INTRODUCTION

Oral cancer ranks eighth in the cancer incidence ranking worldwide. The World Health Organization anticipates a worldwide escalation in the incidence of oral squamous cell carcinomas (OSCC) in the subsequent decades [1]. Despite the steady improvements in treatment modalities, the 5 year survival rate of OSCC is about 55% and it continues to stand poor. This implies the need for early and accurate detection of OSCC in order to bring down the associated high morbidity and mortality rates [2]. Upon its onset, OSCC progresses imperceptibly, becoming evident only in a dangerously advanced state. This may be due to patients’ negligence as it initially presents with no remarkable symptoms, or due to inappropriate diagnostic and prognostic assessment on the behalf of the clinician and the pathologist. The latter may be a result of lack of availability or utilization of reliable techniques for the same. The current scenario urges an enhanced comprehension of the tumor behavior.

The intrinsic capacities of limitless replicative potential and self-renewal are characteristics of cancer cells. Uncontrolled proliferation indicates that the cells are capable of acquiring further cellular alterations that contribute to full malignant phenotype. The expression of the human Ki-67 protein is strictly associated with cell proliferation and is present during all active phases of cell cycle (G1, S, G2, and mitosis), but is absent from resting cells (G0). Thus it represents the growth fraction of the tumor and serves as a reliable nuclear marker for cell proliferation in malignancies [3]. Cell proliferation serves as a guide for prognostication of malignancies as a high proliferative activity is associated with a poor prognosis [3].

Histological examination is regarded as the gold standard for identifying and diagnosing OSCC[4]. However, there is wide variation among pathologists regarding the subjective evaluation of histopathological features. This necessitates the use of a more sophisticated technique of computer-assisted morphometry to investigate the cellular and nuclear changes in correlation with the clinical behavior of the lesions. There are many variables observable in microscopic images which are acquiscent to morphometrical analysis and the outcome is more objective, reliable and reproducible[5]. The morphometrical criteria considered in this study are: nuclear area, cell area, nuclear perimeter and cell perimeter.
Fairly extensive research has been conducted on the application of Ki-67 in head and neck cancer establishing its use as a proliferative marker.[2,3,6,7] Morphometry has also been employed in a few studies in relation to the head and neck cancer.[4,5]. However, they have not been studied in conjunction before, for their implication in diagnosis and prognosis of head and neck squamous cell carcinoma. In this study, we attempt to determine the association between the Ki-67 expression and the morphometrical values as a useful adjunct to the routine histopathological examination. Our attempts are also directed to find out whether this association can enable us to use morphometrical values alone as an accurate and cost effective diagnostic tool for the underprivileged Indian oral cancer patients. To the best of our knowledge, such an association has not yet been established in the literature in relation to the head and neck squamous cell carcinoma.

MATERIAL AND METHODS

A retrospective cohort study
Ethics: Institutional ethics committee approval was obtained to carry out the study (IEC 526/2012)
Inclusion criteria: Only the cases which were histologically confirmed cases of OSCC of the buccal- alveolar complex and the floor of the mouth and the patents in whom the treatment (radiotherapy or chemotherapy) had not begun at the time of initial diagnosis were included.

Exclusion criteria: Cases of oral cancer other than oral squamous cell carcinoma, cases of oral squamous cell carcinoma undergoing treatment, cases with history of recurrence of oral squamous carcinoma and cases of oral squamous cell carcinoma with systemic conditions were excluded.

Methodology
A total of 105 formalin fixed paraffin embedded (FFPE) cases of OSCC were studied. 35 cases each of well, moderate and poorly differentiated OSCC of bucco- alveolar mucosal complex and floor of the mouth were studied. The selected cases were diagnosed and treated at the University Hospital from the year 2008 to 2013. 5 tonsil tissue specimens were used as controls along with 5 cases of healthy oral mucosal tissues. Relevant clinicopathologic details including the tumor staging, the histologic grading, and the development of recurrence or metastasis were attained from the medical record files.

