

# Comparison of Cytological versus Histopathological Grading of Invasive Ductal Carcinoma of the Breast with Correlation of Lymph Node Status

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## Abstract

**Background:** Invasive ductal carcinoma is one of the most common type of tumors in females, constituting a single large group of breast cancers. There are various prognostic factors, of which tumor grade, size and axillary lymph node metastasis are among the important ones. Fine needle aspiration cytology is a commonly used diagnostic technique in the initial evaluation of breast lumps. In the era of neoadjuvant chemotherapy, the material obtained from fine needle aspiration cytology is often the only baseline morphology available for future evaluation. Therefore the assessment of cytological grade in fine needle aspiration cytology samples is useful. In addition, correlating cytological grade with axillary lymph node status is indicative of tumor aggressiveness. This study correlates the cytological grade with histological grade and axillary lymph node status.

**Methods:** Patients with cytological diagnosis of ductal carcinoma who underwent subsequent resection and axillary clearance over a period of five years were included in the study. Fine needle aspiration cytology smears were graded by Robinson's method and compared with the Modified Bloom Richardson's histopathological grading and axillary lymph node metastasis.

**Results:** There were a total of 98 cases of invasive ductal carcinoma. Patients' ages ranged from 28 to 98 years with a mean of 52.17 years. In terms of cytology, there were 22 (22.4%) grade I cases, 61(62.2%) grade II, and 15 (15.3%) grade III. For histopathology, 22 (22.4%) were grade I, 56 (57.1%) were grade II, and 20 (20.4%) were grade III. In 76 (77.5%) cases the cytological grade correlated with histological grade, but did not show any significant positive correlation with axillary lymph node metastasis.

**Conclusion:** Cytological grade can be used as a predictor of histological grade, but may not predict axillary lymph node metastasis in patients with invasive ductal carcinomas.

**Keywords:** Axillary lymph node metastasis, Cytological grading, Ductal carcinoma, Robinson's grading

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## Introduction

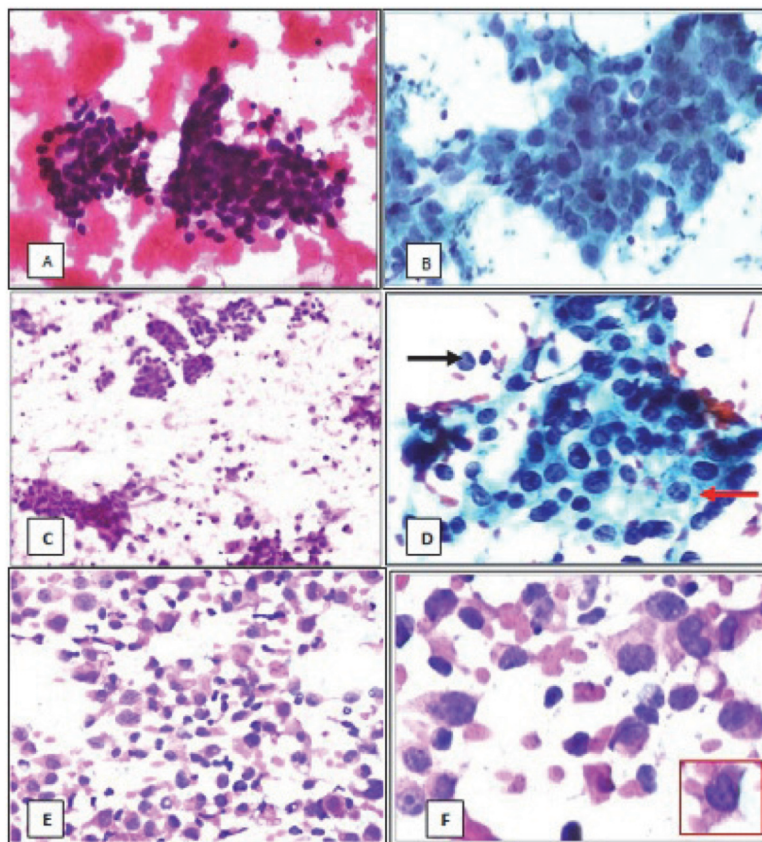
Invasive Ductal Carcinomas (IDC) are a single large group of primary breast carcinomas. There are various prognostic factors that predict tumor behavior and assess survival in patients with breast cancer. The three most important predictors are primary tumor size, histological grade and axillary lymph node (ALN) metastasis. Of the three, ALN metastasis is known to be the single most important predictor of disease-free and overall survival in breast cancer patients.<sup>1</sup>

