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Correlation of AgNOR with Cytopathological Grades in Oral Squamous Cell Carcinoma: A Potential Prognostic Marker

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is a major global health issue, particularly in regions with high tobacco and alcohol use, and accounts for 95% of oral cancers. The AgNOR staining technique, which identifies nucleolar organizer regions (NORs) associated with ribosome production, is used to assess cellular proliferation.
Objective: This study aims to explore the potential of AgNOR quantification as a prognostic tool for OSCC.
Materials and Methods : This retrospective study analyzed 44 cases of oral squamous cell carcinoma (OSCC) from Khartoum Dental Hospital, classifying them into well-differentiated, moderately differentiated, and poorly differentiated based on cytological diagnosis. AgNOR staining was performed, and the percentage of AgNOR-positive cells was recorded. Nucleolar organizer regions (NORs) were counted using a ×100 oil immersion lens, with ten fields randomly selected for analysis and areas with necrosis or artifacts excluded. Statistical analysis was conducted using SPSS version 21, presenting results as percentages and frequencies. Ethical approval was obtained from Al-Neelain University, and informed consent was provided by all participants, although the ethical approval number was lost due to the recent conflict in Sudan.
Results :The study involved 44 patients diagnosed with oral squamous cell carcinoma (OSCC), consisting of 30 males (68.2%) and 14 females (31.8%), with a mean age of 53.5 years. Cytological samples were collected from various tumor sites, including the floor of the mouth, tongue, lower lip, palate, and lower jaw. Tumors were classified as well-differentiated (50%), moderately differentiated (31.8%), and poorly differentiated (18.2%). AgNOR percentages varied across the samples, ranging from 25% to 97%, with a mean of 65%. Well-differentiated OSCC exhibited AgNOR dot percentages from 25% to 95%, moderately differentiated OSCC ranged from 43% to 97%, and poorly differentiated OSCC showed percentages from 35% to 87%.
Conclusion : Well-differentiated OSCC showed the lowest AgNOR expression, reflecting lower proliferative activity, while moderately differentiated OSCC exhibited higher AgNOR counts, indicating increased proliferation. Poorly differentiated OSCC had variable AgNOR expression, possibly due to genetic instability. Although AgNOR count did not correlate significantly with prognosis, it may serve as a useful prognostic tool, particularly when histopathological differentiation alone is insufficient. The study highlights the need for larger cohorts and additional markers to confirm these findings and further explore AgNOR's role in OSCC progression.
Key words: Oral squamous cell carcinoma, OSCC, AgNOR, Cytopathological grade, cell proliferation, prognostic marker, cytopathology.

Introduction

Oral squamous cell carcinoma (OSCC) is a major global health issue, particularly in areas with high rates of tobacco and alcohol use (Eloranta, et al.,2024). Head and neck cancers (HNC), including OSCC, rank among the leading causes of cancer-related mortality worldwide (Ferlay et al., 2010). Approximately 95% of all oral cancers are squamous cell carcinomas, with the remaining 5% comprising sarcomas, lymphomas, and salivary gland tumors (Montoro et al., 2008). In Sudan, oral cancer was identified as the seventh most common cancer in men and the eighth in women (Salah and Mohamed, 2017). Despite improvements in diagnostic and therapeutic approaches, the overall survival rate for OSCC remains poor, based on the stage at which the disease is diagnosed (Jin et al., 2016; Badwelan et al., 2023; Suri et al., 2024)

Nucleolar organizer regions (NORs) are chromosomal areas containing genes responsible for ribosome production. These regions contain proteins that have an affinity for silver ions, allowing them to be visualized as black dots under a light microscope after silver staining. The silver-stained NORs, along with their associated proteins, are collectively known as AgNORs

(Trerè, 2000). AgNORs are found within nucleoli during interphase, and the quantity of AgNORs in cells is an important indicator of cellular kinetics, particularly as it relates to the rate of cell proliferation (Trerè, 2000).

The AgNOR staining technique is widely regarded as an effective and cost-efficient method for assessing the proliferative activity of tissues. The number of AgNORs per nucleus correlates with cell cycle speed and is used as a marker of cell proliferation (Chandrashekar et al., 2020). This method has been extensively studied in pathology, with over 1,000 publications documenting the diagnostic and prognostic significance of AgNORs across various tumor types (Trerè, 2000)

This study aims to investigate the relationship between AgNOR percentage and the tumor grade of OSCC in cytological samples from oral cancer, with a focus on understanding the potential of AgNOR quantification as a prognostic tool.

