Evaluation of Nuclear Morphometry and DNA Ploidy Status for Detection of Malignant and Premalignant Oral Lesions: Quantitative Cytologic Assessment and Review of Methods for Cytomorphometric Measurements

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Purpose: Detection of a precancerous or cancerous lesion when small is one of the most important factors to improve 5-year survival rates of oral cancer. Although surgical biopsy is the most definitive method for diagnosing oral lesions, it is impractical to routinely subject large numbers of patients to biopsy. Recently, cytomorphometric assessments improved by advanced computer-assisted image analysis systems have gained importance. This study was established to evaluate the efficacy of nuclear cytomorphometric analysis and DNA ploidy status for the detection of oral malignancies. Methods used for cytomorphometric analysis were also reviewed.

Patients and Methods: Oral mucosal smears (n = 44) were obtained from patients (n = 22) presenting with various oral lesions using a cytobrush immediately before biopsy. Cytomorphometric measurements and nuclear Feulgen DNA content analysis were carried out after the Feulgen staining procedure. Smears from the lesion site constituted the study group whereas contralateral healthy mucosal sites served as control.

Results: DNA ploidy analysis revealed 20 diploid (90.9%) and 2 aneuploid DNA patterns (9.1%) sampled from the lateral margin of the tongue and floor of the mouth. When only malignant lesions were considered, aneuploidy rate was 16.7% whereas a diploid pattern was indicated for 83.3% of the sample. With cytomorphometric measurements, a statistically significant difference was shown for nuclear perimeter, area, diameter equivalent to circle, minimum and maximum Feret, intensity, DNA content (c) and DNA index values.

Conclusions: Cytomorphometric analysis via oral brush biopsy is a valuable adjunct to biopsy for identification of premalignant and early stage cancerous oral lesions as a rapid and minimally invasive procedure with high specificity and sensitivity rates, requiring no topical or local anesthetic.

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There has been a significant increase in the early diagnosis and survival rates for cancers of the breast, colon, prostate, and melanoma over the last 3 decades[1] although the 5-year survival rate is still less than 50% for oral and oropharyngeal cancer despite recent major advances in surgery, radiotherapy, and

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chemotherapy.[2,3] The 5-year survival rate for patients with early, localized lesions is nearly 80% whereas it is 19% for those with distant metastasis.[4] Despite the fact that the oral cavity and oropharynx are easily accessible for visualization, more than 50% of patients with oral cancer display evidence of spread to regional lymph nodes and metastases at time of diagnosis.[5] Early detection of the cancer lesion when small is one of the most important factors that has been shown to influence 5-year survival with an improvement in patient's quality of life by minimizing extensive, debilitating treatments and hence the reduction of high mortality rate of oral squamous cell carcinoma (SCC).[6]

Surgical biopsy remains the most definitive and reliable method for diagnosing oral lesions. However, the high prevalence of oral abnormalities of 5% to 15% detected as a result of oral screening programs and the difficulty of routinely subjecting large numbers of patients to a surgical biopsy makes the procedure impractical for the assessment of early lesions and recurrences in oral cancer.[7] Sampling of individual epithelial cells has been proposed with the ample data revealing the initial changes that give rise to a clinically visible lesion that are thought to occur within the epithelium. Given that 90% to 95% of oral cancers are squamous cell carcinomas arising from surface epithelium, evaluation of individual cells may give the opportunity for detection of early dysplastic changes.[7] Application of cytologic techniques to oral mucosal smears was established to facilitate the early diagnosis of oral SCC analogous to the screening procedure based on cytologic analysis for cervical cancer[8] and many articles were published between 1955 and 1975.[9] Since then, the method has eventually fallen from favor because of false negative findings due to inadequate sampling of lesions and the subjective nature of its interpretation.[10] Today, the application of quantitative techniques together with the recent progression in image and computer analysis systems improve the potential accuracy of cytomorphometric studies.[11] Combination of DNA measurements with nuclear morphometric variables was considered to enhance the accuracy and reproducibility of cytologic diagnosis.[12]

The aim of this study was to carry out nuclear morphometric and nuclear DNA analysis with image cytometry and to evaluate the efficacy of these analyses for the detection of morphometric alterations in epithelium. Methods used for cytomorphometric analyses were also reviewed. For this purpose, oral brush biopsy was carried out to obtain dissociated epithelial cells from patients with suspicious oral lesions before incisional biopsy.

