Original Article:

Cytomorphometry in Oral Lesions - An Objective Approach to Cytological Diagnosis

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Abstract: Objectives: The present study focuses on cytomorphological evaluation of the scrapings and cytological appearance of a spectrum of lesions of the oral cavity. Morphometric nuclear and cytoplasmic parameters were used to distinguish between benign, premalignant and malignant lesions. Material and Methods: The samples were obtained by scraping the lesion firmly till tiny pin point bleeding points were seen to ensure adequate sampling. Adequate sampling was defined as 50 cells consisting of transepithelial cytology specimen (superficial, intermediate and basal cells). Results: A total of 80 patients were evaluated with predominance in males and mean age of presentation was 45.05±12.8 years. Cytomorphometric analysis revealed an increasing trend in nuclear parameters as the spectrum of oral lesion moved from benign to malignant category while a decline was observed in cellular parameters. A significant p value of <0.0001 was found between cytomorphometrical parameters and histopathological subtypes. Sensitivity and specificity of conventional exfoliative cytology in conjunction with cytomorphometry was 100% and 94.74% respectively. Diagnostic accuracy of cytology in predicting malignancy taking histopathology as gold standard was 97.0.

Conclusion: Grading and scoring of oral cytosmears according to morphology showed a positive correlation with histopathological diagnosis. In addition, cytomorphometry increases the diagnostic accuracy of oral conventional exfoliative cytology and hence is seen to have an impact on early detection of cellular alterations and prompt treatment. Therefore, it is a sensitive and useful adjunct to monitor the cellular transformation.

Key Words: Oral cancer, Exfoliative cytology, Morphometry, Tobacco, Early detection

Introduction:
Oral carcinoma is one of the highly prevalent cancers worldwide and among the leading causes of mortality in countries like India, Sri Lanka, Pakistan and Bangladesh. [1] The usual sites of involvement are the tongue, followed by gum, floor of mouth, soft palate and buccal mucosa. Majority (90%) of the cases reported of oral cancer are attributed to tobacco consumption in various forms. [2] Other risk factors for lip, oral cavity and pharyngeal cancers include betel quid chewing with or without tobacco (oropharyngeal and oral cavity cancers) the consumption of nitrosamine-rich foods, including salted fish, and infection, e.g., Epstein-Barr virus (EBV) (nasopharyngeal cancer). [3,4]

Oral premalignant lesions have the potential to transform into malignancy. In 2005, the World Health Organization (WHO) recommended the term oral potentially malignant disorders (OPMDs) for a variety of lesions, such as oral leukoplakia, erythroplakia, oral lichen planus and oral submucous fibrosis (OSMF). The timely evaluation and treatment of these premalignant lesions, preventing their progression to oral cancer is vital. [5] Biopsy of the oral lesions has been the gold standard technique for the initial diagnosis of oral carcinoma. Unfortunately, over the last 30 years, there has been an insignificant improvement in the 5-year survival rate of patients with oral cancer treated with multimodality contemporary therapy and current survival rates for all stages range from 50% to 55%. [6] Thus, there is a need for a non-invasive or minimally invasive diagnostic tool which can be easily performed and can segregate benign, premalignant and malignant lesions. [7]

Cytological screening for cervical cancer and premalignant cervical lesions with the Bethesda classification method represents one of the great success stories in cancer prevention in modern times. However, a unique grading system in oral cytology is yet to be universally adopted. Cytomorphometry is an objective computer aided image-based analysis of cells which has been found to be useful screening tool for the early detection of oral cancer. [8]

We studied the cytological appearance of a spectrum of lesions of the oral cavity. Cytomorphological evaluation of the scrapings was done using a novel grading and scoring system. [9] Morphometric parameters such as nuclear area (NA), cellular area (CA), nuclear perimeter (NP), cellular perimeter (CA), nuclear diameter (ND), cellular diameter (CD) and
nuclear: cytoplasmic ratio (N:C ratio) were used to distinguish between benign, premalignant and malignant lesions.

**Material And Methods:**

The present study was conducted at the Hakeem Abdul Hameed Centenary Hospital, New Delhi over a three-year period. A total of 80 cases with oral lesions attending the Otorhinolaryngology and Head & Neck Surgery, Out-patient department were the subjects of this study. Data on the age, personal history and presenting clinical features were retrieved from the accompanying laboratory request forms and patients’ medical records.

