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Computerised planimetric and stereological analyses of histological and cytological parameters in dysplastic atrophic epithelium and carcinoma

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Summary
This report analysed two lesions, of the Oral cavity; (atrophic dysplastic epithelium and oral squamous cell carcinoma) using computerized planimetry and point counting stereological methods. Three cytological, and histological parameters were analysed at a time. Results indicate a significant difference between the cell size, and nucleocytoplasmic ratio's of the two lesions when analysed ($P<0.0001$). The nuclear area measurement did not differ when the figure for the two lesions were compared. Study confirms the efficacy of objective analyses of tissue pathology of the oral cavity.

Keywords: *Quantitative, cytological, histological, features, computerised, analysis.*

Résumé
Le but de ci report est l'analyse de deux lesion de la cavite ovale; (development atrophique de l'epithelium et al tumeur malique orale des cellules epithelium) Nous avons utilise la planimetrie informatisee et la methode stereologicale des points comples. Trois parametres cytologiques et histologiques ont ete analyses a la fois. Les resultats ont montre une difference significative entre la taille de la cellule, et la proportion nucleo cytoplasmique des deux lesions ($P < 0,0001$) la mesure de la region nucleaire n'a pas change lorsque les figures des deux lesions etaient comparees. Cette etude a confirme l'efficacite de l'analyse objective des tissus pathologiques de la cavite orale.

Introduction

Oral Carcinomas are preceded by certain histological and cytological changes. These changes are usually subjectively interpreted by the pathologist, and classified as mild, moderate or severe dysplasia. Because, the subjective interpretation of tissue can vary from observer to observer, classification of dysplastic changes will also vary. Some authors have advocated an objective approach to tissue interpretation [1. 2,3] and have reported studies on experimental animals.

The aim of this study was to measure four histological and cytological features in dysplastic atrophic oral epithelium, and carcinoma, and to compare the findings as a predictable objective reference point for diagnosis. Three features were analysed; nucleo-cytoplasmic ratio, nuclear size, and density. Alterations to these features are generally known to accompany most oral epithelial premalignancy. Computerized planimetry and point counting stereological techniques were used.

Material and method

Selection of Cases

The cases for this study were those from which biopsies were received in the Oral Pathology Diagnostic Service of the Glasgow Dental Hospital. In selecting the cases, the main objective was to select cases of dysplastic epithelium adjacent to Squamous Cell Carcinoma. The cases represented primary incisional biopsies from various locations in the oral cavity (Table. 1) Ten cases each were selected for the dysplastic epithelium and carcinoma. The author subjectively categorized 7 of the dysplastic epithelium as demonstrating, mild, dysplasia and the remaining 3 as demonstrating moderate dysplasia.

Table 1: Clinical data on cases of dysplastic atrophic epithelium adjancent to carcinoma.

Case no.	Age	Sex	Site	Dysplasia
1.	62	F	Side Tonge	Mild
2.	72	M	Anterior Pillar/ Floor of mouth	Mild
3.	83	F	Junction Hard/ Soft palate	Mild
4.	78	F	Buccal mucosa	Moderate
5.	50	M	Floor of mouth	Moderate
6.	81	F	Floor of mouth	Mild
7.	54	M	Floor of mouth	Moderate
8.	78	F	Hard Palate	Mild
9.	72	M	Alveolar ridge/ Buccal mucosa	Mild
10.	83	M	Ventral Tongue/ Floor of mouth	Mild

Tissue Preparation

Tissue blocks were mounted on the microtome and sections were cut at 4um thickness. Three consecutive ribbons from each of the 10 blocks selected were mounted on slides and stained with Mayer's haematoxylin and eosin. The same slide was used to analyse both the dysplastic epithelium and carcinoma.

Selection of Section Fields for Quantitative Analysis

In the case of dysplastic lesion, due to thickness of the epithelium which was in most cases greater than one field, only the middle of the five columns of each field was analysed. This was made possible because the square in the eyepiece graticule (fig.1) was equally divided into five columns. All nuclei within that column and those intersecting the left edge of the column were measured. The section was then moved upward and vertically at right angles to the surface to allow the full thickness of the epithelial column to be measured. Five columns, running through the full epithelial thickness were measured per case using stratified random sampling.