Immunohistochemistry
Thin sections of 4µm were cut from FFPE tumor blocks. The section were mounted on amino-propyl-tri-ethoxy- silane (APES) coated glass slides and stained with monoclonal antibody against ki67 (RTU - Ki67 – MM1: Novacastra) using indirect streptavidin-biotin immunoperoxidase technique. Tissue sections obtained from tonsil tissue specimens were taken as positive controls for Ki-67 immunohistochemistry. As a negative control for immunohistochemical procedures the primary antibody was replaced with normal mouse IgG in appropriate concentration[2].

Evaluation of Ki-67 expression: The immunoreactivity of Ki-67 was nuclear. The nuclei with clear brown color, regardless of staining intensity, were regarded as positive. The positive cells of the tumor cells were evaluated in 5 representative fields at 40x magnification. The histological sections with uniform and good intensity staining were assessed. (Figure 1 a,b) The histological section of tonsil tissue stained with Ki-67 was used as positive control and confirmed against a negative control (Figure 2a,b). The ki-67 labelling index was calculated using the formula [8]: (Ki67-positive) / (Ki67-positive + Ki67-negative) x 100

Based on the labeling index, the sections were scored from 1 to 3 for ki-67 expression as follows [9].

<table>
<thead>
<tr>
<th>Ki-67 expression</th>
<th>Extent of proliferation</th>
<th>Percentage of positive cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 High</td>
<td>&gt;50 %</td>
<td></td>
</tr>
<tr>
<td>2 Moderate</td>
<td>30 to 50%</td>
<td></td>
</tr>
<tr>
<td>3 Low</td>
<td>&lt;30%</td>
<td></td>
</tr>
</tbody>
</table>

Morphometry: Tissue sections of 5µm thickness were cut from FFPE tissue blocks, stained with Harris’s Haematoxylin and Eosin and subsequently subjected to morphometric analysis. Only clearly defined cells were measured. The 4 morphometrical parameters considered were: Nuclear area (NA), cell area (CA), nuclear perimeter (NP) and cell perimeter (CP)

The scale for morphometrical analysis was standardized using an eye piece graticule and a stage grid in 40X magnification. For each case, pictures of 3 fields were taken under 40X magnification. Ten clearly defined cells were analyzed from each field. A total of 3150 cells were morphometrically analyzed. Image analysis was done using – “Image J 1.34 software”[10] available at website: http://rsb.info.nih.gov/ij/

In order to assess the slides in image J, the images were captured onto the hard drive of the computer, following which they can be opened in Image J for evaluation, using the various tools provided in the panel [8,11].

Statistical analysis: SPSS (Statistical Package for Social Sciences) version 16.0 for windows was used. P-value (p) <0.05 was considered significant for all statistical analysis.

- Inter-class correlation (ICC) carried out for inter-observer reproducibility between two observers.
- Chi square test was done to study the association between ki67 labelling index and Ki-67 expression.
- One- way ANOVA (Analysis of Variance) was used for comparing the mean Ki-67 labeling index among the different grades. Comparison of the mean ki-67 labeling index between groups was made using multiple comparison test by Tukey-HSD procedure.
- One- way ANOVA (Analysis of Variance) was used for comparing the morphometrical parameters for multiple groups. Comparison of the mean nuclear and cellular area and diameter values between groups was made using multiple comparison test by Tukey-HSD procedure.
- Pearson Correlation test was done to analyse the association between ki-67 expression and each of the 4 morphometrical parameters.
- Chi-square test was applied to study the correlation between Ki-67 expression and OSCC grades.
Chi-square analysis was done subsequently to correlate the Ki-67 expression with the disease recurrence.

RESULTS

In the test group which included 105 OSCC cases, positive Ki-67 expression in the nuclei of proliferating tumor epithelial cells was found positive in all the cases (100%). 5 tonsillar cases were used as controls. 5 cases of normal oral mucosa were also used for comparative assessment of staining. The inter observer reproducibility was analysed with inter-class correlation which revealed a good reproducibility between the 2 observers (ICC=.925).

