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## RESEARCH ARTICLE

# DIAGNOSTIC ACCURACY OF ROBINSON'S CYTOLOGICAL GRADING ASSUMING MODIFIED SCARFF BLOOM RICHARDSON'S HISTOLOGICAL GRADING AS A GOLD STANDARD IN MALIGNANT NEOPLASMS OF BREAST

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### ABSTRACT

Breast carcinoma is one of the leading cause of malignancy in females. The diagnosis of breast carcinoma is often made by fine needle aspiration cytology, which is quick and non invasive procedure to assess in vivo tumor grade. Cytological grading in aspiration of breast malignancies is a useful tool for surgical maneuver and prognosis. The aim of present study was to assess the diagnostic accuracy of the Robinson's cytological grading of breast carcinoma assuming the Elston's modification of Bloom Richardson's histological grading as a gold standard and to assess the importance of cytohistological grading in predicting prognosis of breast carcinomas. The fine needle aspiration smears were stained with papanicolaou and H&E stains & graded using the Robinson's cytological grading system and correlated with modified scarff bloom Richardson's histological grading (Assuming as gold standard). The present study showed that the cytological grade correlated well with the histological grading ( $p < 0.01$ ), diagnostic accuracy was 75.71% and higher grade was associated with nodal metastasis. Hence the cytological grading and typing should be routinely incorporated in the cytology report and they can be of great value in guiding the choice of the treatment protocols.

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## INTRODUCTION

Breast cancer is the second most common malignancy among Indian females next to cervical cancer. (National Cancer Registry Programme, 1992) with heterogenous prognosis (Khan *et al.*, 2009). It is both ironic and tragic that a neoplasm arising in a readily accessible organ for self examination and clinical diagnosis continue to exert such a heavy toll (Lester, 2010). Assessment of biological aggressiveness of the cancer without removing it, will be valuable. The value of the histological typing and grading has been well established (Meena *et al.*, 2005). Cytological grade provides relevant information on the aggressiveness of invasive ductal carcinoma & its tendency to produce regional metastasis. This cytological grade also corresponds well with the histological grade (Ohri *et al.*, 2006). It is a useful parameter to take into consideration for selecting neoadjuvant therapy as primary medical treatment of breast cancer (Robles- Frias *et al.*, 2005).

Including pre-operative chemotherapy and tamoxifen is becoming increasingly common for early breast cancer. Hence, it is desirable to grade tumors before surgery, so that most appropriate medical regimen can be selected hence, much attention is focused on grading tumour on FNAC. Such grading would allow assessment of tumor in-situ, and the morbidity associated with over-treatment of low grade tumors could be avoided (Robinson *et al.*, 1994, Taniguchi *et al.*, 2000). Faster growing tumors respond more to chemotherapy than low grade slow growing tumor, which may be better suited to pre-treatment with tamoxifen (Ohri *et al.*, 2006). FNAC is helpful in diagnosis, treatment planning as well as in prognostication of the tumor factors like the nuclear grading, the mitotic index, the hormone receptor status and the DNA content (Vidya Vasudev *et al.*, 2013).

In the present study efforts were made to study the accuracy of cytological grading and typing of malignant breast lesions with histopathological correlations & assessment of their lymphnode status.

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## MATERIALS AND METHODS

Present prospective study was conducted in the Department of Pathology, SMS Medical College, Jaipur from 1<sup>st</sup> June 2013 to 30<sup>th</sup> Nov. 2014 (i.e. for 1.5 year duration) we have studied 70 cases of cytologically proven breast carcinoma which had histopathological correlation. Fine needle aspiration of breast lump was performed with 22 gauge needle with 10 ml syringe attachment. Samples obtained were smeared onto glass slides and fixed in 95% ethyl alcohol. Wet fixed FNAsmeared were stained by papanicolaou (Koss *et al.*, 1968) & H&E stain. Papanicolaou stain allows better assessment of nucleus, nuclear membrane & chromatin pattern (Scultze *et al.*, 1987). Cell size was measured by comparing with size of red blood cells (Hunt *et al.*, 1901) present in the smear. The cytological grading was done by Robinson's method in which the cell dissociation, nuclear size, cell uniformity, nucleoli, nuclear margins and the chromatin patterns were studied (Mckee *et al.*, 2001; Frias *et al.*, 2005; Vidya *et al.*, 2013). All the FNA smears and corresponding biopsy were examined by two observers independently.

