Ashdin Publishing
African Journal of Pathology and Microbiology
Vol. 2 (2013), Article ID 235672, 4 pages
doi:10.4303/ajpm/235672

Research Article

A Study of Imprint Cytology with Cytomorphometry as an Important Diagnostic Tool in Oropharyngolaryngeal Lesions

Arijit Majumdar, Asim Kumar Manna, Angshuman Jana, Swapan Pathak, Anirban Jana, Soumali Biswas, and Shakya Bhattacharya

Department of Pathology, IPGME&R, Kolkata, India
Address correspondence to Arijit Majumdar, arijitmajumdar23@gmail.com

Received 17 December 2012; Accepted 13 June 2013

Abstract *Objective.* Non-neoplastic and neoplastic mucosal lesions from orophayngolaryngeal region constitute a major health problem in developing countries. Carcinoma of this region is the commonest neoplasm in male in our country. The study was aimed at testing for the accuracy of cytological diagnosis as well as morphometry analysis and compared with histopathology in order to see whether the correlation exists between the three of them. The overall goal is the early diagnosis of these lesions in a cost-effective manner. *Material and methods.* A total 40 cases were selected having symptoms of ulceration and growth in these regions. The tissues were collected at the time of operation for imprint cytology and morphometry of the epithelial cells. Thereafter, the tissues were processed routinely for histopathology and immunohistochemistry (p53 and PCNA). Findings from cytology, cytomorphmetry were compared to those from histology with immunohistochemisty *Results.* Statistically significant difference was found between non-neoplastic, premalignant, and malignant lesions. Cytological diagnosis was confirmed by histological findings along with immunohistochemistry. p53 expression, and PCNA staining was more in malignant lesions. *Conclusion.* The imprint cytology and morphometry can be an important tool for early diagnosis and determination of therapeutic protocol in oral, pharyngeal, and laryngeal lesions, especially if they are correlated with histology and immunohistochemistry.

Keywords oropharyngolaryngeal lesions; imprint cytology; histology; immunohistochemistry

1. Introduction

The oral cavity, pharynx and larynx are the parts of the aerodigestive tract, one of the main portals into our body. A number of non-neoplastic as well as neoplastic lesions are very frequent in these sites. Oral cancer is a major problem in India and accounts for 50% to 70% of all cancers diagnosed [14]. Cancer of the larynx is also prevalent in India. Compared to western races, Indians are more affected [6]. The malignancies arising out of the mucosa of the oropharyngeolaryngeal region are squamous cell carcinoma in more than 90% of the time [18]. The imprint cytology is one of the upcoming methods that can be used in the diagnosis of malignant, and benign lesions in shorter period though the histopathology remains the gold standard [13]. 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 may be the value of cellular morphometry [20]. Cowpe and Longmore advocated the idea of using morphometry for enhanced diagnosis of oral lesions using cytological techniques [5]. Sometimes the distinction between some cases of premalignant dysplasia and carcinoma in situ or minimally invasive carcinoma is difficult in routine haematoxylin and eosin (H&E) stained sections. Application of immunohistochemistry has been found to be an important tool to resolve the problems in microscopic diagnosis of these gray zones in histopathology. The antibody 34βE12 stains selectively the keratins of basal cells; it has been used in the differential diagnoses between different carcinomas and the benign lesions that simulate it. The oral squamous cell carcinoma occurs due to multiple genetic changes. The interaction between the activated oncogenes and the mutations in the tumor suppressor genes is one of the driving force directing normal cells to uncontrolled growth and invasion. Mutation of p53 genes occur in 39–100% of head-neck cancers [17]. In the normal or hyperplastic squamous mucosa, expression of proliferating cell nuclear antigen (PCNA) are limited to the basal cell layer; whereas in dysplasia, they are often expressed also in supra basal level. PCNA seems to be the most consistent and therefore the most potentially useful for the identification and grading of these disorders [3,4,16].

Specific objectives of this study

(1) To know the profile of oral, pharyngeal, and laryngeal lesions by imprint cytology.

(2) To study the morphometry parameters of the cells from these lesions.

Table 1: Distribution age and sex of the cases ($n = 40$, as per final diagnosis).