Among the 105 cases of squamous cell carcinomas in 49/105 (47%) 30-50% of tumor cell expressed Ki 67. Among the 35 cases of well differentiated tumors in 13/35 (37%) 30-50% of tumor cells expressed Ki 67 while in 17/35 (49%) cases <30% of cells were positive for the biomarker. Among the 35 cases of moderately differentiated tumors in 20/35 (57%) 30-50% of tumor cells expressed Ki 67 while in 1/35 (2%) cases >50% of cells were positive for the biomarker. Among the 35 cases of poorly differentiated tumors in 16/35 (46%) 30-50% of tumor cells expressed Ki 67 while in 17/35 (46%) cases <30% of cells were positive for the biomarker. On statistical analysis with chi-square test, a highly significant correlation was found between Ki-67 expression and the advancing grades of OSCC. (p<.001)(Table 1)

The Ki-67 expression and labelling index was assessed in the 105 cases of oral squamous cell carcinomas. The mean labeling index among the well, moderate and poorly differentiated OSCC was 29.84 ± 13.04; 48.10 ± 13.41 and 32.015 ± 13.89 respectively. A one way Anova test showed that there was a significant (p<.001) variation in mean Ki-67 labeling index between the tumor grades. A post hoc test with Tukeys HSD test showed that there was a significant (p<.001) association between Ki-67 labeling index of well differentiated and moderately differentiated and that between moderately differentiated and poorly differentiated tumors.(Table 2 and Table 3)

Ki-67 expression and Ki-67 labelling index also showed a highly significant association through Chi-square test (p<.001).

In addition, an analysis of the ki-67 expression and the intra-oral site of OSCC was also done. Ki-67 expression of nine different sites of the oral cavity was assessed and its mode was calculated. The tongue (dorsal/ventral aspect), vestibule and floor of the mouth and buccal mucosa with alveolus show lowest ki-67 expression[1], whereas angle of mandible, alveolar region and buccal mucosa show moderate ki-67 expression[2] and tongue (lateral border), alveolar region and lips show highest ki-67 expression[3] and least degree of tumoral differentiation. (Table 4)

Nuclear area: The mean nuclear area of poorly differentiated OSCC was found to be 162.04±². One Way ANOVA test showed the value of mean nuclear area to be statistically significant among the grades of OSCC (p= 0.025). Comparison between the groups using Post-Hoc test with Tuckey HSD method showed the value of poorly differentiated OSCC to be significantly lower than well differentiated OSCC (p= 0.05) and moderately differentiated OSCC (p= 0.042).

Similarly, the mean cell area (p<.0001), mean nuclear perimeter (p= 0.27) and mean cell perimeter (p<.0001) were found to be statistically significant among the grades of OSCC. Comparison between the groups using Post-Hoc test with Tuckey HSD method has been depicted in Table 5 and 6

However no statistically significant correlation was seen between ki-67 and any of the 4 morphometrical parameters. (Table 7)

Follow up and recurrence analysis:

Among the 105 cases, 62 were available for follow up, out of which 8 patients showed loco-regional recurrence (12.9%). After their morphometrical analysis it was seen that nuclear area, cell area and nuclear perimeter were higher in the recurrent cases as compared to the non recurrent ones. (Figures 3 and 4)

Recurrence was also correlated with Ki-67 expression with chi-square analysis, which indicated it to be statistically insignificant.