A value which was between 1 & 3 were given to each factor which was analysed. The scores of each of the 6 cytological features were added together to give a total score for each case. In each case, the final score range between 6 & 18. The scores were added & the grading was done. Grade 1; 6-11, Grade 2; 12-14 & grade 3; 15-18. The cytological typing was also done. The subsequent mastectomy specimen which were received in the dept of pathology, were processed and the blocked sections were, subsequently stained with H&E stain. The histopathological typing and grading was done by the modified scarff's bloom Richardson criteria, taking into consideration, the tubule formation, nuclear pleomorphism and mitotic count.

Histological grading includes (a) score 3-5 grade I (well differentiated) (b) Score 6-7 grade II (Moderately differentiated) (c) Score 8-9 grade III (Poorly differentiated) (Vidya *et al.*, 2013). The total cytological scores were compared with the final histopathological grade.

The statistical analysis was performed using SPSS statistical software package version 16 and EPI – Info Version 6. Correlation between cytological grading & histological grading was established using the non parametric spearman's correlation coefficient (rho value). Also chi square ( $\chi^2$ ) test is done to determine the association of cytological grade & histological grade with the presence of axillary lymph node metastasis.

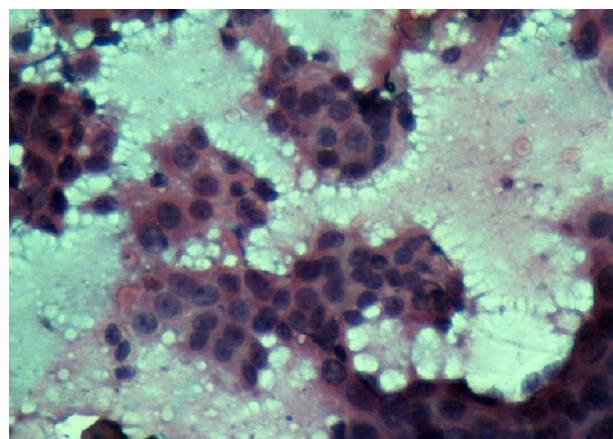
## RESULTS

In the present study Out of 70 cases 68 were females and 2 were males in the age group of 12-80 years, mean age in female was 49 years while that in male subject was 43.5 years. Grading of aspirated sample was done according to Robinson's categories and results obtained were following Out of 70 cases, 25 cases (35.71%) were grade 1 (Table 1; Fig. 1 with inset). 34 cases (48.57%) were grade II (Table 1, Fig. 3 with inset) and 11 cases (15.7%) were in grade III (Table 1, Fig. 5 with inset).

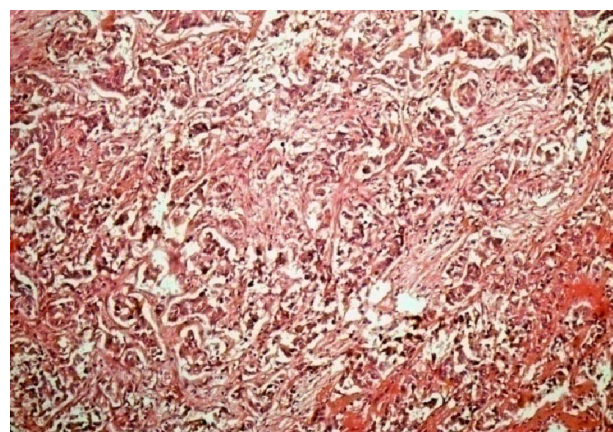
**Table 1. Comparison of the cases showing distribution between the Cytological grade and Histological grade**

| Cytological grade | Histological grade |             |             | Total       |
|-------------------|--------------------|-------------|-------------|-------------|
|                   | I                  | II          | III         |             |
| I                 | 21 (75%)           | 3 (11.11%)  | 1 (6.66%)   | 25 (35.71%) |
| II                | 6 (21.42%)         | 23 (85.18%) | 5 (33.33%)  | 34 (48.57%) |
| III               | 1 (3.57%)          | 1 (3.70%)   | 9 (60%)     | 11 (15.71%) |
| Total             | 28 (40%)           | 27(38.57%)  | 15 (21.42%) | 70          |