Cytological evaluation of a spectrum of oral lesions ranging from benign, premalignant and malignant was done. The samples were obtained by scraping the lesion firmly till tiny pin point bleeding points were seen to ensure adequate sampling, after which the material was smeared on clean glass slides. The slides were fixed immediately in both 95% alcohol and also air dried. Alcohol fixed smears were stained by Papanicolaou stain (PAP), Hematoxylin and Eosin stain (H&E). Air dried slides were stained with Giemsa stain. The first step in the microscopic evaluation was to confirm adequacy. Adequate sampling was defined as 50 cells (less than 50 cells/slide) They were excluded from further analysis.

Of 80 cases (7.5%) were inadequate on cytological parameters. Six out of the patients ranged between 19 years to 78 years & in this study. Oral scrapings were obtained from 80 patients with oral lesions who reported to our Otorhinolaryngology and Head & Neck Surgery, Out-patient department.

**Results:**

Eighty patients with oral lesions who reported to our institution over a period of three years were the subjects of this study. Oral scrapings were obtained from the lesions and cytological and morphometric evaluation was done. The age of the patients ranged between 19 years to 78 years & in this study. 86 (85%) were male & 12 (15%) were female. Six out of 80 cases (7.5%) were inadequate on cytological parameters (less than 50 cells/slide) They were excluded from further analysis.

Oral carcinoma can occur at variable sites in the oral cavity. Most common site is the tongue followed by floor of mouth and gingiva. In Asian countries, due to high incidence of betel quid chewing, the commonest site is the buccal mucosa. In our study, a spectrum of oral lesions including oral carcinoma was studied and the most common site was the buccal mucosa constituting 40% (32 out of 80 cases) followed by tongue (27.5%) and gingivo-buccal sulcus (GBS, 8.75%). Less commonly involved sites were lower lip, hard palate, retromolar trigone, lower alveolus and angle of mouth.

Smears were cytologically graded from A to F according to the novel cytology grading system. [9] Distribution of diagnostic categories were as follows: 14.86% (11) cases were normal, 12.16% (9) cases were in reactive category and 22.97% (17) cases were atypical probably reactive/low grade. 5 cases (6.76%) were categorized as atypical probably high grade while 17 cases (22.97%) were high grade squamous intraepithelial lesion. 20.27% (15 out of 74) cases were in Grade F i.e., invasive squamous cell carcinoma. (Table 1)

**Table 1: Distribution of Cases According to Cytological Grade**

<table>
<thead>
<tr>
<th>Cytological Grade</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Normal)</td>
<td>11</td>
<td>14.86%</td>
</tr>
<tr>
<td>B (Inflammation, Infection, Repair)</td>
<td>9</td>
<td>12.16%</td>
</tr>
<tr>
<td>C (Atypical- Probably Reactive / Low grade)</td>
<td>17</td>
<td>22.97%</td>
</tr>
<tr>
<td>D (Atypical - Probably High Grade)</td>
<td>5</td>
<td>6.76%</td>
</tr>
<tr>
<td>E (High Grade Squamous Intraepithelial Lesion)</td>
<td>17</td>
<td>22.97%</td>
</tr>
<tr>
<td>F (Invasive Squamous Cell Carcinoma)</td>
<td>15</td>
<td>20.27%</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>100%</td>
</tr>
</tbody>
</table>

Scoring of all the smears according to the novel cytological scoring system, described by Afrogheh A et al also was done [9] The characteristics as enumerated, i.e., irregular nuclear membrane, irregular chromatid distribution, prominent and irregular nucleoli, abnormal cell shape and cytoplasmic fragments, any parakeratotic cell, syncytial groups or evidence of necrosis were looked for and documented. [9] Benign lesions had a score below 3 while premalignant and malignant lesions scored from 3-8. Number of cases in each category has been shown in Table 2.

**Table 2: Distribution of Cases According to Cytological Score**

<table>
<thead>
<tr>
<th>Cytological Score</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>14.86%</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>12.16%</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>22.97%</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>6.76%</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>5.41%</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>21.62%</td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>16.22%</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>100%</td>
</tr>
</tbody>
</table>

The cases were distributed according to the cytomorphological evaluation of lesion as shown at Table 3. Of the cases, 32 (40%) were categorized as malignant according to cytological features while 22 (27.5%) cases were premalignant, 20 (25%) of the cases were categorized as benign lesions.