Materials and Methods

This retrospective study involved 44 cases of oral squamous cell carcinoma (OSCC). Cytopathological data were collected from patient files in Khartoum Dental Hospital. Samples categorized into well-differentiated, moderately differentiated, and poorly differentiated OSCC based on the cytological diagnosis. AgNOR staining was carried out following the method described by Trerè (2000), and the percentage of AgNOR-positive cells was recorded.

The assessment of nucleolar organizer regions (NORs) followed the criteria outlined by Siddiqui et al. 1999. Accordingly, ×100 oil immersion lens was used to count the stained NORs in both uni- and multinucleated cells. For each case, 10 fields were randomly selected for analysis. In each field, brown or black stained regions within the nucleus were identified, and regions where stains overlapped or were connected to the nucleolus were counted as one. Areas showing necrosis, significant inflammation, or artifacts were excluded from the analysis (Sadri et al., 2010). Results were then presented in percentages. Statistical analysis was conducted to examine the relationship between AgNOR. The data were analyzed using the Statistical Package for Social Sciences (SPSS), version 21, with results presented as percentages and frequencies.

The research was approved by the Al-Neelain University Ethical Board, and all participants provided informed consent. However, due to the recent conflict in Sudan, the ethical approval number has been lost.

Results

The study cohort comprised 44 patients previously diagnosed with oral squamous cell carcinoma (OSCC). Of these, 30 (68.2%) were male and 14 (31.8%) were female, male to female ratio ≈2.14:1, with ages ranging from 30 to 83 years, and a mean age of 53.5 years. Cytological samples were taken from various tumor sites, including the floor of the mouth, tongue, lower lip, palate, and lower jaw. Cytopathological analysis classified 22 cases (50%) as well-differentiated squamous cell carcinoma (SCC), 14 cases (31.8%) as moderately differentiated and 8 cases (18. 2%) as poorly differentiated (Table 1).

The AgNOR percentage across all cases ranged from 25% to 97%, with a mean of 65%. When analyzed by tumor grade, distinct variations in AgNOR dots percentages were observed. In well-differentiated OSCC, AgNOR dots percentages ranged from 25% to 95%. In moderately differentiated OSCC, AgNOR dots percentages ranged from 43% to 97%. Poorly differentiated OSCC exhibited AgNOR dots percentages ranging from 35% to 87 (Figure 1).

Table 1: The cytopathological diagnosis of the OSCC

Type of OSCC	**Number of patients (%)**
Well differentiated	22 (50%)
Moderate differentiated	14 (31%)
Poor differentiated	8 (9%)
Total number of patients samples	**44**

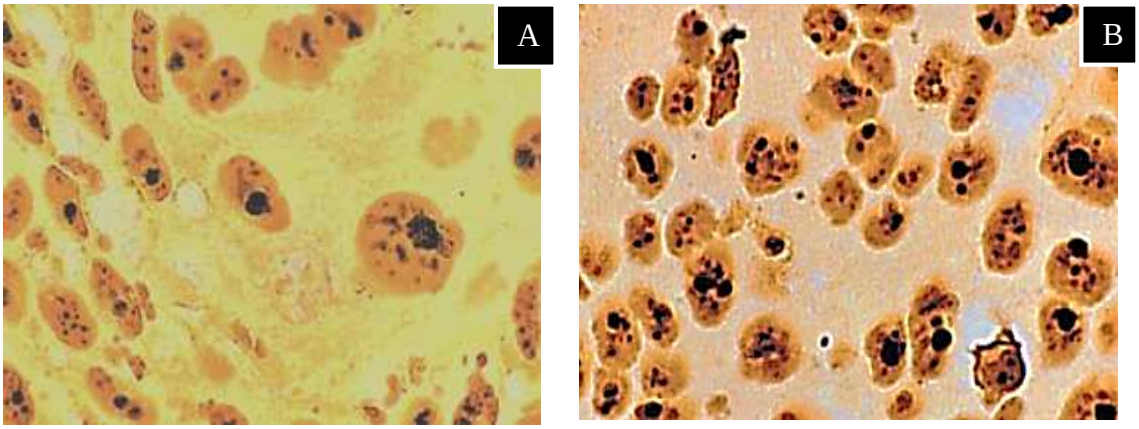

Figure 1: **A.** Well differentiate oral squamous cell carcinoma stained with silver staining showing X40 (AgNOR) (the black dots are NOR dots) in the cell nucleus. **B.** Poorly differentiate squamous cell carcinoma stained with silver staining showing AgNOR (the black dots are NOR dots) in the cell nucleus..