Fine needle aspiration cytology (FNAC) is a simple, cost-effective, and highly accurate method in the initial evaluation of breast lumps.<sup>2</sup> Tumor grade is a known prognostic factor, therefore grading cytological smears will provide accurate prognostic information about the tumor at the initial evaluation.

With the increasing use of neoadjuvant chemotherapy (NACT) as treatment for breast cancers, the FNA material is often the only diagnostic material available from the untreated patients, as the tumor characteristics tend to change after NACT. This necessitates evaluation of tumor grade on FNAC samples. This study therefore evaluates the accuracy of grading on cytology by comparing it with standard histological grade and prediction of ALN metastasis based on cytological grade.

## Materials and Methods

Invasive ductal carcinomas over a five year period from January 2004 to March 2009 were studied. We included patients with breast lumps diagnosed as ductal carcinoma on FNAC who had subsequent resection with ALN dissection in



**Figure 1.** A: Photomicrograph showing cytological grade 1 ductal carcinoma with monomorphic tumor cells arranged in clusters with nuclear size 1-2 times the size of RBCs (H&E, 400 $\times$ ). B: Tumor cells with vesicular nuclei, smooth nuclear margins, and indistinct nucleolus (Pap, 400 $\times$ ). C: Cytological grade II ductal carcinoma with mildly pleomorphic tumor cells arranged singly and in clusters (H&E, 200 $\times$ ). D: Tumor cells with nuclear size 3-4 times the size of RBCs (black arrow), mildly pleomorphic, noticeable nucleoli (red arrow) (Pap, 200 $\times$ ). E: Cytological grade III ductal carcinoma showing singly scattered tumor cells with nuclear size >5 times the size of RBCs and marked nuclear pleomorphism (H&E, 400 $\times$ ). F: Nuclear clefts (arrow) (H&E, 400 $\times$ ). Inset showing nuclear budding (H&E, 400 $\times$ ).

**Table 1.** Robinson's cytological grading of breast carcinomas on fine needle aspiration (FNA) material.<sup>3</sup>

Cell features	Score 1	Score 2	Score 3
Dissociation	Cells mostly in clusters	Mixture of single and cell clusters	Cells mostly single
Nuclear size	1-2 x RBC size	3-4x RBC size	>5x RBC size
Cell uniformity	Monomorphic	Mildly pleomorphic	Pleomorphic
Nucleoli	Indistinct	Noticeable	Prominent or pleomorphic
Nuclear margin	Smooth	Slightly irregular/folds/grooves	Buds or clefts
Chromatin	Vesicular	Granular	Clumped and cleared

Grade 1: Score 6-11, Grade 2: Score 12-14, Grade 3: Score 15-18

St. John's Medical College Hospital. Excluded were cases where the aspirates were inconclusive due to low cellularity or there was only lumpectomy without axillary clearance.

We obtained the clinical and demographic details from the patients' medical records. The patients were classified into three groups based on their age: pre- and peri-menopausal (<47 years), menopausal (47-52 years) and post-menopausal (>52 years). Also recorded were the tumor location (laterality and quadrant) and whether the patient received NACT.

