Nuclear pleomorphism-based cytopathological grading in human oral neoplasm

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Abstract: Objective — The objective of the present study is to record the nuclear pleomorphism in various stages of oral carcinogenesis and to analyse their utility in cytopathological grading for early detection of human oral cancer. Material and Methods — In this hospital based case-control study, oral site, age-group and sex-matched 272 subjects (136 cases and 136 normal healthy individuals) were included. Scraped exfoliated cytosmears were collected from the affected oral site of the subjects and smearing was done in the pre-cleaned-coded glass-slides. Two such slides were prepared from each subject. The cytosmears were immediately fixed in aceto-alcohol (1 part of glacial acetic acid: 3 part of absolute ethyl alcohol) fixative. One set of the slide was stained with Papanicolaou’s stain and the other set was counter-stained with Giemsa’s Solution for cytopathological analysis. Test of proportion (z-test) was followed and the critical ratio (z-value) was calculated for the test of significance. Results — Nuclear pleomorphism in the form of round, oval, spindle, elongated fiber as well as irregular shapes were mostly observed in oral squamous cells during different stages of carcinogenesis. Appearance of such nuclear pleomorphism in human oral neoplasm may be considered as a sign of cellular alternation in general and index of oral carcinogenesis in particular. In the present study, the frank malignant cases mimic to be either premalignant lesions or benign/carcinoma in situ were detected on the basis of nuclear pleomorphism-based cytopathological grading and so an increasing trend was observed from precancerous lesions to malignant cases due to shifting of numbers. Diagnostic tests also indicated that the Sensitivity was calculated to be 83.5%, Specificity was 100%, positive predictive value (PPV) was 100%, negative predictive value (NPV) was 30% and the accuracy was found to be 84.6%. Therefore, the nuclear pleomorphism-based cytopathological grading system makes itself an ideal screening test for early detection of human oral cancer. Conclusion — Pattern of nuclear pleomorphism corresponding to various cytological atypias is a common feature observed during different stages of oral carcinogenesis and thus, it has a practical implication in grading and early detection of oral cancer.

Keywords: nuclear pleomorphism, cytological atypias, cytopathological grading, oral neoplasm


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Introduction

Oral cancer is a long-latency multi-stage pathological event. A progressive gross clinical features in the form of leukoplakia, erythroplakia, benign and malignant tumours are characterized by pleomorphic cytological alteration and a number of nuclear anomalies. Being nucleus is the genetic store-house of the cell [1], any alteration at nuclear level leads to nuclear anomalies in general and the genetic components in particular. Ultimately, the deformity of nucleus has been observed in the cytological atypias during oral carcinogenesis which lead to nuclear pleomorphism. Nuclear pleomorphism coupled with changes in chromatin amount and distribution in the nucleus, remain the basic microscopic criteria for a cytologic diagnosis of cancer. Moreover, in several cancer types, e.g. in breast cancer, cervical cancer nuclear pleomorphism is graded and correlates with clinical aggressiveness and patient outcome [2-4]. Bussolati et al. (2008) have stated that evaluation of nuclear pleomorphism represents a novel parameter of interest in pathological staging and grading [5].

Wang et al. (2015) have pointed out that several nuclear membrane proteins such as emerin, lamin A/C, lamin B, and lamin-associated polypeptide 2 (LAP2) are the architectural components of the nuclear membrane which play an important role in maintaining nuclear structure and coordinating cell activity [6]. Various workers have reported that altered lamin expression or localization and disrupted stoichiometry between A- and B-type lamins can change the elastic properties of the nuclear envelope (NE), which renders it unable to withstand cytoskeleton- and chromosome-based forces. Consequently, these lead to alteration of nuclear morphology followed by nuclear pleomorphism which results in an inheritable disease called laminopathy. Therefore the nuclear lamina alterations might directly account for the cancer-related changes in the nuclear morphology [7-13].

Gadiwan et al. (2014) have reported that nuclear morphology reflects the biological potential and proliferative activity of the cell. They have also confirmed that the nuclear morphometry forms a reliable and reproducible tool that provides an
opportunity to quantify the nuclear changes associated with dysplasia and affords an objective basis for grading dysplasia to predict their malignant potential [14].

