. Proteins associated with some AgNOR are detected by specific silver staining (4).

The values of AgNOR have been the subject of numerous investigations, for example, on malignant melanoma (5), lung cancer (6), and prostate carcinoma (7). Piffko et al. (1997) investigated intraoral carcinomas, nearby mucosa and precancerous lesions, and found the corellation between the number and size of AgNOR and invasivness of malignant tumours located intraorally, especially in the tumor front region (8,9). In the investigation associated with AgNOR, the simplicity and practical use of method is emphased (10).

The aim of this study was to estimate the AgNOR number and size in oral carcinomas with different clinical course.

MATERIAL AND METHODS

At Department of Maxillofacial Surgery, University of Niš, 50 cases of intraoral squamous cell carcinoma (37 male, and 13 female, mean age 51 years) were surgically treated.

To demonstrate the AgNORs, the silver staining procedure was performed on 5 m thick paraffin sections. The silver colloid solution for staining of NOR was prepared by dissolving gelatin in 1% aqueous formic acid in the final concentration of 2%. This solution was mixed in 1:2 volumes, with 60% aqueous silver nitrate to obtain the working solution. The staining time was 45 min and silver reaction was carried out in the dark. A 100x oil immersion objective was used for quantization of AgNORs. In each carcinoma, 100 nuclei were analyzed by an image analyzer MicroImage 3.0 (Olympus, Tokyo, Japan), at objective 40x on Swift microscope with digital camera Mustek w300 and projecting objective x 3,3.

The patients were treated by surgical excision of tumors and lymph node dissection, with consecutive radiotherapy.

For survival analysis, Kaplan-Meyer method was used. Statistical significances of the relationship between survival rate and AgNOR values were obtained by regression analysis.

RESULTS

AgNOR Analysis

The majority of the patients came with the disease already developed, the largest number in T3 stage (*Table 1*).

Table 1. T- staging in patients with intraoral carcinomas

Tumor stage	T1	T2	T2	T4
Number of patients	2	12	25	11

The mean AgNOR count and AgNOR area for all patients with intraoral carcinomas were $1,81\pm0,42$, and $3,34\pm1,2 \,\mu\text{m}^2$, respectively (*Table 2*).

Table 2. AgNOR number and size in all patients

	Mean + SD	Median	Min	Max
AgNOR number	1.81 <u>+</u> 0.42	1.71	1.1	2.86
AgNOR area (μm^2)	3.34 <u>+</u> 1.2	3.08	1.84	7.50

The largest number of AgNOR was found to be in T2 stage, and the smallest AgNOR area was found in T3 stage (*Table 3*). All differences were not statistically significant.

Table 3. AgNOR number and size and T-stage

Tumor stage	T1	T2	T2	T4
AgNOR number	1.83±0.57	1.92±0.61	1.76±0.49	1.87±0.72
AgNOR area (µm ²)	2.63±0.91	3.32±1.03	3.43±0.99	3.25±1.12

Survival analysis

The survival rate of patients with intraoral squamous cell carcinoma was extremelly poor (*Figure 1*).

staining of nucleolar organizer region-associated proteins (AgNOR) was widely used in tumor pathology both for diagnostic and prognostic purposes. The relation between AgNOR values and



Figure 1. Survival of patients with intraoral carcinoma

The vast majority of patients died within first 9 months, only 10% of patient suvived a 5-year period (Table 4).

Table 4. Survival in 50 patients withintraoral sqamous cell carcinomas

	Mean <u>+</u> SD	Median	Min	Max
Survival (months)	14.33+13.41	11	1	60

By regression analysis, the relationship between AgNOR size and number and survival rate was not found (beta=0.21, p=0.18).

DISCUSSION

Beltrami (1992) (3) found a 5-year survival rate less than 35% in his investigated group of patients with intraoral carcinomas. The vast majority of these pateints were in T2 stage. In contrast, in our study, the 5-year survival rate was 10 %; however, the majority of patients was in T3 stage.

Histological criteria have been extensively evaluated in the past to determine their prognostic implications. Grading the squamous cell cancer remains a common practice, although many authors will agree that as a solitary criterion it is unreliable as a predictor of behavior. In the last decade, silver biological characteristics of intraoral cancer behavior have been the subject of some investigations. Rajendran and Nair (1992) (11), Migaldi (1998) (12), Piffko et al. (1999) (13), and Waranakulasurya and Johanson (1993) (14) examined the relation of cellular alteration, expressed in AgNOR values, for early diagnosis of intraoral carcinomas. Most of the authors, like Costa et al. (1999) (15), Kobayashi et al. (1996) (16), Piffko et al. (1995) (17) and Yue et al. (1999) (18) found the correlation between the values of AgNOR and proliferate activity of tumor cells. Sano et al. (1991) (19) found that five-year survival rate of the cases with high AgNOR count (greater or equal to 6.5) was significantly lower than that with low AgNOR counts (less than 6.5).