The tumor size was assessed on gross specimen, then classified into three groups: i) <2 cm, ii) 2-5 cm and iii) >5 cm in greatest dimension. The FNAC smears were graded based on cell dissociation, nuclear size, cell uniformity, nucleoli, nuclear margin and chromatin. Each feature was assigned a score of 1-3; the final score ranged from 6 to 18 which was translated into grades as follows. Grade 1 (score 6-11), grade 2 (score 12-14), and grade 3 (score 15-18) according to the Robinson grading system (Table 1).<sup>3</sup> The cytological grade was then compared with the histological grade [modified Bloom Richardson (MBR)] and ALN metastasis. We classified patients into two groups based on ALN metastasis - those with one or more positive nodes and those with no positive nodes. The cytological grade and ALN metastasis were also correlated with the patient's age, tumor size and location.

### Statistical analysis

Spearman correlation coefficient was used to establish a correlation between histological and cytological grades and the association between cytological grade and ALN metastasis was analyzed by Pearson's chi square test. The degree

of correlation between tumor size, location and metastasis was calculated independently using the chi square test.  $P \leq 0.05$  was considered statistically significant.

### Results

There were 98 patients included in the study. Patients' age ranged from 28 to 98 years with a mean of 52.17 years. There were 42 (42.9%) patients who were pre/peri menopausal and post-menopausal and 14 (14.3%) were menopausal. The tumors were located in the outer quadrant in 59 cases, the inner quadrant in 23 and central quadrants in 14 cases. In 2 patients the tumor was large, involving all quadrants. In 69.4% of cases the tumor size was between 2 to 5 cm whereas 18.4% had a tumor size <2 cm and 12.2% had a tumor >5 cm.

According to Robinson's cytological grading system, the majority of tumors (62.2%) were grade II. Grade I tumors comprised 22.4% of cases and there were 15.3% who had grade III tumors. The most commonly observed cytological features in grade I IDC were predominance of cell clusters, monomorphic or mildly pleomorphic nuclei, nuclear size that approximated the RBC size, smooth nuclear margins, and indistinct nucleoli (Figure 1A and 1B). In grade II tumors the cells were mostly arranged singly and in clusters (Figure 1C). The cells exhibited mild-to-moderate pleomorphic nuclei, indistinct to noticeable nucleoli, and slightly irregular nuclear margins (Figure 1D). Some cases showed nuclear grooves. In most cases the chromatin pattern was coarse and granular.

In grade III tumors, the cells were predominantly singly scattered, pleomorphic and large (Figure 1E). Abnormal nucleoli were noted

in some cases. Clefts and buds from the nuclear membrane were observed in a few cases of grade III ductal carcinomas (Figure 1F). The chromatin pattern was variable. Clumping and clearing was observed in a few cases. On histopathological grading, there were 22 grade I cases, 56 grade II cases and 20 grade III cases. We observed ALN metastasis in 51 cases; 47 cases had no nodal metastasis.

The cytological grade of the tumor was correlated with histological grade and lymph node status. Overall, a significant correlation between cytological grade and histological grade was obtained in 76 (77.5%) cases; there was a discrepancy in 22 (22.4%). In 9 out of 22 (41%) cases, the tumors were downgraded on histology and in 13 cases the tumors were upgraded on histology. There was a statistically significant correlation between cytological and histological grades ( $P < 0.0001$ ). Lymph node metastasis was seen irrespective of the tumor grade. There was no significant association between cytological grade and ALN metastasis ( $P = 0.36$ ).

The cytological grade did not show a significant correlation with patient's age, tumor size and location ( $P > 0.05$ ). Axillary lymph node metastasis was more commonly seen in tumors that were located in areas other than the inner quadrant ( $P = 0.006$ ) and size  $> 2$  cm ( $P = 0.03$ ).

There were 17 cases that received NACT. A comparative evaluation of the tumor grades before and after administration of NACT showed 3 out of the 4 (75%) cytological grade I cases upgraded to grade II on histology, whereas the majority of cytological grades II (60%) and III (66.7%) did not show a change in grade after NACT.