Diagnosis	< 20		20–40		41–60		61–80	
	M	F	M	F	M	F	M	F
Inflammatory	1	1	8	2	1	—	—	—
Neoplastic	—	—	4	1	9	1	11	1

(3) To know the profile of oral, pharyngeal, and laryngeal lesions by histopathology.

(4) To test the accuracy of cytological diagnosis as well as morphometry analysis and compare with histopathology to establish a correlation among all of them.

The ultimate goal of our study was to offer the patients earliest possible diagnosis by a rapid, reliable, cost-effective, safe procedure which can provide proper management at an earlier date (in the same sitting) with better chances of survival.

2. Materials and methods

A total 40 cases were selected from patients attending the Department of Surgery as well as ENT with the complaints of pain inside mouth, difficulty in swallowing, hoarseness of voice, foul-smelling breath, etc. Detailed clinical history with special reference to the habit of tobacco (both smoking and chewing) and/or alcohol consumption was taken. The clinical examination was done particularly noting the presence of ulcer, growth or even a slight alteration in the thickness of the mucosal surface found on routine examination and most importantly the cervical lymph node swelling. Only the lesions arising from the surface mucosa were taken in the study. After admission, the lesions were operated and each case was studied by imprint cytology, histopathology, and immunohistochemistry. Immediately after obtaining the biopsy specimen, a direct imprint was prepared and the slides were immediately fixed in 95% ethyl alcohol or air-dried and then stained with Papanicolaou stain and MGG stain [19]. The morphometry was done on the epithelial cells obtained in the imprint smears ($\times$400 magnification). The cytology from the areas adjacent to the diseased sites was taken as the controls. For the histopathology, tissue obtained were fixed in formalin, processed, and embedded in paraffin wax block. One section of 3 micron thickness from each block was affixed on egg-albumin-coated slide, and three sections of 3 micron thickness from each block were affixed on poly-l-lysine coated slides. The former slide was stained by H&E staining, and the later group was used for p53 and PCNA nuclear staining. For staining by p53 and PCNA, the kit literature of the manufacturer was followed [1,2]. p53 staining was evaluated by the positive cases (the percentage of cases showing positive staining) and p53 positivity (the percentage of nucleus showing positive staining out of total nuclei counted) [10]. PCNA staining was evaluated by PCNA labeling index (PCNA LI%—the percentage of nucleus showing positive staining out of total nuclei counted) [23]. Finally, a large chart was produced tabulating histopathological diagnosis, p53 nuclear positivity, and final diagnosis. Statistical analysis was done by unpaired Student's "t"-test and P-values were obtained. A level of significance of 5% ($P < .05$) is chosen, for no better reason than that it is conventional [7,21].

Table 2: Distribution of cases according to cytological and histological diagnosis ($n = 40$).

Cytological diagnosis	Histopathological diagnosis	
Inflammatory (09)	Hyperplasia without dysplasia	8
	Dysplasia	1
Dysplasia (20)	Dysplasia	11
	Carcinoma	7
	Hyperplasia without dysplasia	2
Carcinoma (06)	Carcinoma	6
Unsatisfactory (05)	Hyperplasia without dysplasia	3
	Carcinoma	2

3. Results

Most of the patients with neoplastic lesions in our study were older males (> 60 years) (Table 1). We see from Table 2 that 8 out of 9 cases (89%) cytologically diagnosed as inflammatory lesions were in consistent with the histopathological diagnosis. Out of 20 cases of dysplasia diagnosed on cytology, 7 cases were found to be carcinoma on histopathological diagnosis, and 2 were found to be hyperplasia without dysplasia. All cases (100%) of cytological diagnosed carcinoma were in consistent with the results of histopathological diagnosis. Three of the five unsatisfactory cytological smears were found to be hyperplastic on histopathology, and the rest were carcinoma. The mean value of the nuclear diameter, nuclear area, cell diameter, cell area, and ratio between the nuclear area and cytoplasmic area of the 40 cases were given in the Table 3. The P-values between the different groups showing the statistically significant difference can be seen in Table 4. Table 5 shows the interpretation of the p53 nuclear staining of the cases. The P values of them showing the statistically significant difference is given in Table 6.