Although different histologic grading systems such as Broder’s, Anneroth’s, Bryne’s and Jakobsson’s grading systems are used, cytopathology has been used as a primary tool for screening of oral squamous cell carcinoma (OSCC) for many decades. Ignoring invasive punch biopsies for histopathology, the patients usually prefer to non-invasive exfoliative cytology for oral cancer detection and diagnosis. Modified version of exfoliative cytology like fine needle aspiration cytology (FNAC), brush cytology have also been extensively used for the stated purpose and also used as an adjunct to histopathology [15].

It is important to note that both cytology and histopathology are nucleo-centric. On the basis of architectural configuration of the nucleus, the real state of the concerned cell can be determined. Least number of papers has been published on nuclear pleomorphism, to date, so far as human OSCC is concerned. Furthermore, works on nuclear pleomorphism-based grading system are very rare, inadequate and confined to the micronucleus assay only. In this regard, credit goes to Palve and Tupkari (2008) who have found that the percentage of micronuclei was uniformly elevated in all histologic grades of OSCC, suggesting a strong cytogenetic damage of the oral epithelium [16]. Recently, Namala et al. (2016) have studied on micronuclei frequency and reported that there was 60% correlation between the cytological grade and histological grade and the difference between them was found to be insignificant. They have also concluded that cytological grading can be used as an alternative to histological grading in grading of OSCC [17]. Therefore, a humble attempt was undertaken to record a broad spectrum of the nuclear pleomorphism in various stages of oral carcinogenesis, among addicted and non-addicted groups and to analyse their utility in cytopathological grading for early detection of human oral cancer.

Material and Methods

Subjects

In a hospital-based study, out of 136 oral cases, 82 (60.3%) were males and 54 (39.7%) were females registered at the Out-patient Department (OPD) of Acharya Harihar Regional Cancer Centre (AHRCC), Cuttack, Odisha during May 2007 to May 2009 were included in this study. A written consent from the patient was obtained in our self-designed proforma as well as the detailed case-history (including the nature and types of addiction) of each individual was recorded prior to the collection of samples. Considering the nature of addiction, out of 136 cancer-affected individuals, 126 (92.6%) were addicted to different forms of tobacco and alcohol for more than 15 years and 10 (7.4%) were non-addicted.

Out of 82 males, 33 (40.3%) patients belong to 30-49 years, 34 (41.5%) were between 50-69 years and 15 (18.3%) patients were under 70-89 years. Out of 54 females, 11 (20.4%) were between 30-49 years, 36 (66.6%) were grouped under 50-69 years and 7 (13.7%) belong to 70-89 years of age group.

Age-group and sex-matched 136 non-addicted healthy individuals were also included in this study as Control group. Thus, a total of 272 subjects were taken into account for this study.

Collection of samples and staining

Prior to the collection of sample, written consent of the respective subject was obtained. Two scalpels -scraped exfoliated cytosemms were collected from the affected site of the oral cavity on the pre cleaned-coded glass-slides. Collected cytosemms were fixed in 1:3 aceto-alcohols (1 part of glacial acetic acid and 3 parts of ethyl alcohol) immediately. A set of smears was stained with Papanicolaou’s stain and the other set was counterstained with Giemsa’s stain for cytological analysis. Photomicrographs were taken out as the supporting evidences by using computer assisted Bio-Catalyst Cat Cam 1.30 microscope camera (Manufacturer: Catalyst Biotech, Maharashtra, India).

Statistical analysis

Out of 1,000 observed cells, nuclear pleomorphism among the various cytological atypias were recorded. Microsoft Excel as well as Software package PAlaentological STatistics (PAST), Version 2.17 was used for statistical analysis. Test of proportion (z-test) was followed and the critical ratios (z-values) were calculated for the test of significance.

Ethical considerations

This study was approved by the Subject Research Committee (SRC) of Utkal University, Bhubaneshwar, Odisha, India and necessary permission from the Director, AHRCC, Cuttack, Odisha, India was also obtained for the same purpose.

