Morphometric analysis of basal cell layer in oral premalignant white lesions and squamous cell carcinoma

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SUMMARY The size and shape of the cells in the basal cell layer of the oral epithelium in 100 specimens from oral mucosa were studied by using an interactive image analysis system (IBAS-1). Four groups of white lesions (traumatic keratosis, lichen planus, leucoplakia, and a "risk group") in addition to two control groups (normal mucosa and squamous cell carcinoma) were studied retrospectively. The results showed a progressive increase in the dimensions (area, perimeter, and maximum diameter) of the nuclei from normal mucosa through traumatic keratosis, lichen planus, leucoplakia and the "risk group" to carcinoma, with considerable differences. The nucleus in squamous cell carcinoma was twice as large as in normal mucosa. A substantial increase in the dimensions of both the cell and the nucleus was found in the "risk group." The nucleo:cytoplasmic ratio, contrary to what might have been anticipated in risk lesions, did not show considerable differences between the diagnostic groups. Furthermore, it was slightly decreased in the risk group compared with the normal mucosa. The shape factors (form PE and contour index) seemed to be less helpful in the identification of the "risk group." The size of the basal cell and its nucleus can be of diagnostic value for lesions with a high risk of malignant transformation.

Oral squamous cell carcinoma may be preceded by premalignant lesions. The most common of these lesions is leucoplakia which is defined as a white patch that cannot be attributed to any other disease.[1] The rate of malignant change in leucoplakia ranges between 3 and 6%.[2] This rate may be higher in particular variants of leucoplakia—for instance, candidal leucoplakia has been reported to show 30% malignant change.[3] Oral lichen planus carries little or no risk of malignant transformation, although it is suggested that the atrophic or erosive form may. The reported rates of malignant transformation in patients with oral lichen planus range between 0 and 2%.[4 5]

In the examination of oral mucosal lesions that may be premalignant histopathologists have problems not only in differentiating between various lesions but also in determining the risk of malignant change in the individual case. The histopathological diagnosis of oral premalignant lesions is based on the subjective evaluation of epithelial dysplasia, but the wide variation between observers in the subjective evaluation of epithelial dysplasia has been commented on by several authors[6–8] and more objective means of evaluation are constantly sought for.

In the assessment of dysplastic and neoplastic lesions, a great emphasis is placed on the changes in nuclear and cellular size and shape. To overcome the unreliability in the subjective examination of these features a more objective approach would be the value of computer based image analysis techniques.

The keratinocytes of the basal cell layer of the oral stratified squamous epithelium represent the progenitor cells that are responsible for the production of other cells making up the various layers of the epithelium. Changes in the basal cells may have serious implications on future cell behaviour, including malignant transformation. The measurement of their size and shape in white lesions may be an important prognostic marker.

Material and methods

A retrospective study was made on 100 specimens taken from the buccal mucosa of 97 patients. The

specimens were selected from the records in the department of oral pathology at this hospital. These comprised 10 specimens of normal mucosa, 14 of traumatic keratosis or frictional keratosis, 20 of lichen planus, 29 leucoplakias (including nine candidal leucoplakias), 16 squamous cell carcinomas, and 11 white lesions which were classified as the "risk group." The diagnosis of the lesions was made by two of the authors (KWL and AHMS) using the World Health Organisation criteria.[1] Candidal leucoplakia was diagnosed according to the criteria of Cawson and Binnie.[3] The risk group comprised eight patients with oral squamous cell carcinoma who either had white lesions that underwent malignant change or who had lesions coexisting with carcinomas at first examination. Eleven biopsy specimens were taken, nine being from sites other than those of the carcinomas; one was taken from the site that underwent malignant change and the other was taken from the buccal mucosa adjacent to the carcinoma. The diagnosis was leucoplakia in five, lichen planus in one, and in the five other specimens the appearances were intermediate between lichen planus and leucoplakia. Table 1 shows the age and sex of the patients in each group.

The follow up for the patients ranged between one and 23 years (mean 5·3) for the group with leucoplakia, one and 12 years (mean 4·9) for the group with lichen planus, and one and five years (mean 2·7) for the cases in the risk group with lesions preceding the appearance of cancer. The follow up was not recorded for the other groups. In the group with carcinoma lesions showing a cell layer equivalent to the basal cell layer of the stratified squamous epithelium were selected. Accordingly, these were well and moderately differentiated tumours; only one specimen in this group was poorly differentiated. All specimens were fixed in 10% formol saline and 5 μm thick paraffin sections were cut and stained with haematoxylin and eosin.