**Table 3: Distribution of Cases According to Category of Lesion on Cytology**

<table>
<thead>
<tr>
<th>Category Of Lesion According To Cytology</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>20</td>
<td>25.00%</td>
</tr>
<tr>
<td>Premalignant</td>
<td>22</td>
<td>27.50%</td>
</tr>
<tr>
<td>Malignant</td>
<td>32</td>
<td>40.00%</td>
</tr>
<tr>
<td>Inadequate</td>
<td>6</td>
<td>7.50%</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

All cytological smears were subjected to morphometry with the help of computer-based software (Motic image 3.0) and assessed objectively. Fifty cells of each smear were assessed for various nuclear parameters like nuclear diameter (ND), nuclear area (NA), nuclear perimeter (NP) and cellular parameters i.e., cellular perimeter (CP), cellular area (CA) and

**Grading system for oral cytology**

A: Normal
B: Reactive (inflammation, infection, repair)
C: Atypical- probably reactive / low grade
D: Atypical - probably high grade
E: High grade squamous intraepithelial lesion
F: Invasive squamous cell carcinoma
G: Other neoplasms specify

**Scoring system for oral cytology**

Irregular nuclear membrane yes (1) / no (0)
Irregular chromatid distribution yes (1) / no (0)
Prominent nucleoli yes (1) / no (0)
Abnormal cell shapes* yes (1) / no (0)
Parakeratotic cells** yes (1) / no (0)
Necrosis yes (1) / no (0)
Syncytial groups (>10 cells) *** yes (1) / no (0)
Irregular nucleoli yes (1) / no (0)
Abnormal cytoplasmic fragments yes (1) / no (0)

*Abnormal cell shapes: Abnormally configured keratotic cells
spindling, tadpole shapes or long cytoplasmic projections.
**Parakeratotic cells: Cells with dense orangeophilic cytoplasm and small hyperchromatic degenerate nuclei.
The nuclear-to-cytoplasmic ratio is low.
***Syncytial groups: Pleomorphic cells seen in three-dimensional clusters.
Oral scrapings were obtained from 80 patients with oral lesions. Histopathology confirmed verrucous leukoplakia and one case of verrucous hyperplasia were erroneously categorized as benign on cytology and two cases categorized as malignant on cytology were reported as oral proliferative verrucous leukoplakia on histopathology. Oral scrapings were obtained from 80 patients with oral lesions in this study. Six out of 80 scrapings were found to be inadequate on cytological parameters and no further evaluation was done. Out of the remaining 74 cases, in 68 cases the cytological impression and grade was positively correlated with the histological diagnosis and no discrepancy was present. In 6 cases, all from the benign category, histopathological evaluation was not done. These included 3 cases of aphthous ulcer, 2 cases of retention cyst, 1 case of mucosal erosion due to trauma by teeth. In our study it was observed that benign lesions had a cytological score of 1 and 2 with an overlap seen between benign (45.45%) and premalignant lesions (11.11%) with a cytological score of 2. Score of 3-6 corroborated with premalignant lesion while malignant lesions had cytological score of 8 mostly. There were 2 cases of each premalignant and malignant lesions with a score of 6. Cytological score was found to be significantly associated (p value <0.0001) with histological subtype of the lesions.

Cytological grading of the smears was done from A to F as described by Afrogheh et al [6] Association of the cytological grade with the histopathologically confirmed categories was assessed. Cytological grade was noted to be significantly associated (p value <0.0001) with the type of lesion. The majority of the malignant cases were in the category E and F (100%) i.e., invasive squamous cell carcinoma while premalignant cases were categorized as B-D. Benign cases were mostly assigned cytological grade A (54.55%) and B (45.45%). Grade B showed overlapping of few cases of benign (5 out of 11 cases, 45.45%) and premalignant (3 out of 27 cases, 11.11%) lesions. Grade E showed overlap between premalignant (2 out of 27 cases, 7.41%) and malignant cases (15 out of 30 cases, 50%).

Diagnostic accuracy of cytology was found to be 97.06% compared to histopathology which is regarded as a gold standard diagnostic test. Sensitivity of cytology in our study was 100% with a confidence interval of 95% with a specificity of 94.74%. We were able to diagnose all malignant and benign lesions accurately with the help of cytomorphometry. The grey zone lesions i.e., premalignant lesions showed cellular and nuclear changes on morphometry which can predict the tendency to transform into malignancy. Hence, overall cytomorphometry had good positive predictive value (93.75%) and 100% of negative predictive value.
Discussion:

