BLOOM-RICHARDSON GRADING:
A MARKER OF CHEMOSENSITIVITY IN INVASIVE BREAST CARCINOMAS

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ABSTRACT:
This was a retrospective and prospective study carried out in the Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi from October 2000 to October 2002. A total of fifty breast carcinoma specimen were investigated comprising of forty invasive ductal carcinomas and ten invasive lobular carcinomas. Ki-67 antigen was immunostained on formalin-fixed paraffin embedded tissue and the positivity index was determined. Thirty three cases showed positive nuclear staining. Eight cases were of ILC showing a mean Ki-67 positivity index of 13.2±5.3% and twenty five cases were of IDC showing a mean Ki-67 positivity index of 15.9±9.8%. The overall Ki-67 index in 33 cases was 15.3±8.9%. Grade-I contained 04 cases out of 33 with a mean Ki-67 index of 6.57±3.8%. Grade-II included 18 cases out of 33 with a mean Ki-67 index of 14.5±7.9%. Grade-III contained 11 cases out of 33 with a mean Ki-67 index of 19.8±9.6%. The results showed that the Ki-67 index was increasing with the increase of histological grade of the tumor and the results were statistically significant. There is a steady increase in proliferative index with loss of differentiation in invasive breast cancers and this association may provide the basis for more intensive chemotherapy and hence a better response with increasing grades of these tumors.

Keywords: Ki-67 Positivity index, Immunostaining, Breast Carcinoma.

INTRODUCTION
Breast cancer continues to be the most common cancer and is the second most lethal cancer in women despite declining mortality rates (Gerald et al., 2006). The histological grading of malignancy is the most classical evaluation method for prognosis in breast cancer patients and it is the simplest method, requiring only hematoxylin-eosin staining (Kansei et al., 2006). Histological grading has long been recognized to have potential value in evaluating prognosis of adenocarcinoma of the breast beyond that of simple nodal staging. The two most widely used systems for microscopic grading are of Bloom-Richardson and Black, the first is based mainly on architectural features and second on the degree of nuclear atypia (Pienta et al., 1991; Van Diest et al., 1991). The Nottingham modification of the Bloom and Richardson system is made by Elston, who used the previous ones in conjunction (Frierson et al., 1995). The point distribution in this scheme is based on tubule formation, nuclear pleomorphism and mitotic count. The result is expressed as a total score of 3 to 9 points, which is translated into the final grade i.e., Grade I, II or III (Rosai, 1996).

The grade is also associated with cell proliferation, a consistent indicator of response to chemotherapy. The determination of an association between grade and responsiveness would be clinically useful (Amat et al., 2002). The possible role of histologic grade in prediction of response to chemotherapeutic regimens and in the selection of these regimens has been less thoroughly investigated (Pinder et al., 1998). Fisher et al. reported that
there was an increase in the survival rate for women treated with L-phenyl-alanine mustard (L-PAM) plus 5-fluorouracil or L-PAM, 5-fluorouracil, and methotrexate when stratified according to histologic grade (Fisher et al., 1983). This was particularly apparent in patients with Grade 3 lesions, was only statistically significant with regard to patients with Grade 3 tumors and 4 or more LN metastases, and was irrespective of age. O'Reilly et al. recorded that premenopausal LN positive patients with all Bloom-Richardson grades of breast carcinoma benefited from 12 cycles of CMF compared with a control group, but they observed no improvement in relapse free survival in the postmenopausal groups defined by histologic grade (O'Reilly et al., 1990). Carcinogenesis is linked to the development of proliferative abnormalities which precede the occurrence of morphological abnormalities and hence their measurement serves as useful biomarker for chemotherapy trials and prognosis (Elias, 1997; Goodson et al., 2000). Many markers of proliferation have been studied in breast cancer, including thymidine labeling, bromodeoxyuridine incorporation, flow cytometry, and immunostaining with the Ki-67 monoclonal antibody (Hall and Levison, 1990; Hall and Woods, 1990; Silvestrini, 1990). This antibody recognizes a nuclear antigen encoded by a gene located on chromosome 10q25 (Fonatsch et al., 1991; Gerdes et al., 1991). The antigen Ki-67 is regarded as a marker for proliferating cells. It was identified as a protein (s) (Pki-67) which exists free or associated with DNA as evidenced by DNA digestion of cells before or after immunolabelling with Ki-67 (Lopez et al., 1994). As Ki-67 identifies the proliferating cells in a tumor, it reflects the percentage of dividing cells. The positivity of the cells correlates with the degree of differentiation, vascular invasion, and lymph node metastasis, and it relates inversely to the presence of steroid hormone receptors (Isola et al., 1990). The current study was designed to evaluate proliferative activity within the tumor cells by immunohistochemical analysis of Ki-67 labelling index and correlate it with the histological grade in invasive ductal and invasive lobular carcinomas. This was based on the hypothesis that the determination of an association between grade and cell proliferation would be clinically useful for predicting the responsiveness to chemotherapy in these patients.

