



Original Article

# Comparative study of cytomorphological Robinson's grading with modified Bloom-Richardson histopathological grading for breast carcinoma attending BPKIHS

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## Keywords:

Breast carcinoma;  
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## ABSTRACT

**Background:** Breast cancer is an impending public health problem for developing countries. Fine needle aspiration cytology is a minimally invasive technique that yields rapid results and is cost-effective. Robinson's cytological grade is simple, easily reproducible, and correlates precisely with the Modified Bloom-Richardson histological grade, which is the gold standard. This study aims to compare Robinson's cytological grade with the Modified Bloom-Richardson histological grade.

**Materials and Methods:** This is a prospective study where 36 patients diagnosed with breast carcinoma cytologically and confirmed histologically were enrolled. The study was carried out from January 2021 to January 2022 in the Department of Pathology, B.P. Koirala Institute of Health Sciences. Comparison of Robinson cytological grade with Bloom Richardson histological grade was done using concordance rate and absolute concordance rate.

**Results:** The concordance rates of Robinson's cytological grades I, II, and III were 77.8%, 62.5% and 54.5% respectively. The absolute concordance rate was 63.89%. There was a significant correlation between Robinson's cytological grade and Modified Bloom-Richardson histological grade ( $p$  value=0.001,  $T_b=0.493$ ).

**Conclusions:** It is possible to grade breast carcinoma on fine needle aspiration cytology preoperatively, as it corresponds well with histological grade. This would allow the assessment of the tumors without any surgical intervention, so that morbidity associated with overtreatment of low-grade tumors or undertreatment of high-grade tumors can be avoided.

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## INTRODUCTION

Breast cancer has emerged as an impending public health problem for developing countries. In Asia, it is the most common cancer diagnosed among females.<sup>1</sup> In 2020, the total number of new breast cancer diagnoses was 2,261,419 globally, and the total deaths caused by it were 684,996.<sup>2</sup> Using the GLOBOCAN 2020 estimates of cancer incidence and mortality, an estimated 1973 new breast cancer cases were diagnosed in Nepal, while 1049 deaths occurred. It is the second most common cancer among women in Nepal.<sup>3</sup> In Asia, it has become the fourth leading cause of cancer-

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related mortality.<sup>1</sup> Fine needle aspiration is used to evaluate palpable breast masses and cysts, as well as non-palpable mammographic abnormalities.<sup>4</sup> The technique is minimally invasive, produces a speedy result, and is inexpensive.<sup>5</sup> Fine needle aspiration cytology (FNAC) is an important part of the triple assessment of a palpable breast lump. Its diagnostic accuracy increases to 99% when combined with clinical and radiological examination.<sup>6</sup> It is a relevant method for the preoperative pathological diagnosis of breast carcinoma in a developing nation.<sup>7</sup>

A preoperative diagnosis relieves the patient's anxiety, saves time, is cost-effective, allowing one-step definitive surgery including lymph node sampling in malignant cases; reduces the need for frozen section diagnosis, and a definitive treatment plan can be prepared and discussed with the patient in advance.<sup>5</sup> The grading of breast cancer on FNAC helps in understanding the biology of the disease, to predict the outcome, and for preoperative prognostication.<sup>8</sup> It is also useful in patients with locally advanced disease, older patients with accompanying chronic diseases, and patients who reject surgery.<sup>9</sup> The National Cancer Institute, Bethesda, sponsored conference had recommended that the tumor grading on fine needle aspiration (FNA) material should be incorporated in FNA reports for prognostication.<sup>10</sup> Cytological grading is recommended to be followed by all cytopathologists, to bring uniformity in the reporting of breast FNAs for grading the malignant lesions.<sup>11</sup>

Among the various cytological grading systems, Robinson's cytological grading (RCG) system is simple, objective, takes little time and effort, is reproducible, and correlates precisely with Modified Bloom- Richardson histological grade (MBRG).<sup>10</sup>

RCG can be used for the selection of neoadjuvant therapy and would allow the assessment of the tumors without any surgical intervention, so the morbidity associated with overtreatment of low-grade tumors or under-treatment of high-grade tumors can be avoided.<sup>10,12</sup> High-grade tumors are faster-growing tumors and more likely to respond to adjuvant chemotherapy after surgery than low-grade slow-growing tumors, which are better suited to neoadjuvant therapy before surgery with tamoxifen.<sup>13</sup>

Robinson's grading uses six different cytological parameters as shown in Table 1.<sup>13</sup> Grade I is given if the total score is 6-11. Similarly, grade II if the total score is 12-14 and grade III if the total score is 15-18.