MORPHOMETRIC TECHNIQUE
The measurements were made by using a semi-automatic image analysis system (IBAS-1, Kontron). The system comprised a microcomputer, a digitiser tablet, a drawing pen (stylus), a video-camera (CCTV, Hitachi) attached to a light microscope (Olympus BH-2), and a dot matrix printer (OKI, DP-125). The measurements were made by tracing the outlines of the cells and nuclei displayed on a black and white monitor by the stylus on the digitiser tablet. The contrast of the image was enhanced by a green filter. An oil immersion objective at a magnification of 100 was used to provide the largest possible magnification so that human error in tracing was minimised. The final magnification was 2000 at the monitor.

For each specimen, one section or more that was most representative of the diagnosis was selected. In the lesional areas normal looking tissue was identified and masked by using a black ink marker. The basal keratinocytes were then measured for the parameters described below, except in squamous cell carcinomas where only the nuclei were measured. Histologically identifiable non-keratinocytes such as melanocytes and inflammatory cells, as well as cells showing degenerative changes, those undergoing mitoses, and multinucleated cells, were not measured.

MORPHOMETRIC PARAMETERS
The sizes of the cells and nuclei were represented by their area, perimeter, and maximum diameter. The shape was measured by "form PE" which assesses the deviation from circularity. By the relations shown in table 2, a circle has a value of 1 and an ellipse or an irregular structure is less than 1. These parameters are part of the videoplan measuring program (x, y). Another shape measuring variable is the Contour Index (CI), which measures the regularity of the particle surface.[9] A value of 3·54 is obtained for a circle by the formula presented in table 2. Higher values are obtained with the increase in the indentations or convolutions of the outline. The contour index and the nucleo:cytoplasmic ratio were calculated from mean values.

Table 1 *Diagnostic groups age and sex of patients studied*

Diagnostic group	No of patients*	Age Range	Age Mean	Sex M	Sex F
Normal mucosa	10 (10)	40 − 67	(49·6)	5	5
Traumatic keratosis	14 (14)	36 − 73	(50·7)	6	8
Lichen planus	20 (20)	30 − 78	(51·8)	10	10
Leucoplakia	20 (20)	33 − 75	(52·4)	10	10
Candidal leucoplakia	9 (9)	31 − 80	(53·4]	6	3
Risk group	8 (11)	27 − 71	(58·9)	6	2
Squamous cell carcinoma	16 (16)	36 − 79	(63·7)	6	10

*No of specimens in parentheses.

Table 2 *Morphometric parameters*

Parameter	Unit and formula
Nuclear area	μm^2
Nuclear perimeter	μm
Nuclear diameter	μm
Nuclear form PE	$4 \times 22/7 \times$ N Area/N Perim^2
Nuclear contour index	N Perim/$\sqrt{}$N Area
Cellular area	μm^2
Cellular perimeter	μm
Cellular diameter	μm
Cellular form PE	$4 \times 22/7 \times$ C Area/C Perim^2
Cellular contour index	C Perim/$\sqrt{}$C Area
Nucleo: cytoplasmic ratio	N Area/(C Area—N Area)

SAMPLE SIZE

The number of cells to be measured from each specimen was determined by the cumulative mean plot[10] and was carried out on six specimens representing the various diagnostic groups. The nearest eight cells and their nuclei to the centre of the monitor from each of 25 fields were selected by moving the microscopic stage to the side while the section was out of focus (to avoid selectivity in sampling and to avoid possible image distortion at the edges of the screen). Several sections were used where the length of the epithelium was less than 5 mm. Using this method, 120 cells and nuclei obtained from 15 fields were found to provide a representative sample size for the various parameters in each specimen. The results were then represented by the mean and standard deviation. No corrections for section thickness or the Holmes effect were applied, so that the measurements represent the morphological image as seen.

STATISTICAL ANALYSIS

The non-parametric Mann-Whitney test was used to evaluate the difference between the distributions of two groups. The Kruskal-Wallis test was used for more than two group comparisons. These rank sum tests were used as the data were often asymmetrical and the standard deviations were also different. A significance level of 5% was used. The difference between group means divided by the pooled standard deviation is given the value (d). The variables with high (d) values discriminate best between groups.[11]

Results

Table 3 shows the results of measuring the basal cells and their nuclei in the diagnostic groups. The results showed that the nuclear area, perimeter, and maximum diameter increased steadily from the normal epithelium through traumatic keratosis, lichen planus, leucoplakia, candidal leucoplakia and the risk group to their highest values in squamous cell carcinoma. The nuclear area of the neoplastic cells was about twice as large as that of the normal cells.