**MATERIALS AND METHODS**

This study was performed on formalin fixed paraffin embedded blocks of cases diagnosed as invasive ductal carcinoma and invasive lobular carcinoma of breast with and without lymph node involvement, in the Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, Pakistan from October 2000 to October 2002. Fifty cases were selected comprising of 10 cases of invasive lobular carcinoma (ILC) and 40 cases of invasive ductal carcinoma (IDC) and were subjected to immuno-staining for Ki-67 positivity. Five-micron thick sections were retrieved for H&E staining. Extra slides were prepared for immuno-staining by cutting 4µm thick sections from representative paraffin embedded blocks and were applied to already positively charged slides. Antigen retrieval was done by trypsin digestion (Zymed Cat No. 00-3003) followed by heat induced antigen recovery. Specific staining is accomplished by localizing the Ki-67 antigen with Ki-67 polyclonal antibody. The antigen/antibody complex is then identified using the LAB-SA biotinylated secondary antibody detection method (Jonstone and Thorpe, 1988). A streptaviden enzyme is then added which binds to the biotinylated secondary antibody. A substrate solution is then added that forms a coloured deposit in the presence of the enzyme that is complexed to the antigen. The location of the antigen is then revealed by the presence of the colored deposit that forms around it. Any nuclear staining was regarded as positive. Positivity index of Ki-67 was determined by counting the number of positively stained nuclei in 1000 tumor cells in at least five representative high power fields across the slide.
The computer package "Microsoft Excel" was used for data feeding and "EPI-INFOR" was used for statistical analysis. The results were given in the text as number and percentage for qualitative variables and mean and standard deviation for quantitative data. To compare the difference between two means, Student t-test was employed. For the comparison of more than two means Analysis of variance (F-test) was performed. In all statistical analysis, only ‘P’ values less than ‘0.05’ were considered significant.

### Table-1
Grade-wise Distribution of Cases in Invasive Lobular Carcinoma and Invasive Ductal Carcinoma of Breast

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Grades</th>
<th>ILC</th>
<th>IDC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>1.</td>
<td>I</td>
<td>02</td>
<td>04%</td>
<td>06</td>
</tr>
<tr>
<td>2.</td>
<td>II</td>
<td>06</td>
<td>12%</td>
<td>21</td>
</tr>
<tr>
<td>3.</td>
<td>III</td>
<td>02</td>
<td>04%</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>10</td>
<td>-</td>
<td>40</td>
</tr>
</tbody>
</table>

Key: L.N. = Lymph node, ILC = Invasive Lobular Carcinoma, IDC = Invasive Ductal Carcinoma

![Graph showing grade-wise distribution of cases in invasive lobular carcinoma and invasive ductal carcinoma of breast](graph.png)

**Fig. 1:** Grade-wise distribution of cases in invasive lobular carcinoma and invasive ductal carcinoma of breast

**Key:** ILC = Invasive lobular carcinoma, IDC = Invasive ductal carcinoma

### STATISTICAL ANALYSIS

In this study, 50 diagnosed cases of human breast carcinoma including invasive lobular carcinoma (ILC) and invasive ductal carcinoma (IDC) were subjected to immunohistochemical staining for Ki-67 antigen. Out of 50 cases, 10 were invasive lobular carcinoma and 40 invasive ductal carcinomas (Table 1, figs.1 and 2). In ILC, maximum number of cases were in grade-II, i.e., 06 and minimum number of cases were in grade-I and III, i.e. 02 cases each, which were 12% and 4% of total cases, respectively. In
Bloom-Richardson Grading

IDC, maximum number of cases were in grade-II, i.e., 21 and minimum number of cases were in grade-I, i.e., 06 which were 42% and 12% of total cases, respectively.

In overall 50 cases, the maximum number of cases were in grade-II, i.e. 27 and minimum number of cases in grade-I, i.e. 08, which were 54% and 16% of total number of cases, respectively.

Out of a total of 50 cases of invasive breast carcinomas, 33 (66%) cases showed positive nuclear staining for Ki-67 antigen. Remaining 17 (34%) cases showed absence of any nuclear staining. Lack of reactivity in these cases could be the result of long-term storage of tissue specimen affecting the stability of Ki-67 antigen or during the processing of the slides the stability of Ki-67 antigen had suffered which could have explained the lack of reactivity.

Table 2, figs. 3 and 4 are showing the details of grade versus Ki-67 positivity in 33 cases of ILC and IDC of breast. Grade-I comprised 04 cases out of 33 with a mean Ki-67 index of 6.57±3.8%. In ILC, 01 out of 08 cases and in IDC 03 out of 25 cases were present in grade-I showing a mean Ki-67 index of 10.5% and 5.26±3.45% respectively. Grade-II included 18 cases out of 33 with a mean Ki-67 index of 14.5±7.9%. In ILC, 06 out of 08 cases were in grade-II, i.e., 27 and minimum number of cases in grade-I, i.e. 08, which were 54% and 16% of total number of cases, respectively.

