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Cytomorphologic Analysis of Papanicolaou Stained Smears Collected from Floor of the Mouth Mucosa in Patients with or without Oral Malignancy

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Abstract: Cytology has been found to be a reliable and accurate technique when used to diagnose early malignant transformation in areas of the body where visual examination is difficult or impossible. The factors initiating neoplastic growth are still not clearly understood. Thus, automated instruments, capable of objective and quantitative cell analysis, have been used in descriptive morphology, for the assessment of tumour cell heterogeneity. Smears were collected from a group of 33 patients presenting with squamous cell carcinoma. Nine females and 24 males presenting with squamous cell carcinoma of the floor of the mouth underwent quantitative cytological assessment. These slides were fixed immediately with cytofix spray and then stained with Papanicolaou stain. Fifty randomly selected cells and their nuclei in each Papanicolaou stained specimen were measured using a X40 objective lens using a Seescan TV Image Analyser. Statistically, NA and CA pairs were compared using a paired t-test. Smears collected from the tumours displayed a statistically significant elevation in mean NA ($p<0.01$) and reduction in mean CA ($p<0.001$) when compared with normal smears from patients with no oral lesions. In conclusion, cytomorphologic assessment of the Papanicolaou stained oral smears collected from suspicious lesions was found to be a significant diagnostic factor in relation to malignancy.

Key Words: Papanicolaou smear, quantitative cytology, Seescan TV image analyser, oral malignancy

Introduction

The factors initiating neoplastic growth are still not clearly understood. Thus since around 1980, automated instruments capable of objective and quantitative cell analysis have been used in descriptive morphology, for the assessment of tumour cell heterogeneity (1,2). Additional information is needed to identify patients with tumours who are at risk of rapid progression or to detect recurrent tumours before they become clinically apparent (3). Descriptive morphology is of considerable importance when differentiating between normal and abnormal cells. There are two main quantitative techniques which have been applied in cytopathology: cytophotometry and cytomorphology.

The aim of this study was to measure and compare the nuclear and cell size of cells present in smears collected from tumours presenting in the floor of the mouth with clinically normal smears from tumour patients and from individuals with no oral lesions. This site was chosen as it is reported to be one of the high risk sites for developing oral cancer (4).

Materials and Methods

Smears were collected from a group of 33 patients presenting with squamous cell carcinoma. Nine females and 24 males presenting with squamous cell carcinoma of the floor of the mouth underwent quantitative cytological assessment. The age range for these patients was 39 to 98 years with a mean age of 64.18 years. There was no relationship between the presence of oral tumours in these patients and their medical history or current medication. The haematological investigations revealed no evidence of anaemia in these patients. A total of 67 Papanicolaou stained smears (37 tumour and 30 contralateral normal sites) were collected from tumours and from oral mucosa which appeared clinically normal in these patients. The smears underwent quantitative cytological assessment using the Seescan TV image analysis system

(TVIAS). A pro forma was completed for each patient each time smears were collected.

Smears were collected by firmly rotating a cytobrush over the site(s) under investigation. The samples were transferred to two dry glass slides, to ensure an adequate harvest of cells for measurement. These slides were fixed immediately with cytofix spray and then stained with Papanicolaou stain (Ortho Diagnostics). The Papanicolaou staining procedure was as follows.

The slides were washed in running tap water for one hour, stained for 4-6 minutes in Harris' Haematoxylin (Ortho Diagnostics, modification). This stain was filtered immediately before use. The smears were placed in distilled water. They were rinsed in successive changes of distilled water until the water remained colourless. The slides were then dipped gently five to ten times in 70% ethanol and then dipped in a 1% solution of HCl in 70% ethanol until the smear displayed a salmon colour. This was followed by rinsing well in two changes of 70% ethanol. The slides were then dipped gently in a 3% solution of ammonium hydroxide in 70% ethanol until the smear took on a blue colour and after this they were rinsed well in two changes of 70% ethanol. The slides were then dipped gently five to ten times in 95% ethanol, followed by staining in Orange G 6 for 1.5 minutes, and were rinsed in two changes of 95% ethanol. The smears were then stained in EA 50 for two minutes and rinsed well in two changes of 95% ethanol, followed by rinsing well in absolute ethanol. Lastly, smears were cleared in xylene and mounted in DPX.

Using this technique the nuclei stained dark purple, basophilic cells stained green or blue-green due to the effect of the EA 50 and the acidophilic cells stained pink to orange. The superficial, totally cornified cells stained orange or yellow and the erythrocytes stained orange-red. All smears were stored at room temperature in the dark ready for measurement.

The measurement of the nuclear and cytoplasmic areas (NA and CA) were performed for 50 randomly selected cells in each specimen using a X40 objective lens. The Seescan TVIAS contains a software program which allows the operator to digitise the cell and nuclear boundaries of each cell. Each smear was projected from an Optiphot microscope, via a monochrome CCD camera, onto the Seescan TVIAS monitor. The individual and the mean NA and CA values were recorded for each specimen using the Seescan TVIAS.

Statistically, NA and CA pairs were compared using a paired t-test. In this comparison, intraclass correlation coefficients (R1) were calculated for pairs because the Pearson correlation coefficient (r) does not consider the changes in mean values. The overall mean NA and mean CA values (±sd) for clinically normal and tumour smears are displayed in the Table.