4. Discussion

In our study, we did the imprint cytology of the biopsied specimen or surgically resected specimens from a total of 40 patients. Among the non-neoplastic inflammatory lesions, the male:female ratio was 1.2:1; and among the neoplastic lesions, the ratio was 4.8:1 (Table 1).

The cytological examination had revealed 9 cases (22.5%) of inflammatory smears of which 1 case was diagnosed as dysplasia and the rest were hyperplasia without dysplasia on histopathology (Table 2). The only false-negative case in our study may be inherent in the procedure.

Table 3: Results of morphometric study in different category from Papanicolaou stained smear.

Cytological diagnosis	ND±SD (μm)	NA±SD (μm^2)	CD±SD (μm)	CA±SD (μm^2)	N:C ratio
Inflammatory	6.89 ± 0.78	37.68 ± 8.55	37.78 ± 0.09	$1,120.98 \pm 58.57$	0.033
Dysplasia	10.55 ± 0.51	87.57 ± 8.42	32.95 ± 0.83	852.79 ± 42.76	0.103
Carcinoma	12.5 ± 0.55	122.86 ± 10.75	28 ± 0.89	615.97 ± 39.32	0.201
Control	7 ± 0.07	38.46 ± 0.78	39 ± 1	$1,199.61 \pm 61.23$	0.03

ND: nuclear diameter, NA: nuclear area, CD: cytoplasmic diameter, CA: cytoplasmic area, N:C: nucleo-cytoplasmic ratio, and SD: standard deviation.

Table 4: P-values showing the significance of differences in cytomorphometry between different categories.

Cytological diagnosis	ND	NA	CD	CA	N:C
Control and inflammatory	0.683	0.791	0.06	0.06	0.347
Inflammatory and dysplasia	4.70917×10^{-8}	2.15939×10^{-10}	5.65946×10^{-9}	3.52487×10^{-8}	3.93333×10^{-12}
Inflammatory and carcinoma	5.24388×10^{-10}	4.93447×10^{-8}	2.62882×10^{-10}	3.94002×10^{-11}	1.61946×10^{-10}
Dysplasia and carcinoma	6.171×10^{-5}	0.000156809	2.63449×10^{-6}	5.45845×10^{-7}	1.29706×10^{-5}

ND: nuclear diameter, NA: nuclear area, CD: cytoplasmic diameter, CA: cytoplasmic area, N:C: nucleo-cytoplasmic ratio, and SD: standard deviation.

Table 5: Results of IHC with staining by monoclonal antibody against p53 and PCNA.

Final histological diagnosis	p53 nuclear positivity (%)	Positive cases (%)	PCNA labeling index
Hyperplasia without dysplasia	5.5 ± 0.95	20	3.58 ± 0.36
Dysplasia	30 ± 5.5	85	29.5 ± 1.00
Carcinoma	80.8 ± 15.2	95	34.0 ± 0.79

Table 6: P-values showing the significance of differences in p53 nuclear positivity (%) and PCNA labeling index between different categories.

Final histological diagnosis	p53 nuclear positivity (%)	PCNA labeling index
Hyperplasia without dysplasia and dysplasia	0.000234425	3.90038×10^{-8}
Hyperplasia without dysplasia and carcinoma	0.000104823	1.10967×10^{-9}
Dysplasia and carcinoma	0.000146996	6.40021×10^{-5}

Out of 20 cases (50%) of dysplasia on cytology, 11 were dysplasia, 2 were hyperplasia without dysplasia, and 7 were carcinoma on histopathological diagnosis. This is because of difficulty in differentiation of epithelial cell dysplasia and well differentiated carcinoma on cytology as Wellman found in his study [22]. Moreover, Hussein et al. said that one of the disadvantages of the imprint cytology is that it can not differentiate between in situ and invasive lesions [9]. All cases of cytologically diagnosed carcinoma (15%) were consistent with the results of histopathological diagnosis. The cause of two false-positive cases (dysplasia in cytology but hyperplasia without dysplasia in histopathology) in our study may be due to interpretation error. The unsatisfactory specimen in the cytology (12.5%) may be due to scant cellularity, air-drying, or distortion artifact obscuring blood or inflammation as found by Herrmann et al. in their study [8].