## Discussion

Invasive ductal carcinomas are the most common type of breast carcinomas and are diagnosed on the initial investigation of FNA. As the grade of breast carcinoma is a known prognostic marker,<sup>4</sup> an attempt to grade these tumors on the FNA sample will provide useful information on the tumor behavior prior to tumor excision. Numerous studies have used various

grading methods to grade cytology and compared them with the MBR grading on histology. Robinson's method of cytological grading was applied in this study as the criteria for tumor grading. This method was simpler and easier to reproduce compared to other methods.

In the present study most tumors were cytological grade II, which was similar to the results of two other Indian studies on IDC by Dash et al. and Chhabra et al.<sup>5,6</sup> Frias et al. studied 100 aspirate samples of IDC with a similar distribution of cytological grades.<sup>7</sup>

When cytological and histological grades were correlated, we observed a statistically significant correlation although a discrepancy was noted in 22.4% of cases. In 9 (41%) cases the tumors were downgraded on histology. This might be due to the fact that in cytological grading the nuclear features were predominantly considered for grading. The degree of tubule formation (degree of differentiation) and the mitotic count (proliferation index) were not assessed on cytology. The second reason might be attributed to large tumor size or tumor heterogeneity. When the tumor size is large, multiple passes may be required to sample the most undifferentiated areas of the tumor, which may not be performed on routine FNAs. The third factor could be observer subjectivity when assigning the grade. Nuclear margins (smooth, slightly irregular, folds, grooves clefts budding) and chromatin pattern (granular, clumping and clearing) are features where observer variability is more likely.

In a study of 50 Indian women, Khan et al. reported a significant correlation between cytological grade and histological grade in 84% of cases.<sup>8</sup> Chhabra et al. observed an overall concordance rate of 65%.<sup>6</sup> The studies by Dash et al. and Mouriquand et al. reported a concordance rate of 77.4 %<sup>5,9</sup> which were very similar to the present study.

Studies in the literature that correlated cytological grade with lymph node involvement found higher grade tumors more likely to metastasize than low grade tumors.<sup>5,10,11</sup> Dash et al. studied 93 cases of IDC and found that 74.2%



of grade III carcinomas had nodal metastasis in contrast to 27% of grade I tumors.<sup>5</sup> Frias et al. reported that 88% of cytological grade III and 64% of cytological grade II carcinomas had lymph node metastasis.<sup>7</sup> In contrast, the present study did not show a significant association between cytological grade and incidence of ALN metastasis.

When tumor grade (cytological and histological) was correlated with patients' age, we observed that grade III tumors occurred more frequently in pre- and peri-menopausal age women, which indicated that breast carcinomas behaved more aggressively in this age group. This finding agreed with a study by Pratap and Shousha who analyzed 229 cases of invasive breast carcinomas in women less than 50 years of age. In their study, a slightly higher incidence of grade III tumors was seen in younger patients.<sup>12</sup> Another study by Gann et al. of 18025 cases showed that tumors in younger patients behaved more aggressively, a finding which was in concordance with the present study.<sup>13</sup>

There was no correlation between tumor size and cytological grade in the present study which was similar to the results obtained by Kim et al. on 62 cases of IDC, although the study utilized Black's nuclear grade to grade the smears.<sup>14</sup> In the present study the number of cases that received NACT was small, therefore no statistical significance could be assigned to this factor.

In conclusion, this study established a significant correlation between Robinson's cytological grade and MBR histological grade of IDC, whereas no correlation was established with ALN metastasis. Higher grade tumors featured more commonly in the pre- and peri-menopausal age group. Cytological grade showed no significant correlation with tumor size or location, whereas tumor located in quadrants other than the inner quadrant and those >2 cm showed increased incidence of ALN metastasis. Neoadjuvant chemotherapy might alter the primary tumor grade, thereby necessitating grading on cytology.

## Conflict of Interest

No conflict of interest is declared.

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