Ki-67 Index in Salivary Gland Neoplasms

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ABSTRACT

Assessment of proliferation is a means of predicting local recurrence and metastatic potential of malignancies. A mitotic count is not an ideal marker for proliferation in certain situations, such as salivary gland neoplasms. Ki-67 expression as a proliferation marker has been investigated in many human tumors. In the present study, Mitotic index (MI) and Ki-67 index were studied in pleomorphic adenoma, basal cell adenoma, mucoepidermoid carcinoma, adenoid cystic carcinoma epithelial myoepithelial carcinoma, carcinoma ex Pleomorphic adenoma and adenocarcinoma of salivary glands. The results were compared.

The MI was similar in benign neoplasms, mucoepidermoid carcinoma and epithelial myoepithelial carcinoma, whereas it was high in carcinoma ex pleomorphic adenoma, adenocarcinoma and adenoid cystic carcinoma. The Ki-67 index was different in basal cell adenoma and pleomorphic adenoma. It was helpful in differentiating high grade and low grade mucoepidermid carcinoma. It highlighted the malignant behavior of epithelial myoepithelial carcinoma.

It was concluded that Ki-67 in benign neoplasms is 5% or less and in malignant ones more than 23% with a few exceptions. In mucoepidermoid carcinoma and epithelial myoepithelial carcinoma, Ki-67 index was found to be a better indicator for aggressiveness. These findings will be presented in this paper, with review of literature.

Keywords: Ki-67 index, Mitotic index, Prognostic marker, Proliferation, Salivary gland neoplasms.


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Conflict of interest: None

INTRODUCTION, AIM, AND OBJECTIVE

Proliferation is one of the most fundamental biological processes of growth and maintenance of tissue homeostasis. Assessment of proliferation is a means of predicting the likelihood of local recurrence and metastatic potential of malignancies. Counting of mitotic figures is the oldest way of assessing proliferation. The ease with which mitoses can be recognized without special equipment has led to the increasing popularity of mitotic index (MI).

A mitotic count does not necessarily provide a good marker of proliferation, especially in certain situations. For example, in salivary gland neoplasms, unlike the more common forms of cancer, it may be impossible to decide by mitoses alone, whether the salivary gland tumor is truly malignant. Ki-67 expression as a proliferative cell and a prognostic marker has been investigated in many types of human tumors including those of salivary gland origin, such as acinic cell and adenoid cystic carcinomas.

MATERIALS AND METHODS

Salivary gland lesions encountered at Dr Pinnamaneni Siddhartha Institute of Medical Sciences and Research Foundation (Dr PSIMS and RF) during the last six and a half years of study (June 2005 to May 2010 retrospectively and June 2010 to September 2011 prospectively) were 93 out of 24,933 histopathology specimens received (0.37% of the total specimens). Of these 65 (69.89%) are neoplasms 45 benign and 20 malignant. These lesions were classified according to site, age, and sex.

Hematoxylin and eosin (H&E) stained sections from the above neoplasms were studied for mitoses and scored per 10 high-power fields (HPFs) by two independent observers. The peripheral portions of neoplasms were preferred as these usually have the highest score.

For Ki-67 immunostaining Biogenix Kit was used. A positive control was selected from a nonmucin secreting adenocarcinoma of colon which proved to have a Ki-67 index of 75%. The negative control was the same as which mitoses could be recognized without special equipment has led to the increasing popularity of mitotic index (MI).
staining was considered nonspecific and was not taken into consideration. These results were confirmed using Image Pro-express Software version 6.0, and the images of the corresponding areas were photographed for manual recount. The overall average was derived as Ki-67 index.

RESULTS

The results of the salivary gland neoplasms at Dr. PSIMS and RF (May 5 to September 11) distributed according to age, sex, and site are shown as bar chart (Graph 1), pie diagrams (Graphs 2 and 3), and (Tables 1 to 3).

