



MORPHOMETRIC ANALYSIS OF NUCLEOLI OF BENIGN AND MALIGNANT SQUAMOUS CELLS: A NOVEL PARAMETER TO DIFFERENTIATE BETWEEN REACTIVE AND TUMOR CELLS.

Pathology

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ABSTRACT

INTRODUCTION: Squamous cell carcinoma (SCC) is the most common malignancy of oral cavity. It is usually secondary to usage of tobacco products, smoking, chronic irritation etc. The most common presentation of SCC is painless ulcer, nodule in the oral cavity. **AIMS AND OBJECTIVES:** 1 Measure the surface area and diameter of nucleoli of benign and malignant cells by morphometric analysis software. 2. Comparison of both values using statistical test and to identify the significance. **MATERIAL AND METHODS:** Total 60 cases are included in the present study. 30 cases are benign and 30 cases are malignant. The images of benign and malignant cells will be taken from each cases by camera attached to microscopes. The morphometric analysis software is available online as free downloads, will be used for measuring the surface area and diameter of nucleoli. Surface area and diameter of nucleoli of both benign and malignant cases will be compared with chi square "t" test and its significance is calculated by "p" value. **RESULTS:** Average size of nucleoli in benign lesions: $13.3 / 30 = 0.44 \mu\text{m}$. Average size of nucleoli in malignant lesions: $79.5 / 30 = 2.65 \mu\text{m}$. P value: < 0.5 , hence significant difference. **DISCUSSION:** According to Lee, the size of nucleoli is around 6 micrometer in case of squamous cell carcinoma. According to Malhotra et al, tumor cells of squamous cell carcinoma have prominent nucleoli which measures about 2-3 micrometer or more. It has 97% of sensitivity with p value < 0.001 . **CONCLUSION:** Other than the routine H&E slide examination, an objective parameters of cell morphology measured by software will be helpful in diagnosis the malignant cases.

KEYWORDS

Nucleoli, squamous cells, diameter

INTRODUCTION:

Squamous cell carcinoma (SCC) is the most common malignancy of oral cavity. It is usually secondary to usage of tobacco products, smoking, chronic irritation etc. The most common presentation of SCC is painless ulcer, nodule in the oral cavity. Few patients have difficulty in opening the mouth also. Early diagnosis of SCC is helpful in early treatment for the better prognosis. Most common modality of investigation is the edge biopsy of the lesion, which is considered as gold standard investigation for the diagnosis of malignancy. The most salient microscopic features of SCC are pleomorphic cells with intra and extracellular keratin, nucleomegaly with prominent nucleoli and evidence of invasion. But in some benign reactive cases secondary to ulcer, the squamous cells will show pleomorphism with nucleomegaly and dense inflammation. In such cases, it is difficult to differentiate between reactive and malignant squamous cells. Hence utilizing the morphometric analysis¹ method by using the software, we can analyse the size of nucleoli in both reactive and malignant cases. It will be helpful in differentiating reactive from the malignant cases. Various authors like Kumaran M², George et al³, ECM looms⁴, have successfully conducted the study on morphometry with reliable and reproducible results. The study conducted by Mukta⁵ et al on squamous cell carcinoma regarding angiogenesis shows statistical significant results. The study conducted by maduresh⁶ et al, Chaterjee⁷ et al, Kshmir⁸ et al provides an objective means for the assessment of epithelial dysplasia and to predict their malignant potential in oral cavity lesions. It will also help in reducing the inter-observer variability and gives an unbiased interpretation.

AIMS AND OBJECTIVE

1. Measure the surface area and diameter of nucleoli of benign and malignant cells by morphometric analysis software
2. Comparison of both values using statistical test and to identify the significance

MATERIALS AND METHODS:

Total 60 cases are included in the present study. Out of 60 cases, 30 cases are benign and 30 cases are malignant one. The H&E stained slides of all 60 cases will be retrieved from pathology archives. The images of benign and malignant cells will be taken from each cases by camera attached to microscopes and saved in jpeg format. The morphometric analysis software is available online as free downloads, will be used for measuring the surface area and diameter of nucleoli of 60 cases. This software will be calibrated accurately. Total 10 nucleoli will be selected in each cases and measurement will be taken. Mean value will be calculated in each cases. Then, surface area and diameter of nucleoli of both benign and malignant cases will be compared with chi square "t" test and its significance is calculated by "p" value.