Results

Oral sites

According to International Classification of Diseases-10th Revisited Edition (ICD-10), six oral sites (lip, tongue, alveolus and gingiva, floor of the mouth, palate and buccal mucosa) are the cancer-prone sites. On the basis of ICD-10, out of 136 oral cases, highest numbers of cases (45.2% males and 48.2% females) were suffering from cancer at buccal mucosa and the lowest numbers of cases (7.3% males and 35.6% females) were recorded as palatal cancer (Table 1).

Degree of pathogenicity

Basing on the degree of gross clinical pathogenicity, 15 (18.3%) males and 14 (25.9%) females were of leukoplakia (21.3%), 17 (20.7%) males and 9(16.7%) females were of erythroplakia, 35 (42.7%) males and 20 (37.0%) females were of benign (40.5%) cases and 15 (18.3%) males and 11 (20.4%) females were of malignant (19.1%) cases (Figure 1).

Nuclear pleomorphism

A progressive gross clinical features in the form of leukoplakia, erythroplakia, benign and malignant tumors has been characterized by pleomorphic cytological alteration. Being nucleus is the genetic store-house of the cell, any alteration at nuclear level leads to nuclear anomalies in general and the genetic components in particular. Therefore, nuclear morphology and nature of chromasia provides the cytopathologists with most of information necessary to make a correct diagnosis.
Table 1. Site and sex-wise collected samples

<table>
<thead>
<tr>
<th>No</th>
<th>Sites</th>
<th>Control group (n=136)</th>
<th>Cancer affected group (n=136)</th>
<th>Grand Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
</tr>
<tr>
<td>1</td>
<td>Lip</td>
<td>5 (6.1)</td>
<td>6 (11.1)</td>
<td>11 (8.0)</td>
</tr>
<tr>
<td>2</td>
<td>Tongue</td>
<td>11 (13.4)</td>
<td>7 (12.9)</td>
<td>18 (13.2)</td>
</tr>
<tr>
<td>3</td>
<td>Alveolus and gingiva</td>
<td>16 (19.5)</td>
<td>6 (11.1)</td>
<td>22 (16.2)</td>
</tr>
<tr>
<td>4</td>
<td>Floor of the mouth</td>
<td>7 (8.5)</td>
<td>6 (11.1)</td>
<td>23 (9.6)</td>
</tr>
<tr>
<td>5</td>
<td>Palate</td>
<td>6 (7.3)</td>
<td>3 (5.6)</td>
<td>9 (6.7)</td>
</tr>
<tr>
<td>6</td>
<td>Buccal mucosa</td>
<td>37 (45.2)</td>
<td>26 (48.2)</td>
<td>63 (46.3)</td>
</tr>
<tr>
<td></td>
<td>All sites</td>
<td>82</td>
<td>54</td>
<td>136</td>
</tr>
</tbody>
</table>

Data presented as number and percentage (from all sites) – no (%).

Figure 1. Sex-wise degree of oral pathogenicity

Figure 2. Nuclear pleomorphism in detected oral cytological atypias: a) NOSC; b) PKSCs and KSC; c) an MNC with a number of micronuclei; d) KTCs, KFC and KRC; e) KTCs, KRC and PKSC; f) KSC-A; g) a bundle of KFCs; h) NMSCs with a number of amitoses.

Figure 3. Age and sex-wise percentage of cytological atypias in control and cancer affected groups

On the basis of their morphological peculiarities and nature of keratinization, a number of pleomorphic cytological atypias were observed in various stages of oral carcinogenesis [18]. In the exfoliated cytosmear of Control group, the cells were mostly normal oral squamous cells (NOSC), few Plump keratinized squamous cells (PKSC) [19] and rarely Micronucleated cells (MNC) were found. The nuclear morphology appears to be either round or oval in shape. In precancerous lesions, as in leukoplakia stage, more numbers of PKSCs and MNCs were scored than the Control group. It has been observed that number of MNCs were observed to be in increasing order with increase in degree of pathogenicity where as the number of PKSCs were found to be in decreasing order gradually [20]. In some of the leukoplakia cases few Keratinized spindle cells (KSC) [21] and Keratinized tadpole cells (KTC) [22] were also observed. Similarly, in erythroplakia stage, few more cytological atypias, like Keratinized tadpole cells (KTC) and Keratinized strap (Anitschkow) cells (KSC-A) [23] were found. In these atypias, the nuclei were observed to be round, oval, elliptical or ribbon like strapped or flattened. In addition to these, Keratinized fibre cells (KFC) [24] and keratinized round cells (KRC) [25] were rarely found among erythro-leukoplakia, but frequently observed in benign / carcinoma in situ (CiS) and malignant neoplasm cases. In KFCs, the nuclei were drastically modified and found to be fibre like elongated. The nuclear-cytoplasmic (N/C) ratios in these cytological atypias were previously reported to be in increased state in comparison to their normal counter-part [18-25].