**DISCUSSION**

Criteria to identify the mitotic cells given by Ankle MR et al and Van Diest et al were used in this study. Standardized evaluation of mitotic activity in tumor tissues has been
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Table 3: Comparison of Ki-67 index and Mitotic index (index of Ki-67 are positive nuclei per 500 cells, while MI is the number of mitoses per 10 HPF). Average for each category are depicted in this table

<table>
<thead>
<tr>
<th>Type of neoplasm</th>
<th>Ki-67 index</th>
<th>Mitotic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleomorphic adenoma</td>
<td>08 (1.6%)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Basal cell adenoma</td>
<td>25 (5.0%)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Warthin tumor</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Epithelial myoepithelial carcinoma</td>
<td>120 (24.0%)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Carcinoma ex pleomorphic adenoma</td>
<td>115 (23.0%)</td>
<td>10</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>250 (50.0%)</td>
<td>25</td>
</tr>
<tr>
<td>Mucoepidermoid carcinoma low grade</td>
<td>11 (2.2%)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Mucoepidermoid carcinoma high grade</td>
<td>40 (8.0%)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Adenoid cystic carcinoma</td>
<td>120 (24.0%)</td>
<td>15</td>
</tr>
</tbody>
</table>

revised by Biesterfeld, and it was found that MI compares well with cytometric and morphometric methods in assessment of tumor cells and is of higher clinical relevance than the conventional histomorphological tumor grading systems. We used conventional histomorphological grading of tumors, and hence we did not follow the evaluation as indicated by Biesterfeld.

The objections against the use of mitotic counts are instability due to prefixation factors (body site, temperature, compromised blood supply, hormones, and circadian biorhythm) and the lack of reproducibility. Digital image processing could be of help in improving reproducibility of mitotic count assessment, by automated selection of the most cellular areas and counting of mitotic figures. Hence we selected the peripheral growing portion of neoplasm for our study. However, we did not use digital imaging technique.

Ki-67 is a monoclonal antibody which reacts with proliferating cells, except for those in G0 period of cell cycle. The close correlation between the frequency of Ki-67 positive cells and the level of malignancy is well established. Ki-67 proliferative index in benign is usually <5% vs malignancy usually being >10%.

In malignancies, patients with Ki-67 values of 15% or less had a better survival than did those with Ki-67 values of more than 15%.

In the present study, among the benign neoplasms, pleomorphic adenoma, basal cell adenoma, and Warthin tumor were taken into consideration. In pleomorphic adenoma the MI was <5, where as the Ki-67 index was 1.6%. As per Anna Kazanceva et al, the Ki-67 value was higher in recurrent pleomorphic adenomas compared with primary pleomorphic adenoma. However, in our study we did not encounter any recurrent tumor in pleomorphic adenomas. Varghas et al and Skálová et al showed that Ki-67 can help to differentiate pleomorphic adenoma with low Ki-67 from adenoid cystic carcinoma (Ki-67 index of 20.5 – 54%). Figure 1 shows the H&E stain, and Figure 2 shows immunohistochemistry (IHC) by Ki-67 for pleomorphic adenoma.

Basal cell adenoma had an MI similar to pleomorphic adenoma in our study but the Ki-67 index was higher (5%). This is in accordance with the study of Batsakis et al who stated that the Ki-67 index is a better marker as is substantiated by the higher rate of recurrence in basal cell adenomas. In our study, there were two basal cell adenomas and both were from parotid. One was canalicular and the other was membranous type. Both showed MI of <5 and a Ki-67 index of 5% which is the upper limit of index for benign neoplasms. The slightly higher Ki-67 of basal cell adenoma is in accordance with the study of Batsakis et al in which basal cell adenoma (the membranous subtype) had a recurrence rate of 25 to 37% (Figs 3A and B).

Ki-67 index in Warthin tumor was not calculated as the epithelial cells did not show positivity, while the

Fig. 1: Pleomorphic adenoma (H&E)  
Fig. 2: Pleomorphic adenoma (Ki-67)
lymphoid cells especially in germinal centers showed nuclear positivity.

Mitotic index<5 was observed in epithelial myoepithelial carcinoma and mucoepidermoid carcinoma. The epithelial myoepithelial carcinoma had a high Ki-67 index of 24% when compared to mucoepidermoid carcinoma (8%). Hence Ki-67 a better indicator of malignancy than MI in this category of tumors (Figs 4A and B) Nagao et al.16 had similar results for Epithelial Myoepithelial carcinoma.