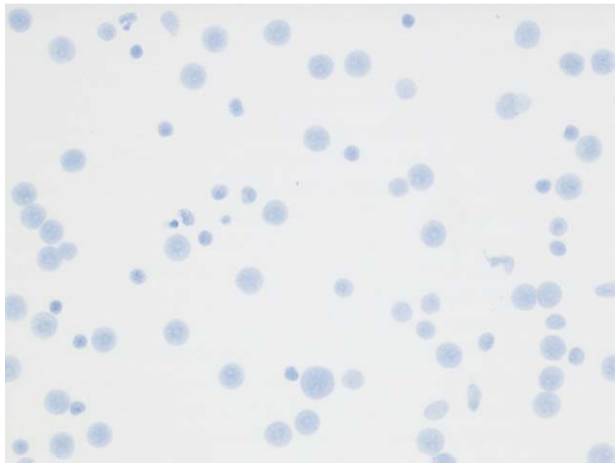

FIGURE 1. Feulgen stained nuclei.

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Patients and Methods

The study was comprised of 22 patients (11 male, 11 female) ranging in age from 22 to 77 years with a mean age of 50 years who had been referred for examination and treatment of various oral lesions. After informed consent, 2 oral mucosal smears (a total of 44) were obtained from each subject using a cytobrush (Rambrush, Ginram, Mirandola, Italy) immediately before biopsy without requiring any topical or regional anesthesia. Pinkness of tissue or pinpoint bleeding at the brush biopsy site proved an adequate sampling as stated by Sciubba.[13] Smears from the lesion site constituted the study group and contralateral healthy mucosal sites served as control. All smears were transferred to clean, dry, glass slides and air dried. For analysis of DNA ploidy and nuclear cytomorphometry in cellular nuclei, tissue samples were stained stoichiometrically according to Feulgen (Fig 1).[14] Measurements directed at cytomorphometric analysis and nuclear Feulgen DNA content were carried out on the images obtained from a colored video camera (Sony, AVT Horn, Tokyo, Japan) equipped with a Zeiss Axioscope (Zeiss, Göttingen, Germany) light microscope. Zeiss Vision KS 400 (version 3.0) image analysis software operating with a 128 MB memory, Pentium 233 MMX processor (Intel, Santa Clara, CA) IBM compatible computer (IBM, New York, NY) was used for nuclear measurements. For every subject, a minimum number of 200 nuclei were chosen from the lesion site and 60 nuclei were selected from control specimens with a X20 plan apochromat, numeric aperture: 0.17 objective. Individual nuclei with definable boundaries were traced whereas overlapping, folded, or clumped ones were avoided. An image analysis macro program was established to determinate cytomorphometric values. With

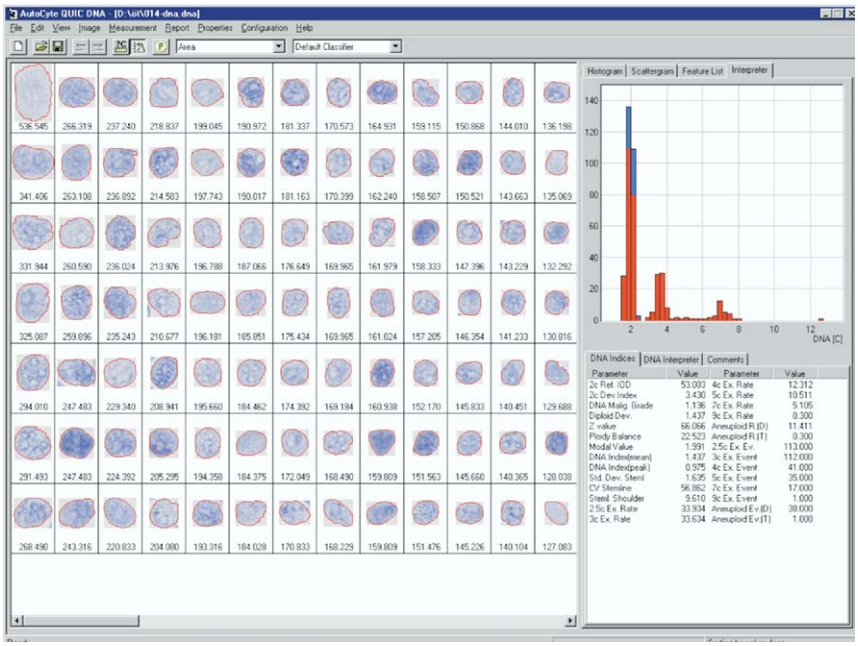

FIGURE 2. Nuclear gallery with nuclear morphometric measurements and DNA histogram. Study, red box; control, blue box.