A unique type of atypia named as Non-keratinized malignant squamous cells (NMSC) was observed in the samples of frank malignant neoplasm cases. These atypias were mostly either round or oval in shape, very small in size and absolutely non-keratinized due to lack of cytoplasm.
<table>
<thead>
<tr>
<th>No</th>
<th>Groups</th>
<th>Age groups, years</th>
<th>Number of samples scored</th>
<th>Number of atypical cells scored</th>
<th>Percentage of atypical cells</th>
<th>Mean percentage of atypical cells</th>
<th>Critical ratio (z-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>30-49</td>
<td>33</td>
<td>11</td>
<td>445</td>
<td>163</td>
<td>1.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50-69</td>
<td>34</td>
<td>36</td>
<td>537</td>
<td>552</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70-89</td>
<td>13</td>
<td>07</td>
<td>271</td>
<td>110</td>
<td>1.81</td>
</tr>
<tr>
<td>2</td>
<td>Pre-cancerous</td>
<td>30-49</td>
<td>5</td>
<td>5</td>
<td>1,722</td>
<td>1,036</td>
<td>21.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50-69</td>
<td>18</td>
<td>14</td>
<td>4,080</td>
<td>2,967</td>
<td>22.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70-89</td>
<td>6</td>
<td>4</td>
<td>1,546</td>
<td>883</td>
<td>25.77</td>
</tr>
<tr>
<td>3</td>
<td>Cancerous</td>
<td>30-49</td>
<td>25</td>
<td>4</td>
<td>1,106</td>
<td>2,408</td>
<td>46.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50-69</td>
<td>16</td>
<td>22</td>
<td>7,897</td>
<td>10,906</td>
<td>47.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70-89</td>
<td>9</td>
<td>3</td>
<td>4,592</td>
<td>2,558</td>
<td>51.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30-89</td>
<td>82</td>
<td>54</td>
<td>31,443</td>
<td>20,746</td>
<td>38.35</td>
</tr>
</tbody>
</table>

* Significant differences (p<0.01) from Control group.

Mohanata et al. (2016) have reported that the small, round or oval nucleus of the NMSC become enlarged and touched the cell boundary (plasma membrane) thereby the nuclear-cytoplasmic (N/C) ratio was found to be 1:1 [26]. They have also reported that except the NOSC and NMSCs, all these atypias get keratinized and appear to be either orange or yellow-orange in color due to acidic (eosinophilic) cytoplasm with Papanicolaou’s stain. Frequent amitoses were observed in these cytological atypias (Figure 2).

The cytospin in various atypias appears to be dense, and keratinized. Tremendous reactive changes were observed during different stages of oral carcinogenesis. From the numerical point of view, the percentages of atypical cells were calculated to be 1.35, 1.56, 1.81 in males and 1.48, 1.53, 1.57 in females in 30-49, 50-69 and 70-89 years in Control group. Thus, the mean percentages of atypical cells were recorded to be 1.52 in male and 1.527 in female. In premenopausal group, the percentage of atypical cells were found to be 21.53, 22.67 and 25.77 in male, whereas in female, these were 20.72, 21.19 and 22.08 in 30-49, 50-69 and 70-89 years of age groups, respectively. The mean percentages of atypical cells were found to be 22.96 in male and 21.24 in female in precancerous group. In cancerous group, the percentages of atypia were 46.42 in male and 40.10 in female respectively in the age group 30-49 years, 49.36 in male and 49.57 in female in the group of 50-69 years and 51.02 in male and 86.27 in female in 70-89 year of age group (Figure 3). The mean percentages of atypical cells were calculated to be 48.19 in male and 51.29 in female in cancerous group. In both precancerous and cancerous groups, the z-values in males and females were observed to be significantly (p<0.01) higher than the normal observed value, where z =2.58 (Table 2).
Table 4. Nuclear pleomorphism-based cytopathological grading in multi-stage oral carcinogenesis