From the results of our study (Table 2), we note that the sensitivity and specificity of detecting malignancy with imprint cytology was 96% and 80%, respectively. Hussein et al. noted that the sensitivity and specificity of 88% and 92%, respectively, in their study [9].

In all these cases, morphometric analysis of the epithelial cells on cytological smear was performed in terms of cell diameter, cell area, nuclear diameter, nuclear area, and nucleo-cytoplasmic ratio (Table 3). In the present study, the mean cytoplasmic area was the highest ($1,120.98 \pm 58.57\,\mu$m^2) for inflammatory smear and gradually decreasing up to squamous cell carcinoma ($615.97 \pm 39.32\,\mu$m^2). Nuclear abnormalities in the form of nuclear pleomorphism, nuclear margin irregularity, hyperchromasia, and high nuclear area:cytoplasmic area ratio (N:C ratio) were more marked in high grade neoplastic lesions. In this study, the N:C ratio was 0.201 for squamous cell carcinoma (SCC), 0.103 for dysplasia, 0.033 in inflammatory cases, and 0.031 in control cases which were also comparable with other studies. Cowpe and Longmore found that tissues undergoing malignant transformation typically show a reduction in cytoplasmic area before a reduction in nuclear area. They also suggested that samples of healthy mucosa from the same patients provide the best controls [5]. Ramaesh et al. found that cytoplasmic diameter was the highest in normal mucosa, lower in dysplasia, and the lowest in carcinoma and the reverse is true for nuclear diameter, i.e., it is the highest in cases of carcinoma [15].

We performed the unpaired Student's t-test with the morphometric parameters to determine the significant differences between the different categories of lesions on cytology. The results are shown in Table 5. The P-value of N:C ratio was highly significant ($P = 3.93333 \times 10^{-12}$ which is $< .001$) between inflammatory lesions and dysplasia and between inflammatory lesions and carcinoma ($P = 1.61946 \times 10^{-10}$). The P-value of N:C

ratio between dysplasia and SCC was also significant ($P = 1.2970610 - 5$). Differences of the nuclear diameter, nuclear area, and N:C ratio between inflammatory and control category was not found to be significant ($P > .001$) as shown in Table 6. According to Ramaesh et al., this is because although the histology of non-dysplastic lesions show superficial keratinization and hyperplasia their cellular features are similar to that of normal epithelial cells and the cytomorphometric measurement also agrees with this [15].

Both nuclear positivity for p53 and mean p53 positivity of each positive case was gradually increasing from hyperplasia to dysplasia to carcinoma (Table 5). A statistically significant difference is found between the different groups (Table 6). Kerdpon et al. in their study showed that positive nuclear staining was found in 36% cases of hyperplasia, 85% cases of dysplasia and 94% cases of carcinoma [11]. Jain et al. showed both the percentage of positive cases and p53 positivity showed a corresponding increase in values with increase degree of dysplasia and carcinoma [10].

The mean PCNA labeling index is the lowest in hyperplasia without dysplasia and highest in carcinoma (Table 5) which corroborates with the finding of the study done by Kurokawa et al. [12]. A statistically significant difference is found between different groups (Table 6).

References

[1] BioGenex Kit, *Anti-p53 Protein [1801], AM240-5M*. BioGenex, 4600 Norris Canyon Road, San Ramon, CA 94583, USA.

[2] BioGenex Kit, *Anti-Proliferating cell nuclear antigen (PCNA) [PC10], AM252-5M*. BioGenex, 4600 Norris Canyon Road, San Ramon, CA 94583, USA.

[3] M. E. Christensen, M. H. Therkildsen, B. L. Hansen, H. Albeck, G. N. Hansen, and P. Bretlau, *Epidermal growth factor receptor expression on oral mucosa dysplastic epithelia and squamous cell carcinomas*, Eur Arch Otorhinolaryngol, 249 (1992), 243–247.

[4] M. D. Coltrera, R. J. Zarbo, W. A. Sakr, and A. M. Gown, *Markers for dysplasia of the upper aerodigestive tract. Suprabasal expression of PCNA, p53, and CK19 in alcohol-fixed, embedded tissue*, Am J Pathol, 141 (1992), 817–825.