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this program, nuclear area (NA) ($\pi.r^2$), perimeter ($2.\pi.r$), form factor ($4\pi.\text{area}/\text{perimeter}^2$), diameter equivalent to circle ($2.\sqrt{\text{area}/\pi}$), minimum Feret (diameter), maximum Feret (diameter), intensity, DNA content (c), DNA index, and entropy values were measured and stored in a computerized folder or "gallery" for each selected nucleus and smears from the healthy mucosa were included as internal controls (Fig 2). For nuclear morphometric evaluation, mean values of these parameters were recorded for individual subjects and considered for statistical analysis. The results were displayed as DNA distribution histograms and used to determine the DNA content. The ploidy status according to Aurer was estimated.[15] A lesion was classified as diploid if there was only 1 peak (that was 2c) during the G_0 or G_1 phase, if the number of 4c nuclei during the peak of the G_2 phase did not exceed 10% of the total, or if the number of nuclei with a DNA content of more than 5c did not exceed 1% of the total. A lesion was defined as tetraploid when there was a 4c peak during the G_0 or G_1 phase together with an 8c peak during the G_2 phase or when the number of 4c nuclei during the peak of the G_2 phase exceeded 10% of the total. A lesion was defined as aneuploid if there were aneuploid peaks (3c, 5c, 7c, or 9c) or if the number of nuclei with a DNA content of more than 5c or 9c exceeded 1% of the total (Fig 3).

Statistical analysis of the data was carried out with SPSS for Windows 9.0 software (SPSS, Inc, Chicago, IL). Student's *t* test and paired *t* test were used for the statistical evaluations whereas Pearson's correlation analysis was used to determine the correlation between cytomorphometric variables.

Results

Demographic features and histopathologic diagnosis of the patients is given in Table 1. Among 22 patients, histologic diagnosis was consistent with SCC in 12 (54.5%) whereas 4 lichen planus (18.2%), 3 leukoplakia (13.6%), 1 erythroplakia (4.5%), 1 erythroleukoplakia (4.5%), and 1 hyperkeratosis (4.5%) constituted the remaining patients.

For SCCs, most common localizations were tongue (n = 7), floor of the mouth (n = 3), and gingiva (n = 2),

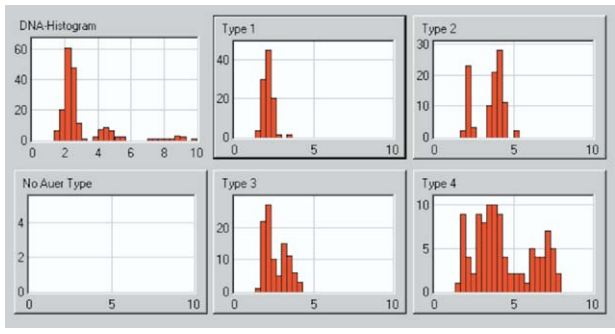

FIGURE 3. DNA profiles for diploid, tetraploid, and aneuploid lesions.

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Table 1. DEMOGRAPHIC DATA AND HISTOPATHOLOGIC DIAGNOSIS

Number	Age (year)	Gender	Site	Diagnosis
1	77	F	Ventral tongue	Squamous cell carcinoma
2	76	M	Floor of mouth	Squamous cell carcinoma
3	48	M	Retromolar region	Lichen planus
4	52	F	Buccal mucosa	Leukoplakia
5	35	F	Palatal mucosa	Leukoplakia
6	54	F	Ventral tongue	Leukoplakia
7	43	F	Buccal mucosa	Erythroleukoplakia
8	61	M	Retromolar region	Squamous cell carcinoma
9	53	M	Lateral tongue	Squamous cell carcinoma
10	62	M	Floor of mouth	Squamous cell carcinoma
11	66	F	Gingiva	Squamous cell carcinoma
12	36	M	Buccal mucosa	Erythroplakia
13	52	F	Lateral tongue	Squamous cell carcinoma
14	57	M	Lateral tongue	Squamous cell carcinoma
15	56	M	Retromolar region	Focal hyperkeratosis
16	48	M	Floor of mouth	Squamous cell carcinoma
17	22	F	Lateral tongue	Lichen planus
18	55	F	Lateral tongue	Lichen planus
19	29	M	Lateral tongue	Squamous cell carcinoma
20	50	F	Buccal mucosa	Lichen planus
21	48	M	Ventral tongue	Squamous cell carcinoma
22	38	F	Ventral tongue	Squamous cell carcinoma