The mean values of the whole cell showed similar trends to those of the nucleus. The cell area, perimeter, and maximum diameter of lichen planus lesions, however, were greater, on average, than those in leucoplakia. Nevertheless, the risk group had the largest cell dimensions.

The nucleo:cytoplasmic ratio of the basal cells did not show significant differences between the diagnostic groups. It was noticed, however, that the nucleo:cytoplasmic ratio was slightly lower in the risk group than in the normal group, the opposite of what might have been anticipated in the risk lesions. This decrease in nucleo:cytoplasmic ratio was attributed to the enlargement of the cell to 1·71 times the normal value, whereas the increase in the nuclear area was 1·68 times. In contrast, the increase in nucleo:cytoplasmic ratio in the group with candidal leucoplakia was mainly attributable to the enlargement of the nucleus to 1·46 times. The corresponding increase in the cell area was only 1·38 times.

The group with carcinoma shows the largest nuclear dimensions of all. The shape of the nuclei was more circular than that found in the other groups with the exception of traumatic keratosis which showed the highest value for the nuclear form PE. The contour index of the nuclei showed that candidal leucoplakia had the most irregular shape and the normal group the most regular. All shape factors did not show increasing or decreasing patterns through the groups in accordance with their premalignant potentialities.

Table 4 shows the variables which had (d) values greater than 1, when comparing the risk group with the other groups of white lesions. All the variables listed in the table showed significant differences. The dimensional variables of both the cell and its nucleus seemed to be important for distinguishing the risk

Table 3 *Morphometric parameters of basal cell in different diagnostic groups (Mean ± SD)*

Variable	Normal (n = 10)		Traumatic keratosis (n = 14)		Lichen planus (n = 20)		Leucoplakia (n = 20)		Candidal leucoplakia (n = 9)		Risk group (n = 11)		Carcinoma (n = 16)		P value
N Area	25·01	(3·41)	29·59	(5·70)	33·10	(3·81)	33·13	(3·30)	36·40	(5·38)	42·06	(8·97)	54·34	(10·80)	‡
N Perim	19·73	(1·43)	21·32	(1·78)	23·12	(1·17)	23·18	(1·13)	24·37	(1·65)	25·81	(2·39)	28·64	(2·46)	‡
N Dmax	7·17	(0·73)	7·41	(0·32)	8·27	(0·47)	8·33	(0·67)	8·91	(0·57)	9·36	(0·78)	10·28	(0·90)	‡
N PE	0·780	(0·026)	0·804	(0·038)	0·781	(0·028)	0·767	(0·046)	0·754	(0·018)	0·768	(0·055)	0·799	(0·042)	*
N CI	3·593	(0·089)	3·944	(0·130)	4·029	(0·128)	4·035	(0·148)	4·051	(0·067)	4·013	(0·168)	3·907	(0·109)	*
C Area	57·63	(8·84)	66·78	(11·10)	81·36	(9·82)	76·13	(9·45)	79·63	(7·67)	98·28	(16·29)	ND		‡
C Perim	31·72	(2·96)	34·03	(2·94)	39·62	(2·57)	37·98	(3·64)	39·14	(1·84)	43·73	(3·81)	ND		‡
C Dmax	11·00	(1·22)	11·94	(0·86)	13·73	(1·04)	13·56	(1·54)	14·19	(0·74)	15·39	(1·15)	ND		‡
C PE	0·703	(0·034)	0·720	(0·046)	0·666	(0·037)	0·672	(0·057)	0·652	(0·028)	0·645	(0·053)	ND		†
C CI	4·185	(0·120)	4·182	(0·198)	4·401	(0·155)	4·355	(0·209)	4·392	(0·111)	4·430	(0·214)	ND		†
N/Cyt	0·784	(0·151)	0·808	(0·156)	0·692	(0·080)	0·809	(0·215)	0·845	(0·117)	0·753	(0·151)	ND		–

* = p < 0·05; † = p < 0·01; ‡ = p < 0·001.

Table 4 *(d) values and significance value of different diagnostic groups compared with those of risk group*

	Traumatic keratosis (d) (n = 14)	p <	Lichen planus (d) (n = 20)	p <	Leucoplakia (d) (n = 20)	p <	Candidal leucoplakia (d) (n = 9)	p <
N Area	1·31	0·001	1·21	0·01	1·23	0·01		
N Perim	1·48	0·001	1·27	0·01	1·26	0·01		
N Dmax	1·72	0·001	1·37	0·001	1·19	0·01		
C Area	1·52	0·001	1·15	0·01	1·37	0·001	1·20	0·01
C Perim	1·64	0·001	1·14	0·01	1·25	0·01	1·23	0·001
C Dmax	1·72	0·001	1·24	0·001	1·11	0·01	1·08	0·05
C PE	1·23	0·05						

group from the other groups of white lesions. The dimensions of the nucleus, however, were less important in the comparison with those in candidal leucoplakia. The shape factor PE was only valuable for distinguishing the risk group from that with traumatic keratosis.