[5] J. G. Cowpe and R. B. Longmore, *Nuclear area and Feulgen DNA content of normal buccal mucosal smears*, J Oral Pathol, 10 (1981), 81–86.

[6] P. L. Dhingra, *Cancer larynx*, in Diseases of Ear, Nose and Throat, Elsevier, New Delhi, 3rd ed., 2004, ch. 62.

[7] R. A. Fisher, *Statistical Methods for Research Workers*, Oliver and Boyd, Edinburgh, 1st ed., 1925.

[8] I. F. Herrmann, H. A. Müller, and K. Foet, *The value of fine-needle-cytology, imprint-cytology and histology in the head-neck-region*, Arch Otorhinolaryngol, 219 (1978), 375–376.

[9] M. R. Hussein, U. M. Rashad, and K. A. Hassanein, *Touch imprint cytologic preparations and the diagnosis of head and neck mass lesions*, Ann Oncol, 16 (2005), 171–172.

[10] A. Jain, V. Maheshwari, G. Mehdi, K. Alam, and S. C. Sharma, *Diagnostic and prognostic significance of p53 protein expression in squamous cell lesions of the oral cavity*, Internet J Otorhinolaryngol, 7 (2008).

[11] D. Kerdpon, A. M. Rich, and P. C. Reade, *Expression of p53 in oral mucosal hyperplasia, dysplasia and squamous cell carcinoma*, Oral Dis, 3 (1997), 86–92.

[12] H. Kurokawa, Y. Yamashita, S. Takeda, K. Miura, T. Murata, and M. Kajiyama, *The expression of proliferating cell nuclear antigen (PCNA) and p53 protein correlate with prognosis of patients with oral squamous cell carcinoma*, Fukuoka Igaku Zasshi, 90 (1999), 6–13.

[13] B. Lončar, M. Pajtler, V. Miličić-Juhas, Ž. Kotromanović, B. Staklenac, and B. Pauzar, *Imprint cytology in laryngeal and pharyngeal tumours*, Cytopathology, 18 (2007), 40–43.

[14] J. E. Park and K. Park, *Epidemiology of chronic non-communicable diseases and conditions*, in Park's Textbook of Preventive and Social Medicine, K. Park, ed., Banarsidas Bhanot Publishers, Jabalpur, 19th ed., 2007, ch. 6.

[15] T. Ramaesh, B. R. Mendis, N. Ratnatunga, and R. O. Thattil, *Cytomorphometric analysis of squames obtained from normal oral mucosa and lesions of oral leukoplakia and squamous cell carcinoma*, J Oral Pathol Med, 27 (1998), 83–86.

[16] J. Reibel, H. Clausen, and E. Dabelsteen, *Staining patterns of human pre-malignant oral epithelium and squamous cell carcinomas by monoclonal anti-keratin antibodies*, Acta Pathol Microbiol Immunol Scand A, 93 (1985), 323–330.

[17] J. Rosai, *Guidelines for handling of most common and important surgical specimens*, in Rosai and Ackerman's Surgical Pathology, Elsevier, New Delhi, 9th ed., 2004.

[18] J. Rosai, *Oral cavity and oropharynx*, in Rosai and Ackerman's Surgical Pathology, Elsevier, New Delhi, 9th ed., 2004, ch. 5.

[19] J. Rosai, *Special techniques in surgical pathology*, in Rosai and Ackerman's Surgical Pathology, Elsevier, New Delhi, 9th ed., 2004, ch. 3.

[20] A. H. Shabana, N. G. el Labban, and K. W. Lee, *Morphometric analysis of basal cell layer in oral premalignant white lesions and squamous cell carcinoma*, J Clin Pathol, 40 (1987), 454–458.

[21] S. M. Stigler, *Fisher and the 5% level*, Chance, 21 (2008), 12.

[22] M. L. Wellman, *The cytologic diagnosis of neoplasia*, Vet Clin North Am Small Anim Pract, 20 (1990), 919–938.

[23] W. J. Zeng, G. Y. Liu, J. Xu, X. D. Zhou, Y. E. Zhang, and N. Zhang, *Pathological characteristics, PCNA labeling index and DNA index in prognostic evaluation of patients with moderately differentiated hepatocellular carcinoma*, World J Gastroenterol, 8 (2002), 1040–1044.