RESULTS:

Benign	Diameter in μm	Surface area in μm^2	Malignant	Diameter in μm	Surface area in μm^2
Case 1	0	0	Case 1	2	3.14
Case 2	0.2	0.03	Case 2	3	7.06
Case 3	1.1	0.95	Case 3	4	12.5
Case 4	2	3.14	Case 4	4	12.5
Case 5	0	0	Case 5	1	0.78
Case 6	0	0	Case 6	2	3.14
Case 7	0	0	Case 7	2.5	4.9
Case 8	0	0	Case 8	0	0
Case 9	3	7.06	Case 9	3	7.06
Case 10	0	0	Case 10	0	0
Case 11	0	0	Case 11	3	7.06
Case 12	0	0	Case 12	2	3.14
Case 13	0	0	Case 13	3	7.06
Case 14	0.5	0.19	Case 14	4	12.5
Case 15	0	0	Case 15	6	28.27
Case 16	0.4	0.12	Case 16	3	7.06
Case 17	0	0	Case 17	2	3.14
Case 18	0	0	Case 18	3	7.06
Case 19	0	0	Case 19	1	0.78
Case 20	2	3.14	Case 20	1	0.78
Case 21	5	19.6	Case 21	0	0
Case 22	0	0	Case 22	3	7.06
Case 23	0	0	Case 23	4	12.5
Case 24	0	0	Case 24	4	12.5
Case 25	0	0	Case 25	4	12.5
Case 26	1	0.78	Case 26	5	19.6
Case 27	0.5	0.19	Case 27	2	3.14
Case 28	1	0.78	Case 28	3	7.06
Case 29	0	0	Case 29	0	0
Case 30	0	0	Case 30	4	12.5

Average size of nucleoli in benign lesions: $13.3 / 30 = 0.44 \mu\text{m}$

Average size of nucleoli in malignant lesions: $79.5 / 30 = 2.65 \mu\text{m}$

Average surface area of nucleoli in benign lesions: $35.98 / 30 = 1.19 \mu\text{m}^2$

Average surface area of nucleoli in malignant lesions: $214.79 / 30 = 7.15 \mu\text{m}^2$

P value: < 0.5 , hence significant difference

Hence there is significant difference between the size of nucleoli in benign and malignant lesions.

DISCUSSION:

Squamous cell carcinoma (OSCC) is most common malignancy seen in the oral cavity. Various risk factors which leads to OSCC are tobacco chewing, smoking, alcoholism, abnormal denture, sub mucosal fibrosis, erythroplakia etc^{9,10}. All these risk factors causes irritation of overlying mucosa causing mutation changes in the DNA structure of lining squamous cells. These process takes years together to develop into full blown squamous cell carcinoma. In between this period, the lining squamous cells undergo dysplastic change, probably for our understanding, classified into mild, moderate and severe dysplasia.

Mild dysplasia: squamous cells show mild nucleomegaly with regular nuclear membrane, absent nucleoli and normal N: C ratio.

Moderate dysplasia: features between mild and severe dysplasia.

Severe dysplasia: squamous cells show frank nucleomegaly with irregular nuclear membrane, absent to prominent nucleoli and high N: C ratio.

Squamous cell carcinoma: Predominant pleomorphic cells with nucleomegaly, high N: C ratio, irregular nuclear membrane, keratin pearls, intracellular keratinization, atypical mitosis and necrosis. Frank invasion into the sub epithelium can be demonstrable¹¹.

In conditions like leukoplakia, oral ulcer, psoriasis etc, there will be proliferation of benign squamous cells admixed with dense inflammation. In such conditions, the reactive squamous cells confuses with malignant lesions. Hence present study has taken to assess the efficacy of morphometric study in differentiating the reactive squamous cells from malignant squamous cells.

According to Lee, the size of nucleoli is around 6 micrometer in case of squamous cell carcinoma¹².

According to Malhotra et al, tumor cells of squamous cell carcinoma have prominent nucleoli which measures about 2-3 micrometer or more. It has 97% of sensitivity with p value< 0.001¹³.

According to Nandini et al, there is significant nucleoli size is seen in tumor cells when compared to benign lesions¹⁴.

Hence, all the above studies mentioned that the size of nucleoli is significantly increased when compared to benign reactive squamous cells.

CONCLUSION

In cases of reactive benign lesions, it is very difficult to differentiate between benign and malignant lesion. The diagnosis of malignancy is the utmost important as it will have the different modality of treatment and prognosis. Hence, other than the routine H&E slide examination, an objective parameters of cell morphology measured by software will be helpful in diagnosis the malignant cases.

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