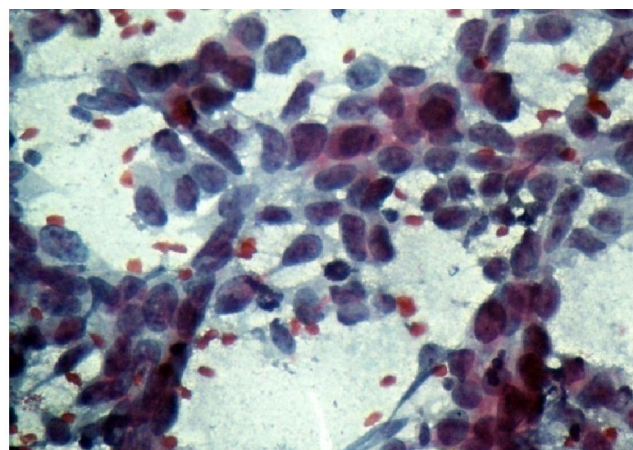
p value = 0.00001(p<0.05, significant)



**Fig. 1. Duct carcinoma cytological grade I (Pap 400X)**



**Fig. 2. Duct carcinoma histological grade I (H&E 100X)  
(Inset HE x 400)**



**Fig. 3. Duct carcinoma cytological grade II (Pap 400X)**

**Table 2. Comparison of concordance rates between each Cytological and Histological grade**

| S.No.                            | Grade | Concordant cases | Number of cases in each Cytological grade | Concordance rate                 | Spearman's correlation coefficient |
|----------------------------------|-------|------------------|---|----------------------------------|------------------------------------|
| 1.                               | I     | 21               | 25  | 84%                              | rho= 0.691<br>p<0.01 (significant) |
| 2.                               | II    | 23               | 34  | 67.64%                           |                                    |
| 3.                               | III   | 9                | 11  | 81.81%                           |                                    |
| Total number of concordant cases |       | 53               | Total number of cases =70                 | Absolute concordance rate=75.71% |                                    |

**Table 3. Comparison of concordance rates in different studies with the present study employing Robinson's Cytological grading system**

| Studies                         | Concordance Rate |
|---------------------------------|------------------|
| Das <i>et al.</i> (2003)        | 71.2%            |
| Chhabra <i>et al.</i> (2005)    | 65%              |
| Sultana and Rehman (2006)       | 86.3%            |
| Khan <i>et al.</i> (2009)       | 89.1%            |
| Sinha <i>et al.</i> (2009)      | 69.5%            |
| Kalhan <i>et al.</i> (2010)     | 88.1%            |
| Rekha <i>et al.</i> (2011)      | 82%              |
| Lingegowda <i>et al.</i> (2011) | 64%              |
| Pandya and Shah (2012)          | 74.57%           |
| Das <i>et al.</i> (2012)        | 69%              |
| Present study (2014)            | 75.71%           |

**Table 4. Comparative table on correlation coefficients between Cytological grade and Histological grade obtained in different studies with our study**

| Studies                      | r value | P value    |
|------------------------------|---------|------------|
| Present study (2013)         | 0.691   | 0.01(S)    |
| Chhabra <i>et al.</i> (2005) | 0.537   | < 0.001(S) |
| Frias <i>et al.</i> (2005)   | 0.774   | <0.0005(S) |
| Das <i>et al.</i> (2012)     | 0.438   | < 0.001(S) |

S-Significant

**Table 5. Number of cases showing Axillary lymph node metastasis**

| S.No. | Axillary Lymph node metastasis | No. of cases | Percentage |
|-------|--------------------------------|--------------|------------|
| 1.    | Present                        | 39           | 55.71%     |
| 2.    | Absent                         | 31           | 44.29%     |
| TOTAL |                                | 70           | 100%       |

**Table 6. Association of each Cytological grade with incidence of axillary lymph node metastasis**

| Grade | Cytological grade |  | Axillary lymph node metastasis |             |
|-------|-------------------|--|--------------------------------|-------------|
|       | No. of cases      |  | Positive                       | Negative    |
| I     | 25 (35.71%)       |  | 10 (40%)                       | 15(60%)     |
| II    | 34 (48.57%)       |  | 22 (64.71%)                    | 12 (35.29%) |
| III   | 11 (15.71%)       |  | 7 (63.64%)                     | 4 (36.36%)  |
| TOTAL | 70                |  | 39                             | 31          |

p=0.139, insignificant(p>0.05)