Oral squamous cell carcinoma (OSCC) is the commonest malignancy of the oral cavity. It is frequently seen in the fifth and sixth decades of life and is predominantly associated with risk factors like smoking, smokeless tobacco and alcohol consumption. [11] Oral cancer poses an overwhelming burden with an alarming increase in incidence. Approximately 2,00,000 cases are diagnosed annually amongst which 1,00,000 succumb to death. [12] Exfoliative cytology is a time-tested technique by which the exfoliated cells are examined under the microscope and has been successfully applied in the early diagnosis of cervical cancer. Similarly, oral exfoliative cytology is a patient friendly method for collecting exfoliated cells from oral lesions. [13] It is a non-invasive procedure and is generally well accepted by patients with oral lesions. Combining cytological evaluation with computer-based image analysis of the exfoliated cells provides an objectivity & gives better results. [14]

In this study, smears prepared from the exfoliated cells from oral lesions were cytomorphologically evaluated. These cells were graded and scored on the basis of a novel system proposed by Afrogheh A et al. [9] Out of 80 cases, 6 smears were inadequate for a definitive opinion and were excluded from further statistical analysis. Remaining 74 cases were cytologically categorized: 20(25%) as benign category, 22(27.5%) as premalignant category and 32(40%) as being in the malignant category.

We observed that most of the benign lesions showed normal mature squamous cell cytological characteristics. Reactive lesions showed mild cells with mild increase in nuclear size along with benign cytological features and inflammatory cells. Afrogheh et al and Jairajpuri et al reported similar findings among their cases in the reactive category. [9,15] In a study conducted in Nepal, Singh described prominent acidoophilic character of the cytoplasm, karyorrhexis and prominent nucleioli as some of the cytologic features of exfoliated cells from leukoplakic patch. [16] Histopathology proven premalignant cases in our study were mostly leukoplakia with few of the cases with mild to moderate dysplasia. These cases on cytology showed mild nuclear size variation with attendant increase in nucleus to cytoplasmic ratio but nuclear borders appeared smooth and chromatin was diffuse. A clinical correlative study on early detection of oral cancer and precancerous lesions described certain features indicative of cellular transformation and distribution and [17] rhomatism, chromatin pattern, and distribution and discrepancy in N:C maturation. Cytological features of malignant cells in our study showed similar morphology to above mentioned study. The cells were arranged in syncytial sheets and clusters and showed anisomucleosis, nuclear hyperchromasia with irregular nuclear borders and prominent eosinophilic nucleioli. Cytoplasm was dense and scanty. Keratin pearl formation and abnormal mitotic figures were seen in few smears from OSCC cases. Few abnormal shaped cells like tadpole cells with cyttoplasmic extension and spindling were also seen.

Cytological grading of the oral smears has been done by several researchers using different methodologies. Gupta S et al graded oral cytological smears according to morphological parameters like enlarged nuclei, nuclear pleomorphism, nuclear borders, nucleocytoplasmic (N:C) ratio, number of nuclei, binucleation, keratinization, tadpole forms, hypochromatism, chromatin pattern, and distribution and discrepancy in N:C maturation and classified the smears into different classes from 0 to 5. They considered class 1 and 2 as negative for malignancy and class 3-5 as positive for malignancy. [17]

Singh A and Kabiraj A et al used Papanicolaou’s classification which graded the smears as normal, atypical, intermediate, suspicious for cancer and malignant. [16,18] Kabiraj A et al examined the cytosmears obtained from OPMDs and found most of the smears had cytological features corresponding to class II i.e., atypical having nuclear and cellular pleomorphism, cellular irregularity, free nuclei, perinuclear halo, intracellular and intracytoplasmic vacuolization and bacterial colonies. [18] In our study the novel cytological grading system of Afrogheh A was adopted [9]. This grading system included nuclear and cytoplasmic features and classified as Grade A to G.

On analyzing, we observed that benign lesions showed cytological features of grade A and B while premalignant lesions showed features corroborating to grade C and D and malignant lesions mainly had cytological features of grade E and F. A similar study on oral lesions reported benign and reactive lesion to have cytological grade of A and B, atypical lesions showing the cytological features of grade C and D while carcinoma in situ and invasive squamous cell carcinoma correlating with features of grade E and F. [15] Precancerous oral lesions can be underdiagnosed as benign lesion or sometimes erroneously be over diagnosed as malignancy on cytology as was observed in our study. Gupta S et al, in their study reported that oral precancerous lesions like leukoplakia and oral submucous fibrosis (OSMF) with benign cytological features were categorized as Benign/negative for atypical cell, whereas verrucous hyperplasia with proliferative growth pattern was over reported as positive for malignancy on cytology. [15] Similarly, in our study few of the premalignant lesions i.e., verrucous hyperplasia and leukoplakia showed cytological features corresponding to lower grade i.e., Grade B. They were falsely categorized as benign on cytology due to their mild subtle changes in the nuclear characteristics with no dysplastic change whereas two cases of oral proliferative leukoplakia in the present study showed high grade changes on cytology and was categorized as malignancy.