Discussion

Well-differentiated carcinomas exhibited the lowest range of AgNOR expression (25% to 95%), likely due to their resemblance to normal epithelial tissue and lower overall proliferative activity. These tumors maintain organized cellular structures, reducing metabolic demand and ribosomal biogenesis, which aligns with fewer AgNOR dots (Jögi et al., 2012; Bahadori, 2019). Moderately differentiated OSCC showed higher AgNOR percentages (43% to 97%) compared to poorly differentiated OSCC (35% to 87%), reflecting their greater proliferative and metabolic activity. Poorly differentiated OSCC, often associated with advanced disease, may exhibit reduced proliferation due to genetic instability, metabolic exhaustion, or a shift toward invasive and metastatic behavior (Crocker, 1987; Hanahan & Weinberg, 2011). Tumor heterogeneity and sample size limitations could influence these findings, emphasizing the need for larger cohorts and additional markers to confirm conclusions. Interestingly, Derenzini et al. (2004) found no significant difference in patient prognosis based on high or low AgNOR counts, suggesting that AgNOR's prognostic value may not directly correlate with ribosome biogenesis or cell proliferation. However, the elevated AgNOR counts in moderately differentiated OSCC may indicate a critical phase in tumor progression where proliferative activity peaks before significant genomic instability manifests (Bakhoum & Cantley, 2018; Li et al., 2023). These findings underscore the potential utility of AgNOR quantification in offering prognostic insights, particularly when histopathological differentiation alone is insufficient. Additionally, the study's mean patient age of 53.5 years and a male-to-female ratio of 2.14:1 align with global OSCC patterns, with common anatomical sites such as the tongue and floor of the mouth further reinforcing the representativeness of the cohort (Sung et al., 2021).

Conclusion

In conclusion, this study highlights the potential of AgNOR quantification as a valuable biomarker for assessing proliferative activity and tumor progression in oral squamous cell carcinoma (OSCC), with differences in AgNOR expression observed between well-differentiated, moderately differentiated, and poorly differentiated tumors. The findings suggest that AgNOR counts may offer additional prognostic insight, particularly when histopathological differentiation alone is insufficient for predicting clinical outcomes. However, the study's limitations, including sample size and tumor heterogeneity, underscore the need for larger, more diverse cohorts to validate these results. Future research should focus on exploring the prognostic significance of AgNOR expression across various OSCC subtypes, incorporating additional proliferation markers, and investigating the role of AgNOR in tumor biology to further elucidate its potential as a reliable prognostic tool.

References:

- Badwelan, M., Muaddi, H., Ahmed, A., Lee, K. T., & Tran, S. D. (2023). Oral Squamous Cell Carcinoma and Concomitant Primary Tumors, What Do We Know? A Review of the Literature. *Current oncology (Toronto, Ont.)*, *30*(4), 3721–3734. https://doi.org/10.3390/curroncol30040283