Results

Smears collected from the tumours displayed a statistically significant elevation in mean NA ($p<0.01$) and reduction in mean CA ($p<0.001$) when compared with normal smears from patients with no oral lesions. There was a statistically significant reduction in mean CA ($p<0.001$), but not for NA, for the tumour smears when compared with normal smears collected from patients with oral tumours. There was no statistically significant variation in mean NA ($p>0.05$) or mean CA ($p>0.05$)

Table. Overall mean NA and mean CA values (±sd) for clinically normasmears from non-tumour and tumour patients, and tumours of the floor of the mouth.

Floor of mouth	No. of smears	Mean nuclear area (NA) μm^2	Mean cytoplasmic area (CA) μm^2
Normal (no lesions)	n=46	84.5 ± 10.3	2516 ± 408
Normal (tumour patients)	n=30	89.0 ± 13.11	2457 ± 560
Tumour	n=37	99.5 ± 29.99	1784 ± 500

between smears collected from clinically normal smears, from patients with no lesions and those with oral tumours.

The Figure displays a scattergraph for the mean NA and mean CA for the smears collected from patients without oral malignancies and for lesions of squamous cell carcinoma. The nuclear area values lie within a narrow range for the group of clinically normal smears. There is a wide range in the NA values for the tumour cells. The group of normal smears and the tumour smears display a broad range of CA values, with a series of much smaller cells being present as would be expected in the tumours.

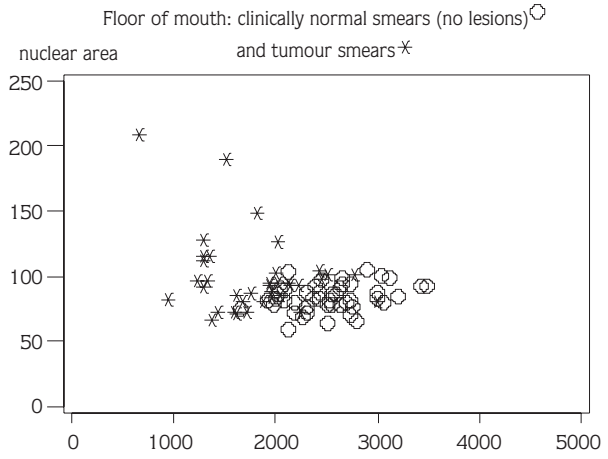

Figure. Scatterplot of the mean NA values versus the mean CA values for clinically normal FM smears collected from individuals with no oral lesions and in patients with oral tumours.

Discussion

Diagnostic and prognostic techniques are continually being developed and refined to detect cancer in its early stages. It is believed that the detection of oral cancer tumours when they are small provides an opportunity for less invasive treatment, thus improving the patient's quality of life and contributing to a better prognosis (5).

Cytology is a simple and relatively pain-free procedure which can be carried out repeatedly with minimum discomfort to patients. If its accuracy could be enhanced then it could provide a valuable adjunct to clinical evaluation of lesions and promote biopsy at the appropriate time. The most common stain used for the cytomorphological assessment of cells present in cytological smears is the Papanicolaou stain (6). When Cowpe and colleagues (7,8) first applied morphometry to oral smears, they used a planimetry method to measure NA and CA in a variety of normal and abnormal oral smears. The planimetry method was then replaced by an image analysis system, the Vids V system (9). Their findings were that all malignancies and some premalignancies displayed a reduction in cell size and an increase in nuclear size.

Tumour smears, in this study, displayed the expected increase in mean NA values and decrease in mean CA values when compared with clinically normal smears (no lesions). This is demonstrated in the scattergraph (Figure). The reduction in NA was significant when compared with both groups of clinically normal smears. However, the tumour smears only displayed a significant reduction in mean CA values when compared with clinically normal smears collected from tumour patients.

In conclusion, cytomorphologic assessment of the Papanicolaou stained oral smears collected from malignant and premalignant lesions in the floor of the mouth could provide an additional diagnostic test for monitoring such lesions and thus detecting oral malignancy at an early stage.

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References

1. Barlogie B, Maddox AM, Johnston DA, Raber MN, Drewinko B, Keating MJ, Freireich EJ. Quantitative cytology in leukemia research. Blood Cells 9:35-55, 1983.
2. Mollaoglu N, Cowpe JG, Potts AJ. Sensitivity of Seescan TV Image Analysis in discriminating between normal, dysplastic and malignant oral smears. Anal Quant Cytol Histol 22:218-222, 2000.
3. Russack V. Image cytometry: current applications and future trends. Crit Rev Clin Lab Sci 31:1-34, 1994.

4. Mollaoglu N. Quantitative assessment of normal and abnormal oral smears using the Seescan TV Image Analysis System [Thesis]. University of Wales College of Medicine, Dental School, Cardiff, 1997.

5. Jullien JA, Downer MC, Zakrzewska JM, Speight PM. Evaluation of a screening test for the early detection of oral cancer and precancer. Community Dent Health 12:3-7, 1995.

6. Sandler HC. Oral cytology. CA Cancer J Clin 16:97-101, 1966.

7. Cowpe JG, Longmore RB, Green MW. Quantitative exfoliative cytology of normal oral squames: an age, site and sex-related survey. J R Soc Med 78:995-1004, 1985.

8. Cowpe JG, Longmore RB, Green MW. Quantitative exfoliative cytology of abnormal oral mucosal smears. J R Soc Med 81:509-513, 1988.

9. Cowpe JG, Ogden GR, Green MW. Comparison of planimetry and image analysis for the discrimination between normal and abnormal cells in cytological smears of suspicious lesions of the oral cavity. Cytopathology 4:27-35, 1993.