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respectively. These results agree with the incidence reports on oral SCC indicated by Silverman and Johnson.[7,16]

DNA ploidy status was evaluated with interpretation of DNA histograms for each sample. These interpretations revealed 20 diploid DNA patterns (90.9%) whereas aneuploidy was detected in 2 SCC lesions (9.1%) sampled from the lateral margin of the tongue and floor of the mouth. When only malignant lesions were considered, aneuploidy rate was 16.7% whereas a diploid pattern was indicated for 83.3% of the sample.

Together with ploidy analysis, cytomorphometric measurements were also carried out for study and control groups in each case. The mean values for each variable were determined and mean values of study and control groups for each cytomorphometric variable were calculated (Table 2). The results and the difference between groups are given in Table 3. A statistically significant difference was shown for perimeter, area, diameter equivalent to circle, minimum and maximum Feret, intensity, DNA content (c), and DNA index values whereas the difference was not statistically significant for form factor and entropy values. Form factor measurements are based on departure from circularity and formulated with 4π *area/perimeter*^{2} values for nuclei. This ratio is calculated as "1" for an ideal circle whereas it approaches "0" in case of deviation from the circle form. Also, entropy determines the heterogeneity of the cells. Entropy value is found to be high for heterogeneous cells whereas homogeneous cells display low entropy values.

Discussion

Detection of early-stage oral cancer is significantly impaired by the absence of classic clinical characteristics of advanced oral cancers including ulceration, induration, elevation, bleeding, and cervical adenopathy for early-stage lesions.[17] In contrast to high sensitivity and specificity rates of visual inspection for the diagnosis of skin cancer, screening has not been considered as a reliable method to detect early stage oral cancers.[18] Also, adjuncts for cancer detection, such as the Pap smear for cervical cancer, mammography for breast cancer, and PSA test for prostate cancer, have been lacking in oral diagnosis. These circumstances may explain the high mortality and morbidity rates remained unchanged for the last 3 decades. From this point of view, cytopathologic assessments and oral brush biopsy could be considered as an effective and valuable adjunct to conventional biopsy.

The contribution of cytologic screening in reducing the incidence and mortality of invasive cervical carcinoma[19] and its subsequent utilization in other sites like upper gastrointestinal tract, pancreas, rectum, lung, and esophagus, has led its application as a method for the detection of early-stage oral cancerous and precancerous lesions. Since then, many authors used cytomorphometric analysis for this purpose. Mollaoglu et al[20] were able to detect the malignant transformation of a lichenoid lesion to SCC using quantitative cytologic assessment. Again, they were able to show significantly elevated nuclear area values