Cytological scoring: A novel cytological scoring system for oral cytology was proposed by Afrogheh A et al in their study as an aid to correctly classify the oral lesions and to distinguish between reactive/low grade lesions from high grade/invasive squamous cell carcinoma. The scoring system was based on nine cytologic characteristics with no dysplastic change whereas two cases of oral proliferative leukoplakia in the present study showed high grade changes on cytology and was categorized as malignancy.

The utility of the novel cytological grading and scoring system of Afrogheh et al was assessed by comparing with the histopathological diagnosis, using statistical methodologies. Significant association was found between the cytological grading and histopathological subtypes with p value <0.0001, compromising a standardized approach towards reporting of oral cytology. The above mentioned nine characteristic features on cytological scoring, reported as either present or absent, made
it easier to classify the oral lesions, thus limiting the bias of interobserver or intraobserver variability. We analyzed the association of cytotological score with histopathological subtypes and found that to be statistically significant with p value of <0.0001 which is consistent with findings seen in a similar study. [9] Cytological scoring also showed a high sensitivity (95%) and specificity (96%) in diagnosing the lesions correctly on cytology and corroborated well with histopathological diagnosis. [9] Overall reproducibility and characterization of these lesions on cytology is thus reliable and can help patient and clinician in early diagnosis and intervention accordingly.

Cytomorphometry is the quantitative measurement by which size of the nucleus and cell is measured and helps to determine whether the cell is undergoing malignant transformation. In literature various studies have used this technique and describe it as a reliable indicator of malignant changes that includes reduction in cellular diameter and an increase in nuclear diameter. [13,19] Nuclear and cellular parameters like nuclear area (NA), nuclear perimeter (NP), nuclear diameter (ND), cellular area (CA), cellular perimeter (CP), cellular diameter (CD) and nuclear-cytoplasmic ratio (N/C ratio) were measured in this study on 50 cells of each cytological smear. It is an objective method and a useful adjunct to the cytolological categorization of oral lesions for better reproducibility of reporting. Many workers have studied oral lesions using cytomorphometry to distinguish between normal mucosal cells, premalignant & malignant lesions. [15,20,21] The morphometrical parameters of benign, premalignant and malignant lesions in our study were tabulated & compared. We observed that as the lesion moves from the spectrum of benign to malignant, the nuclear parameters increased in size while an opposite trend was seen with cytoplasm as it becomes dense and scant in malignancy showing a decrease in size of cellular parameters. Mean nuclear area showed an increasing trend from benign to malignant lesions i.e., 65.48 ± 9.56 to 173.65 ± 40.18. A decreasing trend was seen in mean cellular area from benign (1432.25 ± 753.36) to malignant lesion (477.43 ± 165.67). These findings were consistent with the cytomorphometrical study conducted by Udayshankar U et al in tobacco users to diagnose OSCC. CA was found to decrease in malignant group when compared to control group (2838 ± 275.2 to 2762.1 ± 511.4) whereas NA increased in malignant group (83.88 ± 9.86 to 106.19 ± 13.45). [21] We observed that the association between the cytomorphometrical parameters of benign, premalignant and malignant categories with histological subtypes (benign, premalignant and malignant) was statistically significant with p value <0.0001 which is consistent with the study conducted by previous workers. [15,20]

Conventional exfoliative cytology in conjunction with computer assisted cytomorphometry is a useful diagnostic tool for distinguishing benign from premalignant and malignant lesions. Studies have shown sensitivity and specificity of oral exfoliative cytology ranging from 73.8% to 100%. [22, 23] Navone R reported the sensitivity of 86.5% and specificity of 94.3% for conventional oral cytology. [23] In our study, sensitivity of cytology when compared to gold standard histopathology in predicting malignancy was 100% with a specificity of 94.74%. Positive predictive value was 93.75% while negative predictive value was 100% confirming that no malignant lesion was missed on cytological examination.

**Conclusion:** Oral exfoliative cytology, with a proper sample collection technique, is promising as a useful modality in the early diagnosis of oral lesions, across the entire spectrum of benign, premalignant and malignant lesions. In conjunction with cytomorphometry, the utility of exfoliative cytology is further refined and is beneficial in monitoring the premalignant and malignant transformations in these cases. When applied in a hospital setting, in addition it will help to guide management strategies in various categories of oral lesions.

**References:**