On the other hand, Sarada et al. (1995)(20), Chattopadahyay et al. (1994)(21), Kahn et al. (1993) (22), and Fathy and el Houssary (1994)(23) could not find any statistically significant relation between the values of AgNOR and biological development of intraoral carcinomas. Eslami (2006) found that a significant difference was observed in the number of AgNOR dots between oral SCC and SCC with surrounding dysplastic and normal tissues (24). On the other hand, Elangovan (2008) found that AgNOR quantity was strictly proportional to the proliferative activity of the cell and does not necessarily indicate malignancy. It is the qualitative characteristic of AgNOR that helps to differentiate the hyperplastic, premalignant, and malignant lesions (25). As it is possible that the evaluation of a single histologic criterion as a prognostic indicator may be somewhat simplistic, a multifactorial morphologic evaluation may be more appropriate. In our study, both AgNOR size and number were evaluated, but AgNOR size and number was not statistically associated with both survival time and tumor stage.

Differences between the results obtained by the aforesaid authors may be the consequence of very different factors, e.g. race, geographic position, sampling method, staining procedure, etc. In our investigation, all the parts of tumor tissue could be equally measured for AgNOR size and count, in contrast to Pifko et al. (1997) (9). It may be very difficult to measure AgNOR size and number in tumor periphery only because of limited cell number.

According to our results, AgNOR size and number cannot be used for predicting the biological behavior of intraoral carcinomas, which makes the therapy of these tumors with fast clinical progression more limited. Future investigations on large number of patients are needed.

REFERENCES

1. Ballenger J, Snow J. Otorhinolaryngology. Williams & Wilkins, Baltimore, Philadelphia, London Tokyo, 1996: 254-256.

2. Ildstad S, Tollerund D, Bigelow ME, Remensnyder J. Squamous cell carcinoma of the head and neck at Massachusetts General Hospital: A comparison of biologic characteristics in men and women. Surgery 1986; 99: 7-13.

3. Beltrami CA, Desinan L, Rubini C. Prognostic factors in squamous cell carcinoma of oral cavity. Path Res Prat 1992; 188: 510-516.

4. Kutlešić Č, Mihailović D. Uvod u dijagnostičku kvantitativnu patologiju, Medicinski fakultet Niš, 2000.

5. Mera M. AgNOR values in Callender histopathological typs of malignant uveal melanomas. Rom J Morphol Embryol 1995; 41: 125-128.

6. Chern JH, Lee YC, Yang MH, Chang SC, Perng RP. Usefulness of argyrophilic nucleolar organizer regions score to differentiate suspicious malignancy in pulmonary cytology. Chest 1997; 11: 1591-1596.

7. Mihailović D, Stojanović D, Penev G, Radić S, Dimov D, Milovanović Lj. Broj aktivnih nukleolarnih organizatora (AgNOR) u benignim i malignim tumorima prostate. Libri oncologici 1991; 20 (Suppl 1): 66.

8. Piffko J, Bankfalvi A, Ofner D, Rasch D, Joos U, Schmid KW. Standardized demonstration of silverstanied nucleolar organizer regions –associated proteins in archival oral squamous cell carcinomas and adjacent non-neoplastic mucosa. Mod Pathol 1997; 10: 98-104.

9. Piffko J, Bankfalvi A, Ofner D, Rasch D, Joos U, Schmid KW. Standardised AgNOR analysis of the invasive tumor front intaoral squamous cell carcinoma. J Pathology 1997; 182: 450-6.

10. Bankvalfi I, Piffko J. Prognostic and predictive factors in oral cancer, the role of invasive tumor front. J Oral Pathol Med 2000; 29: 291.8.

11. Rajendran R, Nair SM. Silver binding nucleolar organizer region proteins as possible prognostic indicator in oral submucous fibrosis. Oral Surg Oral Med Oral Pathol 1992; 74: 481-6.

12. Migaldi M, Criscuolo M, Zunarelli E, Lo Bianco L, Martinelli AM, Barbolini G. p 120 and AgNOR nucleolar protein expression: a comparison with nuclear proliferation markers in oral pathology. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 85: 189-196.

13. Piffko J, Bankfalvi A, Joos U, Ofner D, Krassort M, Schmid KW. Immunophenotypic analysis of normal mucosa and squamous cell carcinoma of the oral cavity. Cancer Detect Prevent 1999; 23: 45-56.