Table 2. MEAN VALUES OF STUDY AND CONTROL GROUPS FOR EACH CYTOMORPHOMETRIC VARIABLE

	Perimeter		Area		Form Factor		Diameter Equivalent to Circle		Minimum Feret		Maximum Feret		Intensity		DNA Content (c)		DNA Index		Entrophy	
Group	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C
1	38.29	31.71	95.12	65.53	0.785	0.811	10.78	9.08	10.11	8.49	13.35	11.35	131,039	89,629	2.42	2.00	1.21	1.00	1.20	1.19
2	30.32	27.52	66.04	50.37	0.81	0.82	8.68	7.95	8.28	7.31	10.75	10.09	82,907	65,427	2.89	2.00	1.44	0.99	1.20	1.21
3	35.23	28.62	76.59	49.37	0.76	0.75	9.76	7.90	9.28	7.33	12.25	10.36	103,247	66,312	2.57	2.00	1.28	0.99	1.19	1.20
4	33.76	26.91	71.28	43.42	0.76	0.75	9.34	7.41	8.58	6.71	12.26	10.02	91,760	58,874	3.53	1.99	1.76	0.99	1.19	1.20
5	32.02	26.72	67.75	46.09	0.80	0.80	9.15	7.62	8.36	6.98	11.62	9.81	91,985	65,227	3.27	2.00	1.63	1.00	1.21	1.21
6	33.12	27.88	56.60	51.84	0.80	0.88	8.64	8.20	7.42	7.76	11.20	10.44	81,255	74,248	2.06	2.00	1.10	1.00	1.18	1.17
7	32.14	29.73	57.72	53.13	0.69	0.75	8.47	8.18	7.12	7.37	12.32	11.05	83,754	76,741	2.08	2.00	1.04	0.99	1.19	1.17
8	39.44	31.24	100.66	57.42	0.78	0.73	11.11	8.49	10.56	7.42	13.53	11.60	140,523	83,043	3.52	2.00	1.76	1.00	1.18	1.19
9	30.85	31.82	63.74	63.59	0.79	0.77	8.72	8.91	8.19	8.29	11.01	11.51	85,536	90,197	2.79	1.97	1.39	0.98	1.23	1.22
10	41.17	30.13	109.30	57.83	0.77	0.79	11.47	8.54	10.92	8.05	14.00	10.80	157,126	83,949	3.02	2.00	1.51	1.00	1.17	1.18
11	34.09	21.27	75.78	31.60	0.79	0.85	9.65	6.25	8.83	6.18	12.40	7.73	95,410	36,954	2.90	2.00	1.45	1.00	1.21	1.25
12	36.34	32.79	75.96	58.11	0.71	0.68	9.72	8.55	8.76	7.44	13.07	12.29	110,868	87,658	3.10	1.99	1.55	1.00	1.18	1.20
13	32.26	34.60	69.42	75.35	0.80	0.78	9.17	9.74	8.75	9.20	11.27	12.16	97,352	106,906	2.06	2.00	1.03	0.99	1.25	1.23
14	40.03	28.88	110.77	55.27	0.81	0.81	11.50	8.28	10.83	7.85	13.93	10.40	137,712	66,187	2.87	2.00	1.43	1.00	1.21	1.20
15	32.66	29.77	69.67	56.84	0.81	0.79	9.35	8.43	8.44	7.59	11.97	11.11	95,140	79,247	2.56	2.00	1.28	1.00	1.15	1.18
16	32.77	33.73	66.27	74.08	0.76	0.80	9.08	9.64	7.77	8.60	12.46	12.42	89,826	102,827	2.23	2.00	1.11	0.99	1.18	1.15
17	39.01	29.16	96.29	53.93	0.78	0.79	10.99	8.25	10.19	7.50	13.72	10.73	128,724	66,658	2.08	2.00	1.04	0.99	1.20	1.20
18	37.37	32.14	91.28	64.37	0.78	0.76	10.53	8.91	9.58	7.70	13.35	12.12	102,711	80,818	3.69	2.00	1.84	0.99	1.21	1.19
19	31.40	27.43	70.10	49.95	0.82	0.80	9.02	7.77	8.53	7.27	11.14	9.95	89,560	64,915	2.65	2.00	1.32	1.00	1.19	1.19
20	32.46	28.04	63.84	48.27	0.74	0.76	8.90	7.78	7.73	6.84	12.29	10.84	84,358	63,519	2.38	1.99	1.19	1.00	1.17	1.17
21	35.89	18.25	91.66	22.77	0.80	0.83	10.17	5.30	9.61	5.20	12.50	6.77	127,963	29,066	2.90	2.00	1.45	0.99	1.22	1.26
22	32.29	29.07	65.09	53.45	0.77	0.78	9.04	8.19	8.24	7.43	11.75	10.69	90,990	75,158	2.33	2.00	1.16	1.00	1.23	1.25

Abbreviations: S, study group; C, control group.

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Table 3. MEAN VALUES, DIFFERENCE BETWEEN GROUPS AND *P*-VALUES FOR CYTOMORPHOMETRIC VARIABLES*

Variables	Values for Study Groups (mean ± SD)	Values for Control Groups (mean ± SD)	Difference (mean ± SD)	*P*
Perimeter	34.678 ± 3.26	28.973 ± 3.73	5.705 ± 4.75	.000 $P < .001$*
Area	77.770 ± 16.39	53.754 ± 11.92	24.016 ± 20.28	.000 $P < .001$*
Form factor	0.778 ± 0.03	0.786 ± 0.04	−0.008 ± 0.03	.289 $P > .05$
Diameter equivalent to circle	9.693 ± 0.96	8.153 ± 0.98	1.540 ± 1.36	.000 $P < .001$*
Min feret	8.913 ± 1.09	7.478 ± 0.84	1.435 ± 1.35	.000 $P < .001$*
Max feret	12.370 ± 0.99	10.647 ± 1.35	1.723 ± 1.570	.000 $P < .001$*
Intensity	104,533.91 ± 22,276.14	73,343.64 ± 18,255.24	31,190.27 ± 28,906.61	.000 $P < .001$*
DNA content (c)	2.723 ± 0.50	1.997 ± 0.01	0.726 ± 0.50	.000 $P < .001$*
DNA index	1.362 ± 0.24	0.995 ± 0.01	0.367 ± 0.24	.000 $P < .001$*
Entrophy	1.197 ± 0.02	1.201 ± 0.03	−0.032 ± 0.02	.444 $P > .05$