<table>
<thead>
<tr>
<th>No</th>
<th>Groups</th>
<th>Principal Atypias</th>
<th>Nuclear pleomorphism</th>
<th>Number of cases, no. (%)</th>
<th>CPG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>NOSC, PKSC, MNC</td>
<td>Round, Oval, Regular</td>
<td>82 (100)</td>
<td>54 (100)</td>
</tr>
<tr>
<td>2</td>
<td>Leukoplakia</td>
<td>PKSC, MNC, KSC</td>
<td>Round, Oval, Elliptical, Spindle</td>
<td>5 (6.1)</td>
<td>4 (7.4)</td>
</tr>
<tr>
<td>3</td>
<td>Erythroplakia</td>
<td>PKSC, MNC, KSC-A, KTC, KRC</td>
<td>Round, Oval, Elliptical, Spindle, Elongated flat</td>
<td>12 (14.6)</td>
<td>9 (16.7)</td>
</tr>
<tr>
<td>4</td>
<td>Benign</td>
<td>PKSC, MNC, KSC-A, KTC, KRC, KFC</td>
<td>Round, Oval, Elliptical, Spindle, Elongated flat/Fibre</td>
<td>26 (31.7)</td>
<td>18 (33.3)</td>
</tr>
<tr>
<td>5</td>
<td>Malignant</td>
<td>PKSC, KSC, MNC, KSC-A, KTC, KRC, KFC, NMSC</td>
<td>Round, Oval, Elliptical, Spindle, Elongated flat/Fiber, Regular</td>
<td>39 (47.6)</td>
<td>23 (42.6)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td>82 (100)</td>
<td>54 (100)</td>
</tr>
</tbody>
</table>

Data in parentheses indicate relative percentage with respect to number of cases. CPG, cyto-pathological grading.

Figure 4. Age and sex-wise frequency of cytopathological atypias in control, addicted and non-addicted cancer affected groups

Among the addicted groups, the lowest percentages of atypical cells were recorded to be 20.90, 21.40 and 32.23 in males and 20.73, 23.75 and 33.65 in females in alcoholic group in the age group of 30-49 and 70-89 years, respectively. The percentages of cytopathological atypias in chewers-smokers-alcoholics group were recorded to be the highest and were found to be 2-4 folds to that of alcoholic group. In chewers-smokers-alcoholics group, the percentages of atypical cells are recorded to be 64.15, 51.6 and 53.50 in males in 30-49, 50-69 and 70-89 years of age groups, respectively. No female patients were recorded in the age group of 30-49 and 70-89 years. However, in 50-69 years, the percentage was calculated to be 56.81. In non-addicted cancerous group, the percentages of cytological atypias was recorded to be 42.13 in male and 20.70 in females, in the age group of 30-49 years, 45.20 in males and 27.95 in females in the age group of 50-69 years, 49.60 in male and 43.40 in females in 70-89 years, respectively (Figure 4). The lowest mean percentage of cytological atypias were recorded to be 23.43 in male and 23.20 in female in Smokers group and the highest were found to be 58.11 in male and 56.81 in female in multi-addicted Chewer-smoker-alcoholics group. The test of proportions also clearly indicates that the z-values in each group are significantly (p<0.01) higher than the tabulated values, where z=2.576 (Table 3).

It was also observed that there was an increase in the percentage of atypias from lower to higher age group in both sexes in Control and cancer affected groups. On the other hand, a higher percentage of cytological atypias were marked in 30-49 years followed by 70-89 and 50-69 years in addicted groups. This was probably due to the multi-carcinogenic effect of tobacco and alcohol on the buccal mucosa of younger generation.