In our study while the MI did not help in differentiating low- and high-grade mucoepidermoid carcinoma, the high-grade type had a higher Ki-67 index (8%) and low-grade type had a lower Ki-67 index (2.2%). Thus, in addition to the histology, Ki-67 index is a helpful marker for concluding whether a given mucoepidermoid carcinoma is low or high grade. Skalova et al17 showed in a study of 46 cases of mucoepidermoid carcinoma that a low Ki-67 index is always associated with a benign course, whereas a higher index predicted a considerable risk of aggressive clinical behavior. In comparison with the histologic grading system and MI, the Ki-67 index separates mucoepidermoid carcinoma into indolent and aggressive forms which are virtually nonoverlapping17 (Figs 5A to D).

Thus Ki-67 is a better indicator than MI in neoplasms of this category. This strong correlation between Ki-67 index and the histological grade suggests that both assess the proliferative activity. The Ki-67 index measures the proliferative activity directly by assessing the number of proliferating cells, whereas histologic grade measures it indirectly by registering the proportion of solid areas, which are the main sites of proliferation. Thus the Ki-67 index could be used in parallel to histologic grading to predict tumor behavior.17 The Ki-67 index is a more objective grading system and the subjective assessment of H&E sections correlates with Ki-67 index, which was definitely higher in high-grade subtype than in the low-grade.

Carcinoma ex pleomorphic adenoma in our study had a mean Ki-67 index of 23%. According to Di Palma et al,18 carcinoma ex pleomorphic adenoma, including noninvasive/intracapsular variant, the Ki-67 index is
increased (by mean 35%) compared with that of the parent pleomorphic adenoma. In comparison with the results of Ki-67 of pleomorphic adenoma, the Ki-67 of carcinoma ex pleomorphic adenoma (1.6 vs 23%) is showing similar results in our study (Figs 6A and B).

The present study had one case of adenocarcinoma of parotid gland that showed a MI of 25 and a Ki-67 index of 50%. Auclair et al\textsuperscript{19} has reported MI in accordance to the grade of adenocarcinoma. However, no Ki-67 studies of adenocarcinoma of salivary gland were available (Figs 7A and B).

In our study the MI of adenoid cystic carcinoma is 15 and the Ki-67 index was 24% (Figs 8A and B). Skálová et al\textsuperscript{14} remarked that the Ki-67 index of adenoid cystic carcinoma is > 20%, hence the Ki-67 of our study correlates with the above-mentioned study.
CONCLUSION

From this study we arrived at the following conclusion:

- Ki-67 index in benign neoplasms of salivary glands is 5% or less.
- In malignant neoplasms Ki-67 index ranged between 23 and 50%. The highest scores are in adenocarcinoma. The MI was correspondingly high in carcinoma ex pleomorphic adenoma, adenocarcinoma, and adenoid cystic carcinoma, ranging from 10 to 25.
- In contrast, the mucoepidermoid carcinoma of low grade had a low Ki-67 index of 2.2% and a higher value of 8% in mucoepidermoid carcinoma of high grade. Both had a MI of <5. This indicates that in mucoepidermoid carcinoma the Ki-67 index corresponds to the grade rather than the MI.
- In epithelial myoepithelial carcinoma, while the Ki-67 index is very high (24%). The MI, in contrast, corresponded to that in benign cases. This indicates that the MI is an unreliable criterion in epithelial myoepithelial carcinoma, and Ki-67 index will be advantageous since it confirms the malignant behavior in these cases.

Ki-67 is a costly investigation and requires the basic facilities for IHC; however, this is a needed expenditure in grading of mucoepidermoid carcinoma and in epithelial myoepithelial carcinoma of salivary glands. In basal cell adenoma, Ki-67 index is useful in predicting the possible local recurrence. Therefore, Ki-67 index is a useful diagnostic tool in predicting the aggressive behavior of salivary gland neoplasms.

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REFERENCES