The disorganized histological pattern presented by carcinoma made alignment of the epithelial surface along the horizontal line difficult. The danger of overestimation of cell volume was therefore imminent. Selection was therefore car-

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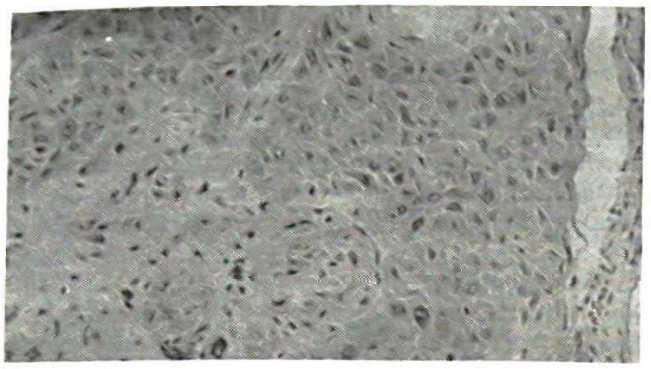

Fig. 3: High power view of squamous cell carcinoma invasion and multiple cytological abnormalities H & E x 420.

Nuclear Size and Tangent Diameter

Dysplastic epithelium

The mean progenitor and maturation cell nuclear size and tangent diameters are presented in Table 2. The progenitor mean nuclear size was 27.58 um² ranging from 18.28-42.14 um², while the mean tangent diameter was 6.09 um ranging from 4.77-7.85 um. The mean value for maturation cell nuclear size was 38.70 um² ranging from 22.39-57 um². The mean value for tangent diameter was 7.83 um ranging from 5.95-9.09 um.

Carcinoma

The mean nuclear tangent diameters and areas for tumour cells are presented in Table 2. The mean tumour cell area was 28.41 um² ranging from 16.11-38.49 um², while the mean tumour nuclear tangent diameter was recorded as 6.68 um ranging from 5.9-7.56 um.

Nucleo-cytoplasmic Ratios

Dysplastic epithelium

The mean nucleo-cytoplasmic ratio values for progenitor and maturation cells are shown in Table 3. The values recorded for inter-cellular spaces were subtracted from the total area in each compartment before deriving the nucleo-cytoplasmic ratio. The figures showed a mean progenitor compartment nucleo-cytoplasmic ratio value of 0.505, with a range of 0.35-0.85. The mean value for the maturation compartment cells was 0.177 ranging from 0.15-0.22.

Table 3: Mean nucleo-cytoplasmic ratios for dysplatic epithelium and carcinoma.

	Dysplatic epithelium N.C. ratios		Carcinoma N.C. ratio
Case no.	Progenitor	Maturation	
1.	0.54	0.16	0.254
2.	0.55	0.21	0.276
3.	0.50	0.15	0.260
4.	0.56	0.22	0.267
5	0.40	0 16	0.263
6.	0.85	0.18	0.278
7.	0.35	0.21	0.235
8.	0.41	0.17	0.197
9.	0.44	0.15	0.217
10.	0.45	0.16	0.363
Mean	0.505	0 177	0.261
S.D	0.140	0.026	0.0444

Table 4: Mean cell area and average cell density for dysplastic epithelium and carcinoma.

	Dysplastic epithelium			Carcinoma		
	Cell size		Cell density	Cell size	Cell density	
Case no.	Progenitor	Maturation	Progenitor	Maturation		
1.	128.09	312.44	6.62	2.89	290.50	3.24
2.	114.31	597.50	7.40	1.59	293.65	3.20
3.	73.56	321.43	9.74	2.72	225.62	3.95
4.	143.93	513.77	5.70	1.68	395.34	2.35
5.	73.80	394.67	12.81	2.50	331.10	2.81
6.	141.02	715.96	5.37	1.40	328.70	2.97
7.	76.86	154.11	8.55	3.97	277.89	3.21
8.	118.26	597.73	7.61	1.57	157.88	5.78
9.	63.04	261.59	14.57	3.69	197.61	4.78
10.	115.96	317.18	7.66	3.11	133.06	7.07
Mean	104.88	418.64	8.60	2.51	263.12	3.94
S.D	30.26	178.81	2.99	0.93	82.99	1.50

Carcinoma

The corrected nucleo-cytoplasmic ratio values for tumour cells is presented in Table.4. The Holmes formula was applied to the raw value. The values recorded for intercellular spaces were subtracted from the total area before deriving the nucleo-cytoplasmic ratio. The recorded mean value for the nucleo-cytoplasmic ratio was 0.261, ranging from 0.197-0.363.