In this case-control study, the number of cytological atypias followed by nuclear pleomorphism was found to be in gradual increasing order from Control/Normal group to malignant group. The frequency of atypias and nuclear anomalies were also observed to be in increased state from lower (30-49 years) to higher age (60-79 years) groups with increase in degree of pathogenicity. It has also been found that more number of nuclear pleomorphism in multi-addicted (chewer-smoker-alcoholic, chewer-smoker) groups than the single addicted (chewers, smokers, alcoholics) groups. Interestingly, least number of pleomorphism was recorded among smoker and alcoholic groups. It is important to note that the frequencies of cytological atypias were recorded to be more in non-addicted cancerous group than the single-addicted smoker- and alcoholic groups which demands further investigation.

**Cytopathological grading**

Based on nuclear pleomorphism, cytopathological grading was done. Cytological atypias such as PKSCs and MNCs with round or oval nuclei were observed to be well differentiated and morphologically resembled with the NOSCs. Therefore, both PKSCs and MNCs were kept under Grade 0. Moderately differentiated atypias including more MNCs and KSCs if found in the exfoliated cytosmears were of Grade I and more atypias, particularly KTCs and KSC-A if observed, were kept under Grade II. Similarly, cytosmears with KFCs and KRCs along with other atypi as were done. Cytological atypias such as PKSC and MNC are well differentiated; KSC, KSC-A, KTC, KRC and KFC are moderately differentiated whereas NMSCs are absolutely poorly differentiated cytopathological atypias. As a result, this novel cytopathological grading, partially corroborates with the Broder’s grading system of cytological differentiation [27].
On the basis of such cytopathological grading, all the normal healthy individuals (82 male and 54 female) were included in Grade 0. But, drastic alteration in number of cases with respect to degree of pathogenicity was observed due to nuclear pleomorphism-based cytopathological grading. More number of cases were included in malignant group/ Grade IV (45.6%) who were earlier included in the premalignant lesion groups (Leukoplakia/ Grade I-6.6% and Erythroplakia/ Grade II-15.4%) and benign group/Grade III (32.4%) respectively (Figure 5). In other words, cases in lower groups with higher malignant potentiality were shifted to the malignant group leading to increase the mean percentage from 19.1% to 45.6% (Figure 6).

**Table 5. Status of oral malignancy potential with respect to cytopathological grading**

<table>
<thead>
<tr>
<th>Disease status</th>
<th>Malignant</th>
<th>Non-malignant</th>
<th>Row Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant</td>
<td>TP = 106</td>
<td>FP = 0</td>
<td>TP+FP = 106</td>
</tr>
<tr>
<td>Non-malignant</td>
<td>FN = 21</td>
<td>TN = 9</td>
<td>FN+TN = 30</td>
</tr>
<tr>
<td>Column Total</td>
<td>TP+FN = 127</td>
<td>FP+TN = 9</td>
<td>TP+FN+TP+FP+TN = 136</td>
</tr>
</tbody>
</table>

TP, true positive cases; FN, false negative cases; FP, false positive cases; TN, true negative cases.

**Table 6. Diagnostic tests for cytopathological grading**

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Calculating formula</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity, %</td>
<td>TP/ (TP+FN)</td>
<td>83.5</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>TN/ (FP+TN)</td>
<td>100</td>
</tr>
<tr>
<td>PPV, cu</td>
<td>TP/ (TP+FP)</td>
<td>100</td>
</tr>
<tr>
<td>NPV, cu</td>
<td>TN/ (FN+TN)</td>
<td>30.0</td>
</tr>
<tr>
<td>Accuracy</td>
<td>(TN + TP)/ (TN+TP+FN+FP)</td>
<td>84.6</td>
</tr>
</tbody>
</table>

TP, true positive cases; FN, false negative cases; FP, false positive cases; TN, true negative cases; PPV, positive predictive value; NPV, negative predictive value.

**Sensitivity, specificity and accuracy of the diagnostic test**

Considering the malignancy potential, 106 cases were found to be true positive (TP) and 9 cases were of true negative (TN). No cases were recorded to be false positive (FP), whereas, false negative (FN) cases were recorded to be 21 in number (Table 5). Therefore, the Sensitivity was calculated to be 83.5%, Specificity was 100%, Positive Predictive Value (PPV) was 100% and Negative Predictive Value (NPV) was 30%. Ultimately, the accuracy of our diagnostic test was calculated to be 84.6% (Table 6, Figure 7).