Discussion

The present results have shown, that there is a steady increase in the basal cellular and nuclear dimensions from normal mucosa through white lesions, which are generally regarded as reactive (traumatic keratosis), those regarded as chronic inflammatory with little or no risk of malignant transformation (lichen planus), and lesions generally regarded as premalignant (leucoplakia), to the lesions having a high risk of malignant change. The nuclei were also the largest in the group with carcinoma. The (d) values of these indicate that the dimensional features of the basal cells are more important diagnostically than the shape features. The nuclear area was greater than $40\,\mu m^2$ in three (10%) of the 29 specimens of leucoplakia, in nine (82%) of the 11 risk specimens, and in 15 (94%) of the 16 carcinomas. None of the normal, traumatic keratosis, or lichen planus cases had a similarly large nuclear area. Although none of the patients with candidal leucoplakia developed carcinoma during the follow up, the three leucoplakia cases with nuclear area greater than $40\,\mu m^2$ were all candidal, representing 33% of the nine cases of candidal leucoplakia. Cawson and Binnie[3] reported a 30% malignant change in candidal leucoplakia.

The present results seem to be compatible with those reported by Boysen and Reith[12] for the basal cell of the nasal mucosa of nickel workers. Their results showed a progressive increase in basal cellular and nuclear area from pseudostratified columnar epithelium, through metaplastic epithelium, to their highest values in dysplastic epithelium.

The increase in nuclear area in the risk lesions and carcinomas in this study may be a reflection of the increase in DNA synthesis.[13] A proportional increase in the nuclear volume and DNA content was found in the epithelial cells in the mucosa of the uterine cervix.

This study showed that there is a progressive increase from the normal mucosa, carcinoma in situ, to invasive carcinoma.[14] In oral leucoplakias an increase in the proportion of cells in the hyperdiploid to the hypertetraploid range has been reported.[15]

One of the main causative factors for the increase in the epithelial cell size seems to be mechanical trauma. Such a change has been reported after repeated friction,[16] in healing wounds,[17 18] after removal of the stratum corneum by Scotch tape stripping,[19] and after hair plucking.[20] Similar changes have been described in epithelia during the menstrual cycle,[21] after applications of turpentine,[22 23] and chemical carcinogens.[24] In a quantitative study of the oral epithelium subjected to friction MacKenzie and Miles found an increase in the total epithelial thickness.[16] This increase was found in both the size and number of the cells—mainly in the stratum spinosum and stratum granulosum rather than the basal cells. It has been suggested that the epithelial responses to a wide range of stimuli may appear similar but that the various cell compartments may contribute in response with varying degrees.[25] In quantitative studies on the hamster cheek pouch epithelium after the application of carcinogens Eveson and MacDonald showed progressive increase in the thickness of the progenitor cell compartment.[25 26] This was due to an increase both in size and number of progenitor cells. The authors found that in a control group (scratch or acetone treated animals) there was no change in the mean cell area of the progenitor or maturational cells compared with those in the untreated group. Although the number of progenitor cells increased in the group treated with carcinogens, the striking feature was the highly significant increase in the size of the progenitor cells expressed by the mean cell area. The maturational cells showed a smaller increase in cell size compared with those in the untreated group. The above study emphasised the potential diagnostic and prognostic value of the cell area in the deeper layers of the epithelium. The changes occurring in the basal cells might indicate an increased metabolic activity prior to the invasion of the underlying connective tissues, as shown ultrastructurally.[27 28]

Previous studies on the assessment of epithelial

changes in premalignant lesions from the uterine cervix,[29] bronchi,[30] and hamster cheek pouch[24] have attributed particular importance to the increase in nucleo:cytoplasmic ratio. In the nasal mucosa of nickel workers, however, the increase in the basal cell area resulted in a decrease in the nucleo:cytoplasmic ratio in the dysplastic epithelium.[28] Our results showed a decrease in nucleo:cytoplasmic ratio of the oral basal cells in the risk group compared with that in the group with leucoplakia, though the difference was not significant.

These results have shown that quantitative techniques can detect features that may be overlooked by routine histological examination. The size of the basal cells and nuclei are not features of epithelial dysplasia. The increase in basal cellular and nuclear size has been shown to be associated with lesions having a high risk of malignant change, and, therefore, their measurement may provide an objective means for the assessment of epithelial dysplasia.

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