**Table 1: Robinson's cytological grading for Breast Carcinoma<sup>13</sup>**

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

Histopathological grading of breast carcinoma is done by the Modified Bloom-Richardson grading system (Table 2)<sup>14</sup>, which is a widely accepted tumor grading system and has been found to have good prognostic correlation.<sup>12</sup> Tumors having a score of 3-5 are graded as grade I, scores of 6-7 as grade II, and scores of 8-9 as grade III.

**Table 2: Modified Bloom and Richardson histological grading for Breast Carcinoma<sup>14</sup>**

Score	1	2	3
Tubule formation	>75% of tumor has tubules	10-75% of tumor has tubules	<10% of tumor has tubules
Nuclear pleomorphism	Minimal, Small regular nuclei, <1.5 times the size of benign epithelial cell nuclei	Mild to moderate, 1.5-2 times the size of benign epithelial cell nuclei	Marked, > twice the size of benign epithelial cell nuclei
Mitotic count 0.55 mm field diameter	<=8 Per 10 hpf	9-17 Per 10 hpf	>=18 Per 10 hpf

The purpose of this study is to grade breast carcinoma on FNAC as per the criteria proposed by Robinson and colleagues (RCG) and compare it with histological grading based on the Modified Bloom-Richardson system (MBRG).

## MATERIALS AND METHODS

In this prospective study, 36 patients diagnosed with breast carcinoma cytologically and confirmed histologically were enrolled. This study was carried out from January 2021 to January 2022. Ethical clearance (IRC/1948/020) was ensured from the institutional review.

All patients diagnosed with carcinoma of the breast cytologically and confirmed histologically in lumpectomy and mastectomy specimens were included in the study. Patients unwilling to give consent, patients having a history of recurrent breast carcinoma, patients having a history of chemotherapy or radiotherapy before mastectomy, and cases without histopathological follow-up were excluded from the study.

FNAC was performed in the cytology department. The smears diagnosed as C4: suspicious for malignancy and C5: Malignant were graded according to Robinson's cytological grading, then compared with the Modified Bloom Richardson grading system on lumpectomy or mastectomy specimens of the same patient.

For cytological examination, two smears were air-dried and stained with May-Grunwald-Giemsa (MGG) stain, and the other two smears were immediately fixed in 95% alcohol and stained with Papanicolaou (PAP) stain. For histological examination, paraffin-embedded section was cut and stained with hematoxylin and eosin. Prepared slides were examined with a Nikon Eclipse E600 microscope under 4x, 10x, and 40x objective lenses.

Data was entered in Excel v 11 and exported to SPSS 2020 for data analysis. For descriptive statistics, frequency, percentage, and mean were calculated along with tabular presentations, as applicable. Kendall's tau-b test was used to measure the strength of agreement between cytological and histological grades. Concordance rate, absolute concordance rate, and chi-square test were used to see the association between RCG and MBRG. Statistical significance was tested with a 95% confidence interval, and p-value of <0.05 was considered significant.

## RESULTS

This cross-sectional study included 36 cases diagnosed as carcinoma of the breast cytologically and confirmed histologically. The mean age of patients at presentation was 51.22 years, with the majority of cases, i.e., 44.5% in the 41-50 years age group. The minimum age of the patient was 33 years, and the maximum age was 75 years. There were no male patients diagnosed with breast carcinoma during the study period. Among the cases, the minimum duration of presentation with a breast lump was 10 days, whereas the maximum duration of presentation was 2 years.

Fifty percent of cases presented with a painful breast lump, while the remaining 50% had a breast lump without pain. Only two patients had a history of carcinoma in their relatives, and one of them was also nulliparous. The tumor was predominantly seen in the upper outer quadrant (UOQ) (50%) followed by upper inner quadrant (UIQ) (25%). The smallest size of the lump was 1.5 cm and the size of largest lump was 25 cm with mean size of 4.38 cm at the time of presentation. Thirty-three cases (91.7%) had irregular margin of lump at the time of presentation. Seventeen (47.2%) of lumps were firm during presentation whereas 16 (44.4%) of cases were hard and 3 (8.3%) of cases were soft. Total of 7 cases (19.45%) were diagnosed as C4: Suspicious of Malignancy and 29 cases (80.6%) were diagnosed as C5: Malignant in FNAC. Most common diagnosis made in histopathological examination was invasive breast carcinoma, no special type with total number of 27 cases (75%).