**Discussion**

Oral cancers are malignant neoplasms that affect the tissues of the mouth. Eventually, the multi-stage transformation of normal oral mucosal cell to a malignant one has been attributed to the consumption of tobacco and alcohol, some of the biological agents (oral lichen planus, Epstein-Barr virus, human papilloma virus), poor oral hygiene, age, gender, familial pre-disposition, changing food pattern, diet and modern life style [29].

The biology of OSCC has been evaluated and descriptively categorized as highly, moderately and poorly differentiated. Broder primarily developed this quantitative grading of cancer in 1920. Lack of correlation between Broders’ grades and the prognosis of OSCC has been explained by the fact that SCCs usually exhibit a heterogeneous cell population with probable differences in invasiveness and metastasis behaviour [30]. Subsequently, multi-factorial malignancy grading systems were developed by Jakobsson et al. (1973) and further improved by Anneroth and Hansen (1986) for application to SCCs in the tongue and the floor of the mouth [31,32]. Histological grading based on Broder’s classification has been a traditional pathologic tool with documented prognostic value, but has not been incorporated into standard therapeutic planning strategies. This is mainly due to the subjectivity of the current grading system and the lack of consensus regarding its prognostic value [33]. Extensive scoring methods have been designed in an attempt to increase the objectivity of these parameters [34-37]. Other ancillary parameters, such as mitotic index, DNA content, Ki-67, proliferation cell nuclear antigen, and bromodeoxyuridine labelling index, may be used in an attempt to identify new prognostic indicators [38, 39]. But, due to financial constraints, availability of advanced parameters along with advanced technology for carrying...
Variable size, shapes and sharp pointed projections in neoplastic cells. Furthermore, they have reported that lower grades of OSCC showed delicate chromatin strands and homogenous chromatin pattern while higher grades exhibited coarse, clumped and heterogeneous chromatin pattern. Normal inconspicuous, regular nucleoli in normal cells turned into very prominent, enlarged, irregular and more in number, with occasional sharp pointed projections in malignant cells which may be attributed to increased protein synthesis [47]. Abnormal mitoses were also frequently noticed and found to be more in higher grades of carcinoma than the lower grades which corroborates with the findings of Francois et al. Bignold (2003) has reported that genetic instability is an early and essential part of tumour development. This instability provides for substantially random cell-to-cell genomic variation (genomic heterogeneity) to arise among cells of individual tumours. Genetically unstable cells then produce ‘successful’ clones of cells with the necessary mutations for malignant transformation [48]. Chewing and smoking of tobacco as well as drinking of alcohol have been reported as the risk factors for oral cancer. Chewing areca quid generates reactive oxygen species (ROS) that might cause oxidative DNA damage to surrounding tissues in the oral cavity [49]. The production and release of ROS occurs under alkaline condition, during the auto-oxidation of areca-nut polyphenols in the saliva of betel-quid-chewers [50]. Prokopczyk et al. (1987) have opined that areca-nut-specific-nitrosamines (ASNA) along with tobacco-specific-nitrosamines (TSNA) are found to be mutagenic, genotoxic, and carcinogenic in nature and are capable of inducing tumors in the oral cavity [51]. The areca nut extract can also impair actin organization that causes fibroblastoid morphologic changes of oral keratinocytes [52]. The genetic susceptibility to such environmental carcinogens and the resulting altered molecular expressions might be potential markers for a diagnosis and prognosis of OSCC [53]. Mohanta et al. (2013) have reported that there has been a detrimental genotoxic effect of tobacco and alcohol on oral mucosa. They have found that chewing and smoking of tobacco as well as drinking of alcohol enhance the rate of formation of micronuclei in MNC along with other cytological atypias followed by OSCC [54]. The combined effects of tobacco abuse and alcohol consumption are found to be multiplicative. Compared with persons who neither drink nor smoke, the risk of developing OSCC is increased 80 fold in persons with the highest level of smoking and alcohol consumption [55]. To be addicted is now become a fashion, a part of modern life style. In our study, out of 136 cancer affected individuals, 126 (92.6%) were addicted to different forms of tobacco and alcohol for more than 15 years and 10 (7.4%) were non-addicted. The frequency of cytological atypias along with nuclear pleomorphism was recorded to be more in multi-addicted (chewer-smoker-alcoholic, chewer-smoker) groups than the single addicted (chewer, smoker, alcoholic) groups. Interestingly, least number of pleomorphism was recorded among smoker and alcoholic groups. Really, the combined effects of tobacco and alcohol trigger genotoxicity followed by nuclear pleomorphism in oral squamous cells. Ultimately, cellular alteration takes place in the respective oral site which accelerates the multi-stage mechanism of oral carcinogenesis. Different diagnostic methods such as routine exfoliative cytology, fine needle aspiration cytology (FNAC), histopathology, and immunohistochemistry are available today. Out of these, exfoliative cytology is, particularly, valuable for...
mass screening of oral carcinoma. Evaluating the quality of cytology as a diagnostic method for OSCC, Fonte et al. (2013) have reported that the sensitivity was 83.1%, the specificity was 100%, the positive predictive value (PPV) was 100%, the negative predictive value (NPV) was 49% and the accuracy was 85.5% [56]. Recently, compared with cytopathological and histopathological diagnoses, Hafez et al. (2014) have also found that the sensitivity was 93.5%; specificity was 96.2%; PPV was 97.7%; NPV was 89.3% with a diagnostic accuracy of 94.4%. According to them, FNAC was found to be highly accurate in the diagnosis of oral lesions. Detailed cytomorphologic examination coupled with clinical data and appropriate immuno-cytoschemical study, in some cases, can lead to an accurate diagnosis of the oral cavity lesions [57]. In the present study, the sensitivity was calculated to be 83.46%, specificity was 100%, PPV was 100%, NPV was 30% and the accuracy of the diagnostic test was found to be 84.55% which corroborates with the findings of Fonte et al. and Hafez et al.