Table 1: Association between tumor grade and Ki-67 expression

<table>
<thead>
<tr>
<th>Tumor Grade</th>
<th>High Proliferation</th>
<th>Moderate Proliferation</th>
<th>Low Proliferation</th>
<th>Total</th>
<th>X2 Value</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well</td>
<td>5 (14%)</td>
<td>13 (37%)</td>
<td>17 (49%)</td>
<td>35</td>
<td>25.050^a</td>
<td>4</td>
<td>.000</td>
</tr>
<tr>
<td>Moderate</td>
<td>14 (40%)</td>
<td>20 (57%)</td>
<td>1(3%)</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>3 (8%)</td>
<td>16 (46%)</td>
<td>16 (46%)</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: One way Anova to compare the mean Ki-67 labelling index

<table>
<thead>
<tr>
<th></th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>.6964</td>
<td>2</td>
<td>3482.066</td>
<td>19.224</td>
<td>.000</td>
</tr>
<tr>
<td>Within groups</td>
<td>18475.855</td>
<td>102</td>
<td>181.136</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>25439.988</td>
<td>104</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Comparison of the mean labelling index among the different grades

<table>
<thead>
<tr>
<th>(I) Grade</th>
<th>(J) Grade</th>
<th>Mean difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well</td>
<td>Moderate</td>
<td>-18.25729*</td>
<td>3.21724</td>
<td>.000</td>
<td>-25.9092 - 9.8188</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>-2.16686*</td>
<td>3.21724</td>
<td>.779</td>
<td>-10.6054 5.4850</td>
</tr>
<tr>
<td>Moderate</td>
<td>Well</td>
<td>18.25729</td>
<td>3.21724</td>
<td>.000</td>
<td>10.6054 25.9092</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>16.0943*</td>
<td>3.21724</td>
<td>.000</td>
<td>8.4385 23.7423</td>
</tr>
<tr>
<td>Poor</td>
<td>Moderate</td>
<td>2.16686</td>
<td>3.21724</td>
<td>.000</td>
<td>-5.4850 9.8188</td>
</tr>
<tr>
<td></td>
<td>Well</td>
<td>-16.0943</td>
<td>3.21724</td>
<td>.779</td>
<td>-23.7423 - 8.4385</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the .05 level.

Table 4: showing the relationship of Ki-67 expression with site of OSCC cases

<table>
<thead>
<tr>
<th>site</th>
<th>Number of cases of Well differentiated OSCC</th>
<th>Number of cases of Moderately differentiated OSCC</th>
<th>Number of cases of Poorly differentiated OSCC</th>
<th>Ki-67 Expression occurring with highest frequency (Mode)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tongue - lateral border (left/right)</td>
<td>2</td>
<td>14</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Tongue - ventral/dorsal aspect</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Buccal mucosa</td>
<td>9</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Buccal mucosa and alveolus</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>Alveolar region</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Retromolar region</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Angle of mandible</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>lips</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Vestibule and floor of the mouth</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total cases (With the details of the site of OSCC)</td>
<td>26/35</td>
<td>31/35</td>
<td>18/35</td>
<td></td>
</tr>
</tbody>
</table>

(Ki-67 expression: 1 = high proliferation, 2 = moderate proliferation, 3 = low proliferation)

Table 5: Statistical analysis using One – Way ANOVA test and multiple comparisons test (Post-Hoc test) with Tukey HSD method (values in micron²)

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Grade of OSCC</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Confidence limit</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear Area</td>
<td>Well differentiated (a)</td>
<td>190.77</td>
<td>54.48</td>
<td>172.05, 209.49</td>
<td>.025</td>
</tr>
<tr>
<td></td>
<td>Moderately differentiated (b)</td>
<td>192.11</td>
<td>46.86</td>
<td>176.02, 208.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Poorly differentiated (c)</td>
<td>162.04</td>
<td>52.57</td>
<td>144.39, 209.98</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cell Area</td>
<td>Well differentiated (a)</td>
<td>542.29</td>
<td>139.91</td>
<td>494.23, 590.35</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Moderately differentiated (b)</td>
<td>553.98</td>
<td>127.48</td>
<td>510.19, 597.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Poorly differentiated (c)</td>
<td>414.72</td>
<td>127.68</td>
<td>370.85, 458.58</td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Statistical analysis using One – Way ANOVA test and multiple comparisons test (Post-Hoc test) with Tukey HSD method (values in micron²)