**Table 7. Multiple regression analysis of Cytological features included in the Robinson's Cytological grading system**

| S. No | Cytological feature | Regression coefficient (B) | t value | Significance (P) |
|-------|---------------------|----------------------------|---------|------------------|
| 1.    | Cell dissociation   | 0.290                      | 5.835   | 0.000            |
| 2.    | Cell uniformity     | .822                       | .803    | .425             |
| 3.    | Cell size           | .234                       | 1.472   | .146             |
| 4.    | Nucleoli            | .326                       | 4.478   | 0.000            |
| 5.    | Nuclear Membrane    | .348                       | 4.233   | 0.000            |
| 6.    | Chromatin           | .341                       | 5.229   | 0.000            |

**Table 8. Distribution of patients according to Correlation of Cytological and Histological Typing**

| Cytological diagnosis | Histopathological diagnosis |         |                   |                     | Total |
|-----------------------|-----------------------------|---------|-------------------|---------------------|-------|
|                       | IDC                         | ILC     | Colloid carcinoma | Secretory carcinoma |       |
| IDC                   | 64(96.96%)                  | -       | -                 | -                   | 64    |
| Lobular carcinoma     | 1(1.51%)                    | 2(100%) | -                 | -                   | 3     |
| Mucinous carcinoma    | -                           | -       | 1(100%)           | -                   | 1     |
| Secretory carcinoma   | -                           | -       | -                 | 1(100%)             | 1     |
| DCIS                  | 1(1.51%)                    | -       | -                 | -                   | 1     |
| Total                 | 66                          | 2       | 1                 | 1                   | 70    |



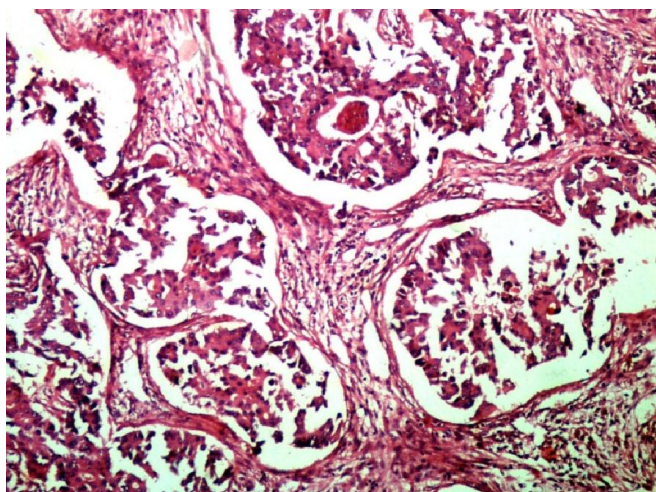
The histopathological grading was done by modified scarff bloom Richardson grading system. 28 cases (40%) were grade I (Table 1; Fig. 2 with inset) 27 cases (38.57%) were of grade II (Table 1; Fig. 4 with inset), 15 cases (21.42%) were of grade III (Table 1; Fig 6 with inset).

Correlation and comparison of both the systems were done and statistical values were calculated.

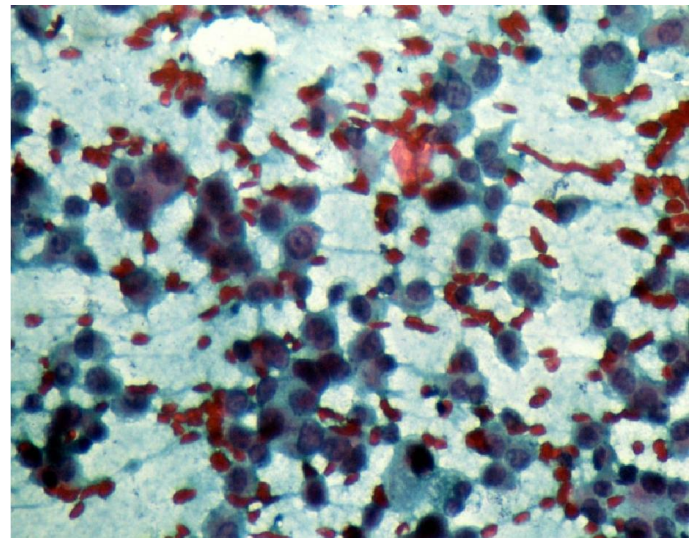
Table 1 demonstrates the relationship between the cytological grade and histological grade. The cytological grading correlation was accurate in 21 (75%) cases of grade I, in 9 cases (60%) of grade III and in 23 (85.18%) cases of grade II. The cytological grade correlated positively with the histological grade. The spearman's correlation coefficient was rho-0.691, the  $\chi^2$  test p value was <0.01 and sensitivity 86.18 & results were compared with other studies (Table 2, 3 & 4).