*Statistically significant.

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for the malignant smears compared to the normal and dysplastic smears.[21] Cowpe et al[22] and Ogden and Cowpe[23] have shown the malignant change with their study based on the assessment of the nuclear and cytoplasmic areas of Papanicolaou-stained smears and DNA profiles of Feulgen-stained smears obtained from epithelial lesions and contralateral normal mucosa. Ramaesh et al[24] measured nuclear and cellular diameters in dissociated epithelial cells obtained from normal buccal mucosa, lesions of oral leukoplakia, and SCC. They reported a significant increase in mean nuclear diameter values during transition from normal mucosal cells to dysplasia and SCC. The mean nuclear diameter was 7.00 to 9.2 μm and 7.17 to 13.75 μm for normal mucosa and SCC lesions respectively.[24] Our study also revealed a statistically significant difference between mean nuclear diameter values for diseased and healthy mucosal sites of 9.69 μm and 8.15 μm respectively. Cowpe and Longmore[25] reported a mean NA of 78 μm^2 for normal oral epithelial cells obtained from 14 healthy individuals in a study that aimed to provide a normal baseline for NA values. This value is much higher than our results, however, indicating a NA value of 55.36 μm^2 for normal smears and an approximate value of 77.78 μm^2 for malignant smears.

Correct and adequate sampling of cells is mandatory for an accurate diagnosis with cytomorphometric analysis. Until now, numerous instruments were used including metal and wooden tongue spatulas, cotton sticks, and brush biopsy instruments.[26] Although there are studies reporting that metal and wooden tongue spatulas are sufficient for cytologic sampling,[9,27] brush biopsy was shown to enable sampling of cells from all layers of epithelium (basal, intermediate, superficial) thus giving the opportunity to detect early precancerous changes[13] in contrast to traditional oral exfoliative cytology that was found to yield unreliable results and high false negative rates.[28,29] On the onset of this study, the use of the blunt end of a scalpel to collect epithelial cells resulted in a failure in sampling and staining so we have decided to utilize cytobrush (Rambrush, Ginram, Mirandola-Italy) and confirmed high accuracy rates. Selection of fixation method for oral smears subsequent to transferring to glass slides is also controversial in the literature, although there are a few studies carried out on this subject. Nevertheless, Ogden et al[30] could not find a significant difference between direct immersion in diethyl ether and ethanol (1:1), spray fixation (Vale Smear Fix), and air drying methods in measuring nuclear and cytoplasmic areas. Hence, we employed air drying throughout this study and observed satisfactory results with favorable staining and imaging properties. The effect of various external and systemic factors on cytomorphometric measurements is also assessed with previous studies.[12,31,32] Standard reference values could not have been established for specific age groups and different oral mucosal sites (hard palate, posterior tongue, ventral tongue, floor of mouth, and buccal mucosa) with DNA analysis and cytomorphometric studies.[9] It was shown that nuclear and cytoplasmic area values were not affected by gender.[24] A significant decrease was noted in mean cytoplasmic and nuclear area values for chronic alcohol consumers when compared to control group.[12] Because none of our patients was a chronic alcohol consumer, this parameter was not taken into account. The influence of radiotherapy on oral mucosa was also investigated by Ogden et al[33] and a significant increase in nuclear and cytoplasmic area together with abnormal DNA values were shown as a result of radiotherapy, returning to within normal limits 1 month after treatment. The specimens were obtained before the onset of chemotherapy or radiotherapy, hence this effect was also overlooked in the present

study. Based on the studies displaying variations in cytomorphometric measurements and DNA content analysis between normal and abnormal tissue in the same patient, and intrapatient comparisons revealing that patient's own corresponding clinically normal site provides a suitable control,[22] we also used the contralateral healthy mucosal site for this purpose in each patient.