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Grade of OSCC</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Confidence limit</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear Perimeter</td>
<td>Well differentiated (a)</td>
<td>48.28</td>
<td>6.47</td>
<td>46.06, 50.51</td>
<td>.027</td>
</tr>
<tr>
<td></td>
<td>Moderately differentiated (b)</td>
<td>48.92</td>
<td>5.86</td>
<td>46.91, 50.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Poorly differentiated (c)</td>
<td>44.88</td>
<td>7.49</td>
<td>42.30, 47.46</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Cell Perimeter</td>
<td>Well differentiated (a)</td>
<td>86.82</td>
<td>12.17</td>
<td>82.63, 91.00</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Moderately differentiated (b)</td>
<td>88.88</td>
<td>9.95</td>
<td>85.46, 92.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Poorly differentiated (c)</td>
<td>76.38</td>
<td>13.08</td>
<td>71.89, 80.88</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>
Table 7: Showing statistically insignificant correlation between Ki-67 labelling index and the morphometrical parameters

<table>
<thead>
<tr>
<th>Morphometrical Parameter</th>
<th>R (Pearson correlation Coefficient)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear area (NA)</td>
<td>.123</td>
</tr>
<tr>
<td>Cell area (CA)</td>
<td>.194</td>
</tr>
<tr>
<td>Nuclear perimeter</td>
<td>.148</td>
</tr>
<tr>
<td>Cell perimeter</td>
<td>.207</td>
</tr>
</tbody>
</table>

DISCUSSION

Oral squamous cell carcinoma (OSCC) is known for its unpredictable progression and severe damage of the tissues involved. Conventionally, OSCC is evaluated with clinical staging and histological grading system, which are essentially subjective and not efficiently reproducible. It is certain that it will take more than just routine histological methods to detect and control this disease before its characteristic progression to serious impairment.

Expression of proliferation markers such as ki-67 for lesions of oral mucosa has been shown to be correlated with the severity of the lesion \cite{12}. The expression of the human Ki-67 protein, a nuclear marker, provides an accurate estimation of the tumour growth rate in a relatively cost and time effective manner \cite{7}.

A study conducted by Dragomir L.P et al. on 34 cases of OSCC, showed that the increased expression of Ki-67 was associated with the decrease of the degree of tumoral differentiation and with high degree dysplasia \cite{13}.

Recently, the findings of Dwivedi N et al. \cite{14} confirmed that the expression of Ki-67 provides an objective criteria for determining the histological grading of OSCC. They also assessed the severity of epithelial dysplasia using Ki-67 expression.

In our study the immunoexpression of Ki-67 was seen in all the 105 cases (100%) of OSCC and our results were closely similar to the observations of Kannan et al. \cite{12}, Premlatha et al. \cite{15} and Bryant et al. \cite{16} On statistical analysis with chi-square test, a highly significant correlation was found between ki-67 expression and the advancing grades of OSCC (p<.001). This indicated that the rate of cell proliferation tends to increase with the decrease in the degree of tumor differentiation in OSCC.

We correlated the Ki-67 expression with the site of OSCC to attain the site with highest ki-67 expression. Nine different sites of the oral cavity were assessed namely Tongue - lateral border (left/right), Tongue - ventral/dorsal aspect, Buccal mucosa, Buccal mucosa with alveolus, Alveolar region, Retro molar region, Angle of mandible, lips, Vestibule and floor of the mouth. Mode was calculated for the ki-67 expression among the different sites. Results showed that tongue (lateral...
border), alveolar region and lips show highest ki-67 expression and least degree of tumoral differentiation. Our study also showed a highly significant correlation between ki-67 labelling index and ki-67 expression (p<.001). Furthermore, the Ki-67 labeling index was found to increase with the advancing grades of OSCC. The moderately differentiated OSCC showed a significantly higher proliferation than well differentiated OSCC (p<.001). Thus higher Ki-67 labelling index could indicate a poor prognosis, as was demonstrated by Maheshwari et al.[9] Our results supported the cogency of Ki-67 as a potential proliferative marker for OSCC. The analysis was quick and easy and there was good reproducibility between the two different observers. (ICC=.925)