MGG stained smears were evaluated in cytology and the tumors were graded based on the grading system described by Robinson et al. Among 36 cases, nine cases (25%) were in grade I, 16 cases (44.4%) in grade II and 11 cases (30.6%) in grade III. Sections after mastectomy were stained by H&E stain and histologically graded according to the Modified Bloom-Richardson system. Ten cases (27.8%) were in grade I, 15 cases (41.6%) in grade II and 11 cases (30.6%) in grade III.

Comparison was done of the Robinson's cytological grade with Modified Bloom Richardson histological grade (Table 3). Among 36 cases, it was found that seven of the cases were in Grade I of both Robinson's cytological grade and Bloom Richardson histological grade. Similarly, ten cases were in Grade II and six cases were in Grade III respectively of both Robinson's cytological grade and Bloom Richardson histological grade. Thirteen of the cases were either under graded or over graded in MBRG. The RCG and MBRG were found to be significantly associated ( $p=0.001$ ). Correlation analysis was also done between RCG and MBRG. It was found that these grading systems were strongly correlated and statistically significant ( $T_b=0.493$ ,  $p=0.001$ ).

**Table 3: Comparison between Robinson's cytological grade and Modified Bloom Richardson histological grade**

Robinson's cytological grading	Bloom Richardson histological grading			Total	p value
	I	II	III		
I	7	1	1	9	0.001
II	2	10	4	16	
III	1	4	6	11	
<b>Total</b>	<b>10</b>	<b>15</b>	<b>11</b>	<b>36</b>	

Concordance rate of Robinson's cytological grade I, II and III is 77.8%, 62.5% and 54.5% respectively and Absolute concordance rate is 63.89% as shown in Table 4.

**Table 4: Concordance of Robinson's cytological grade with histological grade and Absolute concordance rate**

Grade	Number of concordant cases between each cytological and histological grade	Number of cases in each cytological grade	Concordance rate (%)
I	7	9	77.8
II	10	16	62.5
III	6	11	54.5
<b>Total</b>	<b>23</b>	<b>36</b>	
Absolute concordance rate: 63.89%			

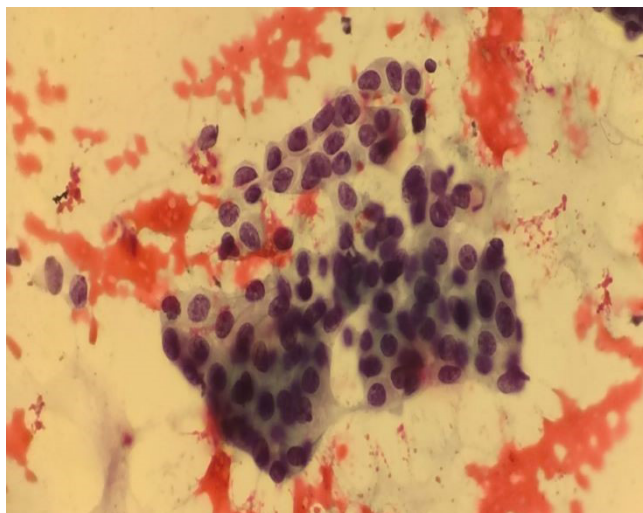
In this study, 15 cases out of total 36 cases (41.66%) showed axillary lymph node metastasis. In RCG grade I, two out of nine cases (22.22%) showed axillary lymph node metastasis. Similarly, in grade II, nine out of 16 cases (56.25%) and in grade III, four out of 11 cases (36.36%) showed axillary lymph node metastasis. A positive correlation was present between presence of nodal metastasis and two of the



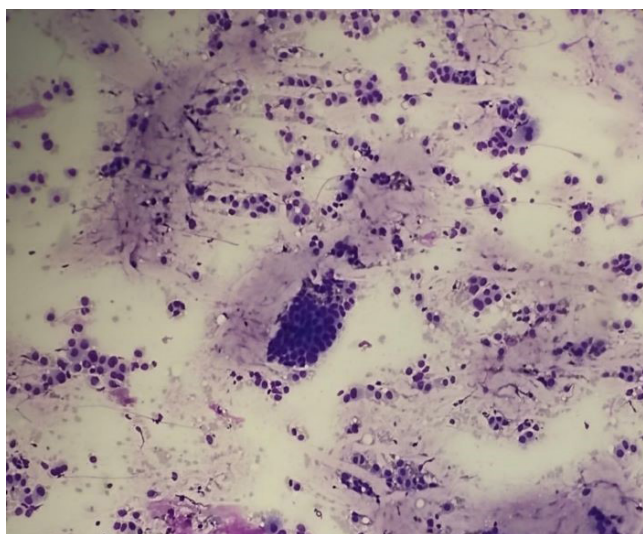
features evaluated in the Robinson grading system— cell dissociation and cell uniformity as shown in Table 5.