In present study Sperrman's correlation coefficient (rho value 0.691) indicates that there is a strong correlation between cytological and histological grade. The high value of coefficient of correlation showed a significant & marked association ( $p < 0.01$ ) between the grades assigned to cytological and histological grades. Out of 70 cases 53 shows agreement between respective cytological and histological grades. Hence absolute concordance rate is (53/70) – 75.71%. In the present study 39 cases (56%) were positive for axillary lymphnode metastasis while 31 cases (44.29%) were negative. The positive cases had ipsilateral lymphnode involvement (Table 5).

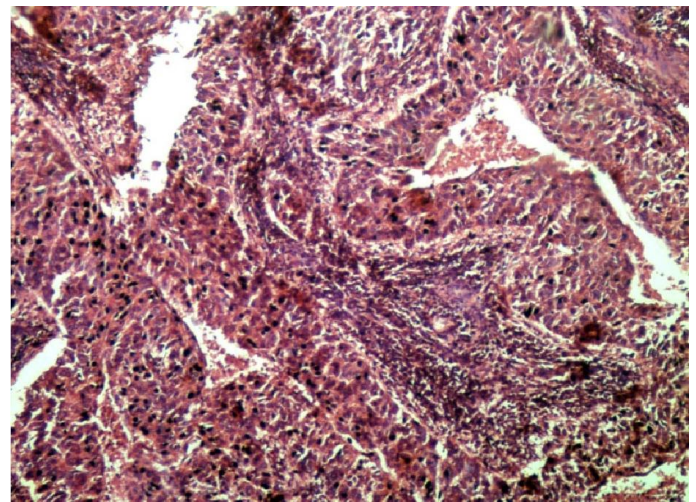
Table 6 shows the relationship between the cytological grade and the nodal metastasis of each cytological feature. Multiple regression analysis of each cytological features was done, as shown in (Table 7). In the present study (Table 8) the cytological typing correlated with histopathological typing in 68 (97%) cases out of 70 cases. The cytological typing was accurate in 64 (96.96%) cases of IDC, 2 cases of lobular carcinoma (i.e. 100%), in 1 case of mucinous carcinoma (i.e. 100%) (Fig. 7 & 8) and in 1 case of secretory carcinoma (100%) (Table 8) (Fig. 9 & 10).



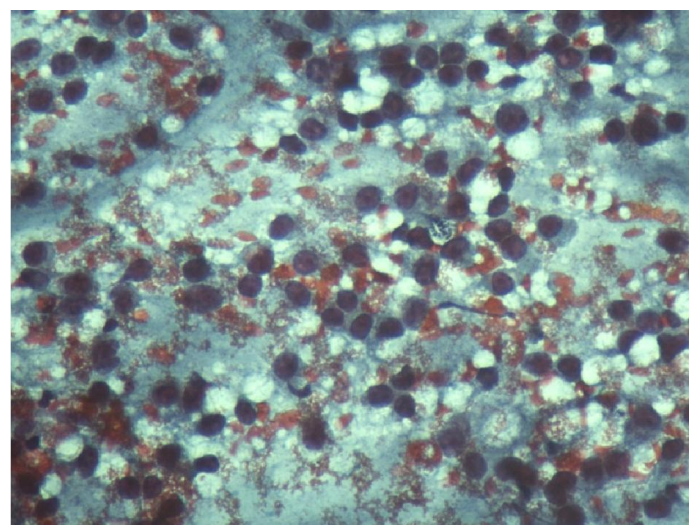
**Fig. 4. Duct carcinoma histological grade II (H&E 100X)  
(Inset HE x 400)**