Table 2
Grade Versus Ki-67 Positivity in Invasive Lobular Carcinoma and Invasive Ductal Carcinoma of Breast

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Grades</th>
<th>ILC No. of Cases</th>
<th>Ki-67 (Mean ± SD)</th>
<th>IDC No. of Cases</th>
<th>Ki-67 (Mean ± SD)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>I</td>
<td>01</td>
<td>10.5%</td>
<td>03</td>
<td>5.26 ± 3.45%</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>2.</td>
<td>II</td>
<td>06</td>
<td>13.7 ± 6.1%</td>
<td>12</td>
<td>14.91 ± 8.86%</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>3.</td>
<td>III</td>
<td>01</td>
<td>13.1%</td>
<td>10</td>
<td>20.43 ± 9.81%</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>4.</td>
<td>Total</td>
<td>08</td>
<td>13.1%</td>
<td>25</td>
<td>P Value</td>
<td>N.A.</td>
</tr>
<tr>
<td>5.</td>
<td>P Value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.04</td>
</tr>
</tbody>
</table>

Key: ILC=Invasive Lobular Carcinoma, IDC=Invasive Ductal Carcinoma, N.A.=Not applicable
in IDC 12 out of 25 cases were present in grade-II showing a mean Ki-67 index of 13.7±6.1% and 14.9±8.86%, respectively. Grade-III contained 11 cases out of 33 with a mean Ki-67 index of 19.8±9.6%. In ILC, only 01 case out of 08 cases and in IDC 10 out of 25 cases were present in grade-III showing a mean Ki-67 index of 13.1% and 20.43±9.81%, respectively.

The results showed that the Ki-67 index was increasing with the increase of histological grade of the tumor and the results were statistically significant (P=0.02). The
results of ILC versus IDC in all grades were statistically insignificant (P>0.05).

**DISCUSSION**

Growth is a characteristic of all true cancers, and the rate of growth is a fundamental determinant of the time required for any cancer to reach threshold size large enough to be detected, to produce symptoms, or to reach lethal proportions (John S Spratt and John A Spratt, 2002). The increasing documentation of the rates of growth of human breast cancers provides insight into the many problems of breast cancer control. Laboratory studies on human breast cancers are yielding a rapid growth of information on the genetic and molecular biologic properties of cells making up breast cancers. Many of these properties are correlated with cell proliferation rates, survival rates, and other traditional histopathologic characteristics of cancers. These correlations may revolutionize systems of anatomic staging.

Measurements of cell kinetics have been found to correlate with clinical course. Biological factors are dependent on cell kinetics and bear important relationship in predicting clinical outcome of the disease and response to the treatment (McGurrrin et al., 1987; aarzanti et al., 2000). There are numerous studies that compared Bloom Richardson grading components and overall grade for prognostic efficacy. Mitotic count and overall grade predicted prognosis equally in one study (Clayton and Hopkins, 1993). Mitotic index contributed to prediction along with another grading component in other four studies (Le Doussal et al., 1989; Parl et al., 1982; Lee et al., 1997; Uyterlinde et al., 1990). Overall grade was more predictive than any of its components in three studies (LeDoussal et al., 1989; Raabe et al., 1997; Aaltomaa et al., 1991). These published results favor mitotic index as the principal prognostic component of Bloom-Richardson grading systems. Studies consistently report that if survivorship is measured from first symptom, the determination of prognosis is a function of the net state of cancer cell increase, i.e., the actual tumor volume doubling time (Fisher et al., 1987). Previous studies have suggested that the Ki-67 indices rise progressively with decreasing differentiation of tumors or with increasing nuclear grade (Isola et al., 1990; Sabin et al., 1991; Sullivan et al., 1993; Wiesener et al., 1998; Barzanti et al., 2000). Lelle and co-researchers (Lelle et al., 1987) have reported a Ki-67 labeling index of 9.7±6.9% in grade-I invasive breast carcinomas, 14.8±9.4% in grade-II, and 24.0±11.3% in grade-III in invasive breast carcinomas. These results were statistically significant (P≤0.05).

In the current study we also found a similar correlation between Ki-67 positivity index and tumor grade, i.e. a mean Ki-67 index of 6.57±3.8% in grade-I invasive breast carcinomas, 14.5 ± 7.9% in grade-II, and 19.8±9.6% in grade-III carcinomas. We found these values to be statistically significant (P=0.02). However, no statistical significance was noted in the results of ILC versus IDC (P>0.05).

**CONCLUSION**

Grade is a strong predictive factor of response to induction chemotherapy in breast cancer, independently of the type of regimen used. In this study we found a steady increase in the proliferative index with loss of differentiation or increasing grade of the tumor. The association between histological grade and cell proliferation may prove valuable for clinicians as they make their decision regarding patient therapy.

**REFERENCES**

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