DNA content analysis of Feulgen-stained nuclei in oral SCC has been investigated recently as a possible indicator of prognosis or to evaluate tumor behavior.[34,35] Image and flow cytometry of DNA have been considered as objective methods for determination of biologic behavior of cancer cells, tumor characteristics,[36,37] and decision for malignancy in borderline lesions, to confirm prognostic and therapeutic information for oral cancer.[38] DNA ploidy analysis has also been used for evaluation of ploidy anomalies in predicting the response to radiotherapy and chemotherapy in SCC of the head and neck.[39,40] DNA aneuploid tumors were found to be more sensitive to irradiation and to cisplatinum chemotherapy, probably due to their high mitotic activity.[41] In this respect, Cowpe et al have developed cytophotometry for the assessment of Feulgen-stained nuclei[9] and revealed diploid DNA distribution for benign lesions whereas malignant lesions had a polyploid configuration.[9,22,25] Sudbo et al[42] evaluated the DNA content as a prognostic marker in 150 patients with oral leukoplakia and with verified epithelial dysplasia. They reported the highest malignant transformation rate in aneuploid lesions (84%) compared to tetraploid (60%) and diploid (3%) ones and considered the leukoplakia lesions with abnormal (aneuploid) DNA content as true carcinomas. The rates of aneuploidy vary widely and have been reported between 30% and 76%.[43,44] Mahmood et al reported an aneuploidy rate of 51% in 90 oral SCCs with flow cytometry[45] and again, Baretton et al[46] determined 58 aneuploid lesions (55%) from 106 oral SCCs. Although non-diploid DNA range profiles have generally been obtained for oral and maxillofacial cancers,[22,23] DNA diploid oral cancers are also known to occur frequently.[39,47,48] Melchiorri et al[49] found a predominance of diploid patterns despite the fact that most of the patients had advanced tumors. They detected 5 aneuploid lesions (20%) in their study that investigated DNA ploidy in specimens obtained from 25 patients with SCC of the oral and maxillofacial region.[49] We also observed that the majority of malignant tumors and all non-malignant lesions (leukoplakia, erythroleukoplakia, lichen planus, and hyperkeratotic lesions) displayed a diploid DNA pattern. The aneuploidy rate was 9.1% when the entire sample was taken into consideration. When only malignant lesions were involved, 2 lesions of 12 cancers (16.7%) were proved to have an aneuploid pattern whereas 83.3% of malignant lesions were reported as diploid. Aneuploid tumors were squamous cell carcinoma of the tongue and floor of the mouth. This wide variety of reported aneuploidy rates might be due to different cytometric methods and tissue preparations that have been applied, the definition of DNA pattern, and the number of samples that have been studied. Irrelevant to the incidence reports, previous studies have revealed a positive relationship with the histologic grade of differentiation, tumor aggressiveness,[49] lymph node metastases,[46] and aneuploidy.

Cytomorphometric assessments improved by advanced computer-assisted image analysis systems have gained importance instead of subjective interpretations based on clinical symptoms and personal judgment and experience of the cytologist. Recent studies were focused on quantitative analysis of cellular alterations to detect early stage malignancies and recurrences, hence the diagnostic values of nuclear morphometry and DNA content analysis were improved with these developments. Our study was able to discriminate between normal, non-malignant, and malignant smears with respect to nuclear morphometric parameters, and confirms the appropriate methods for sampling, preserving, staining, and imaging properties. A statistically significant difference was shown for most parameters including perimeter, area, diameter equivalent to circle, minimum and maximum Feret, intensity, DNA content (c), and DNA index values between study and control groups for both malignant and non-malignant lesions. Furthermore, cytomorphometric measurements were combined with DNA content and pattern analysis to enhance the accuracy and efficacy of cytologic diagnosis.

In conclusion, oral brush biopsy was proved to be a rapid and minimally invasive procedure with high specificity and sensitivity rates, requiring no topical or local anesthetic. It is highly acceptable for patients and can be repeated at every recall visit. It should be kept in mind, however, that cytology is not a substitute for scalpel biopsy, rather it identifies oral lesions that require histologic evaluation. Smears from different sites of a diffuse lesion could determine the most appropriate area for a biopsy. The results of our study suggest that cytomorphometric analysis via oral brush biopsy is a valuable adjunct to biopsy for identification of premalignant and early stage cancerous oral lesions.

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