Cell sizes and Densities

Dysplastic epithelium

The values for total compartment areas were necessary for calculating cell size and densities. In this study compartments were analysed through the point counting system. These points were converted into absolute values for derivation of compartment sizes. The values derived for progenitor and maturation cell sizes and densities after Abercrombie's correction factors are presented in Table 4. The mean value for progenitor cell size was 104.88 um² ranging from 63.04-128.09 um². The mean cell density value for progenitor cells was 8.60 cells per 1000 um² ranging from 5.37-14.57 cells per 1000 um². Maturation cells showed a mean value for 418.64

ried out by first isolating an adequate area of the section under the low power of the microscope and aligning the mean surface horizontally in the microscope. The section was then placed under higher power and fields were analysed. It was not always possible to isolate an area that allowed five consecutive fields to be analysed. Whenever this was the case, the section was again placed under the low power, and other adequate areas of the section were located until five fields per section were analysed.

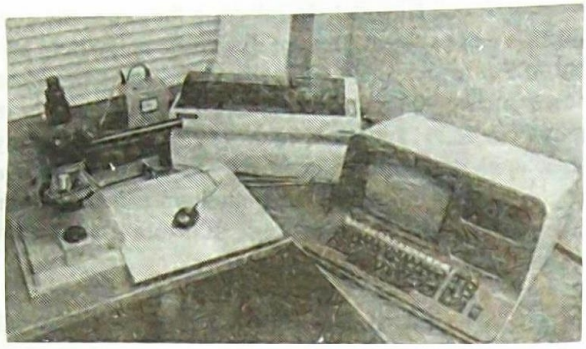

Fig. 1: The elements of the computerized planimetry system used for the compartment and nuclear analyses in the present study.

Measurement of Nuclear Areas and Nuclear Tangent Diameters.

The same procedure of computerized planimetric analysis described under selection of fields for quantitative analysis was used to measure nuclear areas and nuclear tangent diameters. Five fields per case were analysed per lesion through stratified random sampling.

Measurement of Compartment Areas, Nuclei, Cytoplasm and Intercellular Spaces.

Point Counting stereology in conjuction with computerized planimetry were used to analyse compartment areas and intercellular spaces for the dysplastic lesion. After the planimetric evaluation of each microscope field, point counting stereology was introduced. A point counting grid was super imposed on the same field, and number of points falling on nuclei, cytoplasm, and intercellular spaces were counted. Total compartment areas for tumor cells were derived, adding together the absolute conversion values for the counted nuclei, cytoplasm and inter cellular spaces.

Results

Histological Feaures

Dysplastic Epithelium

Except in a case of clear-cut carcinoma, histological labelling of a dysplastic lesion as mild, moderate or severe becomes a purely subjective exercise. The combination of a number of dysplastic features, when present determines the degree of dysplasia. In (Fig. 2) a histological section of an atrophic dysplastic epithelium is shown. The histology shows some of the features mentioned earlier; loss of intercellular adhesion represented by intercellular spaces, anisocytosis, anisonucleosis and basal cell hyperplasia

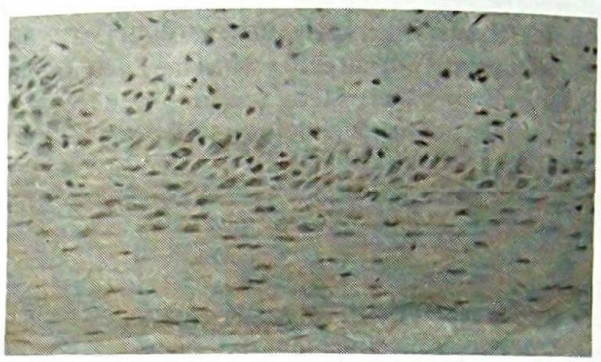

Fig. 2: Dysplatic atrophic epithelium, showing extensive inter cellular spaces. H & E x 415.