- Bahadori M. (2019). New Insights into Connection of Nucleolar Functions and Cancer. *Tanaffos*, *18*(3), 173–179.
- Bakhoum, S. F., & Cantley, L. C. (2018). The Multifaceted Role of Chromosomal Instability in Cancer and Its Microenvironment. *Cell*, *174*(6), 1347–1360. https://doi.org/10.1016/j.cell.2018.08.027
- Chandrashekar, C., Patel, P., Thennavan, A., & Radhakrishnan, R. (2020). Odontogenic keratocyst: Analysis of recurrence by AgNOR, p53 and MDM2 profiling. *Journal of oral and maxillofacial pathology : JOMFP*, *24*(1), 184–185. https://doi.org/10.4103/jomfp.JOMFP_129_19
- Chang J. C. (2016). Cancer stem cells: Role in tumor growth, recurrence, metastasis, and treatment resistance. *Medicine*, *95*(1 Suppl 1), S20–S25. https://doi.org/10.1097/MD.0000000000004766;
- Crocker J, Nar P. Nucleolar organizer regions in lymphomas. The Journal of Pathology. 1987 Feb;151(2):111-118. DOI: 10.1002/path.1711510203. PMID: 2437276.
- Derenzini, M., Ceccarelli, C., Santini, D., Taffurelli, M., & Treré, D. (2004). The prognostic value of the AgNOR parameter in human breast cancer depends on the pRb and p53 status. *Journal of clinical pathology*, *57*(7), 755–761. https://doi.org/10.1136/jcp.2003.015917
- Eloranta, R., Vilén, S. T., Keinänen, A., Salo, T., Qannam, A., Bello, I. O., & Snäll, J. (2024). Oral squamous cell carcinoma: Effect of tobacco and alcohol on cancer location. *Tobacco induced diseases*, *22*, 10.18332/tid/189303. https://doi.org/10.18332/tid/189303
- Ferlay, J., Shin, H. R., Bray, F., Forman, D., Mathers, C., & Parkin, D. M. (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *International journal of cancer*, *127*(12), 2893–2917. https://doi.org/10.1002/ijc.25516
- Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell*, *144*(5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013;
- Hussien, M. T., Mohamed, M. A., Mansor, S. G., Temerik, D. F., Elgayar, S. F., & Abd El-Aziz, E. A. E. (2022). Could Argyrophilic Nucleolar Organizer Regions count mirror DNA Ploidy in Malignant Salivary Gland Tumors?. *Asian Pacific journal of cancer prevention : APJCP*, *23*(6), 1983–1992. https://doi.org/10.31557/APJCP.2022.23.6.1983
- Jin, L. J., Lamster, I. B., Greenspan, J. S., Pitts, N. B., Scully, C., & Warnakulasuriya, S. (2016). Global burden of oral diseases: emerging concepts, management and interplay with systemic health. *Oral diseases*, *22*(7), 609–619. https://doi.org/10.1111/odi.12428
- Jögi, A., Vaapil, M., Johansson, M., & Påhlman, S. (2012). Cancer cell differentiation heterogeneity and aggressive behavior in solid tumors. *Upsala journal of medical sciences*, *117*(2), 217–224. https://doi.org/10.3109/03009734.2012.659294
- Li, S., Wang, W., Yu, H., Zhang, S., Bi, W., Sun, S., Hong, B., Fang, Z., & Chen, X. (2023). Characterization of genomic instability-related genes predicts survival and therapeutic response in lung adenocarcinoma. *BMC cancer*, *23*(1), 1115. https://doi.org/10.1186/s12885-023-11580-0
- Montoro, J. R. M. C., Hicz, H. A., de Souza, L., Livingstone, D., Melo, D. H., Tiveron, R. C., & Mamede, R. C. M. (2008). Prognostic factors in squamous cell carcinoma of the oral cavity. *Brazilian journal of otorhinolaryngology*, *74*(6), 861–866. https://doi.org/10.1016/S1808-8694(15)30146-4
- Sadri, D., Hejazi, M., Jahanbani, J., & Forouzandeh, A. (2010). Quantitative analysis of argyrophilic nuclear organizer regions in giant cell lesions of jaws. *Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*, *39*(5), 431–434. https://doi.org/10.1111/j.1600-0714.2009.00872.x
- Salah, M., Mohamed, E. (2017). Frequency of oral cancers among cancers in Sudanese patients (2009–2016). *J Adv Med Den Scie Res*, 5(9), 26–35.
- Siddiqui, M. S., Soomro, I. N., Kayani, N., Muzaffar, S., & Hasan, S. H. (1999). Assessment of nucleolar organizer regions (NORs) in proliferative conditions of the liver. *Pathology, research and practice*, *195*(6), 421–426. https://doi.org/10.1016/S0344-0338(99)80016-8
- Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: a cancer journal for clinicians*, *71*(3), 209–249. https://doi.org/10.3322/caac.21660

- Suri, S., Boora, G. S., Kaur, R., Chauhan, A., Ghoshal, S., & Pal, A. (2024). Recent advances in minimally invasive biomarkers of OSCC: from generalized to personalized approach. *Frontiers in oral health*, *5*, 1426507. https://doi.org/10.3389/froh.2024.1426507

- Trerè D. (2000). AgNOR staining and quantification. *Micron (Oxford, England : 1993)*, *31*(2), 127–131. https://doi.org/10.1016/s0968-4328(99)00069-4

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