**Fig. 5. Duct carcinoma cytological grade III (Pap 400X)**

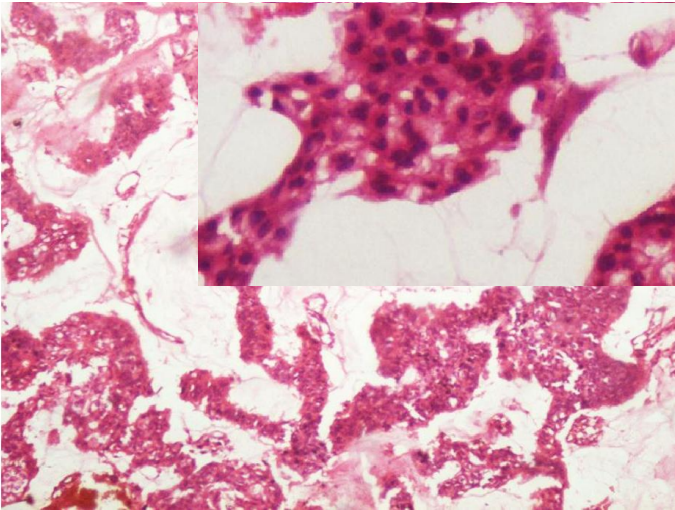


**Fig. 6. Duct carcinoma histological grade III (H&E 100X)  
(Inset HE x 400)**

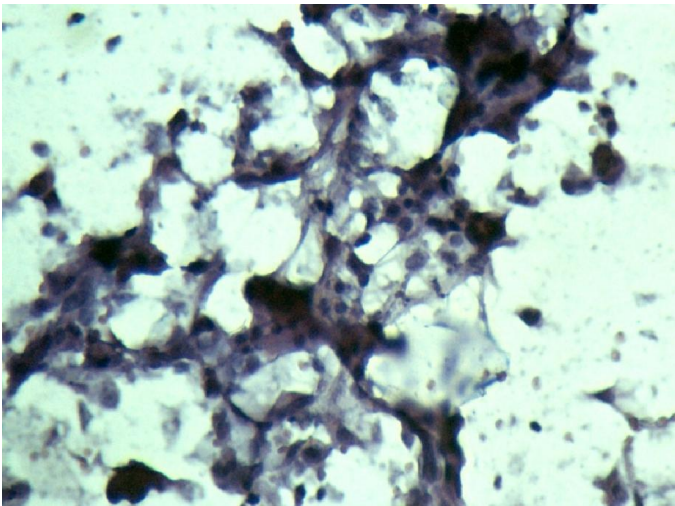


**Fig. 7. Mucinous carcinoma cytology (Pap 400X)**

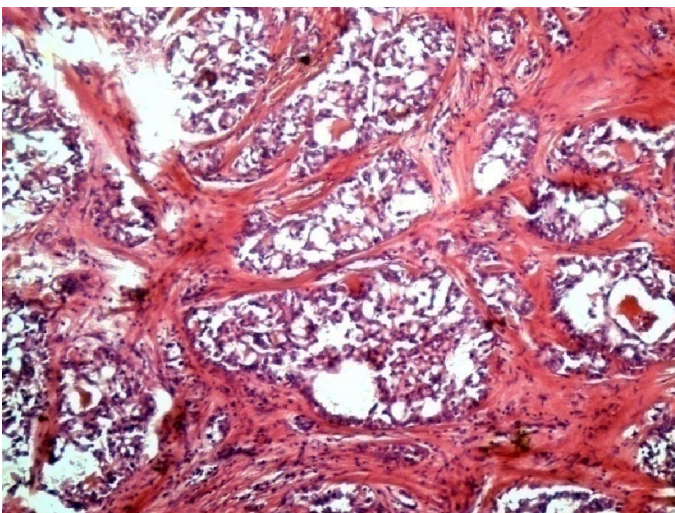




**Fig. 8. Mucinous carcinoma histology (H&E 100X)  
(Inset HE x 400)**



**Fig. 9. Secretory carcinoma cytology (Pap 400X)**



**Fig. 10. Secretory carcinoma histology (H&E 100X)  
(Inset HE x 400)**

## DISCUSSION

Robinson's cytological grading is feasible and reproducible for the evaluation and prognostification of palpable and non palpable malignant breast lesions without any additional morbidity or expenses to the patient (Katze *et al.*, 1994). The aim of cytoprognostic grading is to identify fast growing tumors (grade III) (Chhabra *et al.*, 2005). As fast growing tumors are more likely to respond to chemotherapy than the slow growing low grade tumors in which tamoxifen pretreatment is better suited. Hence assessment of biological aggressiveness by cytological grading without removing the tumor would be valuable (Chhabra *et al.*, 2005). The National Cancer Institute (NCI) Bethesda conference had also recommended that tumor grading on aspirated material should be incorporated in cytological reports for prognostification (Chhabra *et al.*, 2005).