In addition, we studied the expression of Ki-67 in 5 specimen of normal oral mucosa and the expression was found to be present in the basal and parabasal layers. This finding was similar to that made by Rendon et al. [17] and Birajdar et al.[18]

Although certain other immunohistochemical markers such as PCNA, Cyclin D and CENP-F may also be used to assess cellular proliferation, the present study uses Ki-67 due to its intense nuclear staining which is evident in all the phases of the cell cycle except G0 phase and the background staining is nominal[5,7,9]. It can thus be interpreted efficiently with ease.

The present study further attempts to correlate ki-67 expression with computer-aided morphometrical analysis, in order to develop an index which may enable the use morphometrical values alone as an accurate and cost effective diagnostic tool for the underprivileged Indian oral cancer patients. Although several systems are available for morphometrical image analysis. Image J serves as a more cost effective alternative, developed at the National Institutes of Health (NIH)[19,21].The validity of Image J has been demonstrated in our previous study conducted with the same set of cases[22]. The previous study was carried out with 6 morphometrical parameters, out of which the current study has considered 4 parameters for further analysis, namely nuclear area, cell area, nuclear perimeter and a cell perimeter. These parameters have been particularly chosen for the current study as they can be calculated with ease and minimum time consumption using the Image J software. The reliability of these criteria has been emphasized in previous literature, however this is the first study to take account of all the four criteria for analysis.

Giardina et al.[23] conducted a study to highlight the significance of nuclear morphometry in OSCC. They analysed 30 cases to study the relationship between nuclear shape and survival. They established that morphometrical analysis could successfully distinguish patients of the two groups of short term and long term survival with only a 10% error. Similarly, DB Nandini and RV Subramanyam[24] conducted a study using computer-assisted microscopy on nuclear features in oral squamous cell carcinoma and emphasized the reliability of computer-assisted nuclear morphometry in OSCC grading. These studies coordinate with the morphometrical results depicted in our previous study[22]. NA for poorly differentiated OSCC, 162.04 µ2, was significantly lower than both well and moderately differentiated OSCC. Similarly CA of poorly differentiated OSCC was 414.72 µ2 which was significantly lower than both well and moderately differentiated OSCC. NP for poorly differentiated OSCC was 44.88 µ2 which was significantly lower than moderately differentiated OSCC. CP of poorly differentiated OSCC was significantly lower than well differentiated OSCC as well as moderately differentiated OSCC.

These morphometrical values can provide more reliable information to the clinician and are more comprehensive for the patients. However no statistically significant association was observed between ki-67 and any of the 4 morphometrical parameters of the study. This might have been due to less number of cases or a consequence of insufficient patient follow up details. Further research overcoming the limitations of the study could be more effectual.

Taken together, the data from our study adds weight to the growing body of evidence that Ki-67 is a powerful tool to interpret the proliferative potential of the advancing grades of OSCC and that 4 simple yet reliable morphometrical parameters can help in prognostication of OSCC.

CONCLUSION

The study showed Ki-67 to be a reliable proliferative marker for OSCC, along with a strong diagnostic and prognostic significance of four morphometrical parameters in the advancing grades of OSCC. However the association of Ki-67 expression with the computer aided image analysis could not be ascertained. Further studies based on correlating computer aided image analysis and immunohistochemistry may help to develop a simple yet promising adjunct to routine histological examination which helps in diagnosing and understanding OSCC and can be highly valuable tool in predicting an accurate and timely prognosis in order to formulate an effective treatment plan according to the individual treatment needs of the patient.

Acknowledgements Indian Council of Medical Research for the financial assistance.

Conflict of Interest: Nil

REFERENCES


Mahima et al.,