Table 2: Mean nuclear areas and mean nuclear tangent diameters for progenitor and maturation keratinocytes.

Case no.	Dysplastic Epithelium Nuclear area Progenitor	Maturation	Nuclear tangent Progenitor	Maturation	Carcinoma Nuclear	Nuclear Tangent
1.	29.84	28.18	7.85	8.17	30.38	6.83
2.	31.18	54.71	6.17	9.02	33.16	7.03
3.	20.99	31.56	6.11	7.79	24.32	6.29
4.	37.07	52.19	6.45	8.74	38.49	7.38
5.	20.71	36.10	4.77	7.22	35.10	7.56
6.	42.14	57.87	7.22	9.09	31.76	7.29
7.	20.19	22.39	4.59	5.95	32.15	6.36
8.	26.84	48.0	6.44	8.55	19.36	5.90
9.	18.28	25.93	5.19	6.45	23.25	6.41
10.	28.51	30.09	6.15	7.28	16.11	5.72
Mean	27.58	38.09	6.09	7.83	28.41	6.68
S.D	7.84	13.18	1.02	1.08	7.26	0.64

Carcinoma

Carcinomas are microscopically characterized by invasion of the underlying connective tissue. Strands and sheets of epithelial cells similar in general to prickle cells, penetrate the connective tissue. In most oral growths the tumour is well differentiated and the neoplastic cells although showing the stigmata of malignancy such as hyperchromatism, pleomorphism, anisocytosis and other malignant histological features are still recognisably of prickle cell type (Lucas 1976) [4]. In figure (3) the dysplastic features of carcinoma are demonstrated on high power.

um^2 for cell size, with a range of 154.11-597.73 um^2 and a mean cell density value of 2.51 cells per 1000 um^2 with a range of 1.40-3.97 cells per 1000 um^2.

Carcinoma

The raw and corrected nuclear counts and the Abercrombie corrections to nuclear counts were used to derive the mean cell size and density for tumour cells. Table 4 shows the derived values for tumour cell size and density. The mean tumour cell size value was 263.12 um^2 ranging from 133.06-395.34 um^2. The mean value for cell density was 3.94 cells per 1000 um^2 with a range of 2.35-7.07 cells per 1000 um^2.

Discussion

A retrospective study on dysplastic atrophic epithelium and carcinoma is reported. The results indicate an objective analysis which validates expected histological and cytological features in the two lesions. When progenitor and maturation nuclear area measurements of dysplastic epithelia were compared with that of neoplastic cells, no significant differences was recorded. (Table 2) However, there was a significant difference shown when the carcinoma cells were compared with cell size from the progenitor compartment of the dysplastic lesion ($P < 0.001$) Table. 4; the neoplastic cells being larger. The neoplastic cells did not however differ in size from the dysplastic maturation cells. Most studies on objective analysis [5,6] do agree with our findings.

Subjective interpretation of oral tissue microscopy assumes or expects an increased nucleo-cytoplasmic ratio, from normal, through atypical hyperplasia, dysplasia, carcinoma in situ and invasive carcinoma. Comparative results for nucleo-cytoplasmic ratio in our study showed that the neoplastic cells lay between that of the dysplastic progenitor cells and the dysplastic maturation cells, being significantly larger than maturation cells ($P<0.001$), and smaller than progenitor cells ($P<0.001$) Table. 3 Previous report by Regan and Harmonic [2] collaborates this observation, and assertion.

The results obtained for some parameters in our study may not have agreed with those obtained from similar studies at the ultra structural level. It is the impression of the authors that the greater number of cells that were analysed at the light microscope level would have made such disparity inevitable. Also, the procedures utilized for quantitative analysis in our study were tedious, and therefore prove to minor observer error. However, such error could not have altered the results to any great extent. It is therefore, safe, to say that the results obtained have confirmed the authenticy and efficacy of quantitative analysis as an aid to a more objective evaluation of premalignant lesions of the oral cavity.

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