Nuclear grading is considered as one of the most important prognostic factors in breast cancer as well as, it is correlated well with histological section unlike other parameters (tubule formation & mitotic count) (Zoppi *et al.*, 1997). The interobserver & intraobserver reproducibility are the key factors in any grading system (Cajulis *et al.*, 1997) and also in grading of malignant breast lesions and correct diagnosis and grading of malignant breast lesion is also important for further treatment protocols. As neoadjuvant therapy, which includes preoperative chemotherapy and tamoxifen is becoming increasingly common for the treatment of early breast cancer. Grading of tumor before surgery is important so that most appropriate medical regimen can be selected (Robinson *et al.*, 1994). Robinson's cytological grade corresponded well with the established histological grade (Elston modified Bloom Richardson's grading, 1990). In the present study cytological grading was done by using Robinson's grading system. A majority of the tumors were grade II (48.57%) followed by grade I (35.7%) and grade III (15.71%). Many authors i.e. Taniguchi *et al.* (2000), Robinson *et al.* (1994), Meena *et al.* (2006), & Frias *et al.* (2005) had findings in concurrence with our study, while Vidya Vasudev *et al.* (2013) found majority of tumors in grade III followed by grade II & grade I. The corresponding biopsy specimens were graded by Elston modified Bloom & Richardson method (Elston *et al.* 1990). On histological grading 40% cases belong to grade I, 39% cases were of grade II & 21% cases were of histological grade III.

- The cytological grade was correlated well with the histological grade.
- The present study showed overall concordance rate 75.71%.
- Which is comparable to the concordance rate obtained in most of the recent studies as shown in Table 2.

Correlation between cytological grade and histological grade was calculated by Spearman's correlation coefficient (rho-value) that is 0.691, which indicates that there is a strong correlation between cytological and histological grade. The high value of coefficient of correlation showed a significant and marked association ( $p < 0.01$ ) between the grade assigned to cytological and histological specimen overall accuracy of cytological grading system in present study was 81.78% while Vidhya Vasudev *et al.* (2013) found 67.7% and Jayaram *et al.* (2005) found 71% Taniguchi *et al.* (2000) reported 44%,

Robinson *et al* (1994) reported 56.9% and Meena *et al.* (2006) reported 83% accuracy of cytological grade.

The variation in different studies may be due to different techniques used and heterogeneity of degree of atypia within the same tumor and observer subjectivity when assigning a cytological nuclear grade (Das *et al.*, 2012). Multiple regression analysis of various cytological features were used to assess the significance of each cytological feature. The coefficient of regression for the following parameters cell dissociation, cell uniformity, cell size, nucleoli, nuclear membrane and chromatin were 0.290, 0.822, 0.234, 0.326, 0.348 and 0.341. as shown in table-18. For these 6 parameters the p value were 0.000, 0.425, 0.146, 0.000, 0.000 & 0.000 respectively which confirms that all of the features included in Robinson's cytological grading system were statistically significant ( $p=0.000$ ) and all of them equally contributed in determining the cytological grade of tumor.

In the present study, majority (55.71%) of the patient showed metastatic lymphnode deposits and all were in ipsilateral lymphnode and higher positivity rate was associated with higher histological grade. Other studies like Vidya Vasudev *et al.* (2013) (56.7%), Taxin *et al.* (1997) (89%) and Chattopadhyay *et al.* (1995) showed similar observation.

## Conclusion

This prospective study showed that Robinson's grading system of breast carcinoma is less tedious, quick and correlated well with histological grading and typing. Together with lymphnode status, it helps in determining the prognosis. Hence cytological typing and grading should be incorporated in the FNAC report and this can be of great value before choosing the treatment protocol.

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