14. Waranakulasuriya KA, Johnson NW. Nucleolar organizer regions (NOR) distribution as diagnostic marker in oral keratosis, dysplasia and squamous cell carcinoma. J Oral Pathol Med 1993; 22:77-81.

15. Costa A, de Araujo NS, Pinto, de Arujo VC. PCNA / AgNOR and Ki 67/AgNOR double staining in oral squamous cell carcinoma. J Oral Pathol Med 1999; 28: 438-441.

16. Kobayashi I, Matsuo A, Ozeki S, Ohishi M, Ishibashi Y, Sakai H. The proliferative activity in oral epithelial dysplasia analyzed by proliferating cell nuclear antigen immunostaining and argyrophilic nucleolar organizer region staining, Hum Pathol 1995; 26: 907-13.

17. Piffko, Bankfalvi A, Ofner D, Bryne M, Rash D, Joos U, Bocker W, Schmid KW. Prognostic value of histopathology factors (malignancy grading and AgNOR content) assessed invasive tumor front of oral squamous cell carcinoma Br J Cancer 1995; 10: 1543-1546.

18. Yue L, Iwai M, Furuta I. Evaluation of argyrophilic nucleolar organizer regions in tongue squamous cell carcinoma. J Oral Oncol 1999; 35:70-76.

19. Sano K, Takahashi H, Fujita S, Inokuchi T, Pe MR, Okabe H, Tsuda N. Prognostic implication of silver binding nucleolar organizer regions (AgNORs) in oral squamous cell carcinoma. J Oral Pathol Med 1991; 20:53-56.

20. Sarada R, Sankaran V, Ratnakar C, Veileth AJ, Prema V. Application of AgNOR method to distinguish pseudoepitheliomatous hyperplasia from squamous cell carcinoma; Indian J Cancer 1995; 32:169-74.

21. Chattopadahya A, Chawada JG, Doshi J. Silver binding nucleolar organizer regions: study of oral leucoplakia and squamous cell carcinoma. Int J Oral Maxillofac Surg 1994; 23: 374-377.

22. Kahn MA, Mincer HH, Dockter ME. Comparing flow cytometry analysis and nucleolar organizer region enumeration in archival oral premalignant lesion. J Oral Pathol Med 1993; 22: 257-62.

23. Fathy LM, el Houssary A. Variations in nucleolar organizer regions during 9-10 dimethyll-2 benzanthracene induced tongue carcinogenesis in rats. J Egyp Dental J 1994; 40: 795-804.

24. Eslami B, Rahimi H, Rahimi F, Khiavi MM, Ebadifar A. Diagnostic value of silver nitrate staining for nucleolar organizer regions in selected head and neck tumors. J Cancer Res Ther 2006;2:129-31.

25. Elangovan T, Mani NJ, Malathi N. Argyrophilic nucleolar organizer regions in inflammatory, premalignant, and malignant oral lesions: a quantitative and qualitative assessment. Indian J Dent Res. 2008;19:141-6.

VELIČINA I BROJ ARGIROFILNIH NUKLEOLARNIH ORGANIZATORA I STOPA PREŽIVLJAVANJA BOLESNIKA SA INTRAORALNIM KARCINOMIMA

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SAŽETAK

Određivanje budućeg kliničkog toka karcinoma predstavlja objekat brojnih istraživanja koji za predmet imaju uticaj brojnih kliničkih i histopatoloških nalaza na klinički tok tumora. Vrednosti argirofilnih nukleolarnih organizatora (AgNOR-a) predstavljaju jednu od njiih. Odnos njihovog broja i veličine spram kliničkih parametara bio je predmet brojnih istraživanja na različitim tumorima kao i na različitim lokacijama na ljudskom organizmu.

Cilj istraživanja bio je odrediti značaj vrednosti broja i veličine AgNOR-a u odnosu na kliničko ponašanje intraoralnih karcinoma, posmatrano kroz dužinu preživljavanja bolesnika.

Veličina i broj AgNOR-a je analizirana u tumorskom materijalu 50 bolesnika sa intraoralnim karcinomima sa različitim periodima postterapeutskog preživljavanja. Regresionom analizom korelacija između broja i veličine AgNOR-a i dužine preživljavanja kod bolesnika sa intraoralnim karcinomima nije konstatovana.

Spram dobijenih rezultata, može se reći da vrednosti broja i veličine AgNOR-a, kod intraoralnih karcinoma ne koreliraju.

Ključne reči: karcinomi, intraoralni, AgNOR