The presence of typically atypical cells may be correlated with tumour progression. The infiltrating macrophage count was correlated with the progression of OSCC and is a prognostic marker [58, 59]. Cytomorphological alterations indicate the pathological status of the concerned cell. Alizadeh et al. (2016) have reported that shape differences are sufficient to enable a neural network to classify cells accurately as belonging to the highly invasive or the less invasive phenotype. The patterns of shape changes were also reproducible for repetitions of the experiment. They have also strongly suggested that cell-shape may provide a means to read out the phenotypic state of some cell types, and shape analysis can be usefully performed using a Zernike moment representation [60].

In the present study, the broad spectra of detected cytological atypias not only exhibit cytological pleomorphism but also nuclear pleomorphism in different stages of oral carcinogenesis. Thus, nuclear pleomorphism may be considered as a sign of cellular alternation and index of oral carcinogenesis.

Early detection of oral cancer is not an easy task. Most of the malignant neoplasms mimic to be either precancerous lesions or benign ones and vice versa leading to delay in diagnosis and treatment. It is the nucleus that expresses the genotypic changes in individual cells. Our present findings clearly depict the importance of nuclear pleomorphism in the respective cytological atypias in grading of human oral neoplasm and thereby drawing out a simple solution for such difficult diagnostic dilemma in general and early detection of oral cancer in particular.

Conclusion

Nucleus reflects biological potential and general activity of the cell. Being the genetic store house of the cell, any alternation either at gross level or at molecular level, the nucleus and its constituents become reactive and exhibit pleomorphism. Alteration may be due to the induction of mutagenic, genotoxic or carcinogenic agents, nuclear pleomorphism is a real fact. Appearance of nuclear pleomorphism in human oral neoplasm may be considered as a sign of cellular alternation in general and index of oral carcinogenesis in particular. In the present study, the frank malignant cases mimic to be either premalignant lesions or benign/carcinoma in situ were detected on the basis of nuclear pleomorphism-based cytopathological grading and so an increasing trend was observed from precancerous lesions to malignant cases due to shifting of numbers. Diagnostic tests also indicated that the Sensitivity was calculated to be 83.46%. Specificity was 100%, PPV was 100%, NPV was 30% and the accuracy was found to be 84.55%. Therefore, the nuclear pleomorphism based cytopathological grading system makes itself an ideal screening test for early detection of oral cancer.

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