**Table 5: Correlation of cytological features with axillary lymph node metastasis**

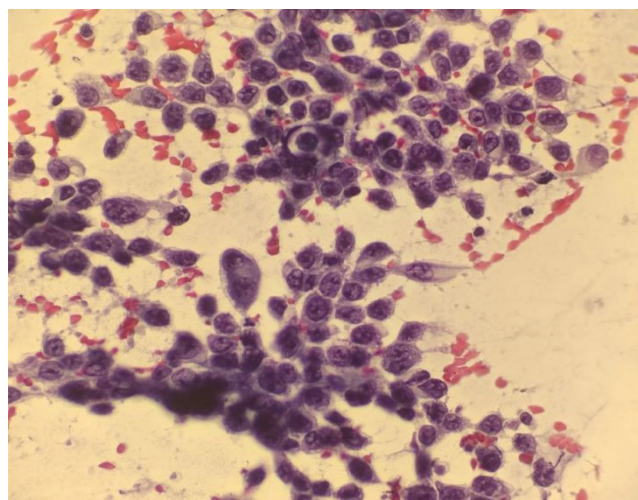
Cytological features	Spearman Correlation Coefficient	
	R value	P value
Cell dissociation	0.435	0.008 (Significant, S)
Cell size	0.297	0.079 (Not Significant, NS)
Cell uniformity	0.470	0.004 (S)
Nucleoli	0.092	0.595 (NS)
Nuclear margin	0.150	0.381 (NS)
Chromatin	-0.63	0.713 (NS)



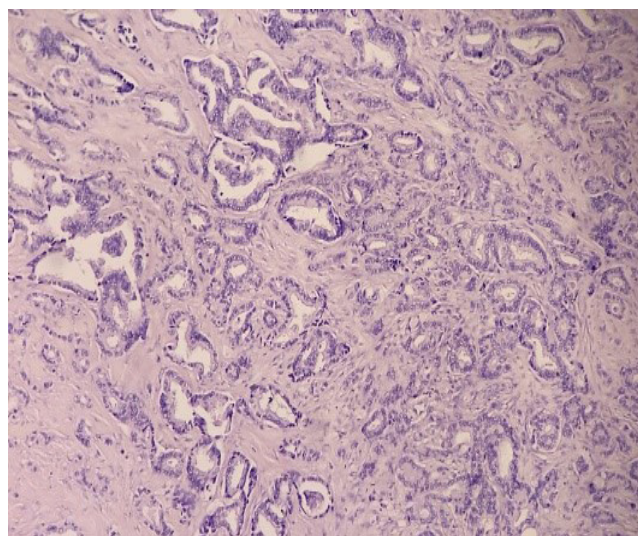
**Figure 1:** Tumor cells with 1-2 x RBCs size (Score 1), mildly pleomorphic (Score 2), noticeable nucleoli (Score 2) and smooth nuclear margin (Score 1) (PAP, x400)



**Figure 2:** Tumor cells arranged as mixture of single cells and clusters – Score 2 (MGG, x100)



**Figure 3:** Tumor cells with size  $\geq 5$  x RBCs (Score 3), pleomorphic (Score 3), prominent nucleoli (Score 3), clefting of nuclear margin (as shown by arrow) - Score 3 and clumped chromatin – Score 3 (PAP, x400)



**Figure 4:** >75% of tumor showing tubule formation (Score 1) (H and E, x100)

## DISCUSSION

In this study, the mean age of presentation was 51.22 years. Agarwal AA et al and Patel NK et al showed mean age of 52.8 years and 50.22 years respectively which is in concordance with this study.<sup>11-15</sup> Similarly, highest number of cases in this study was in age group 41-50 years followed by 51-60 years accounting 44.5% and 27.8% respectively. Agarwal AA et al, Sudhakar G et al, Jayasree KAM et al and Kathed V et al also showed highest number of cases in age group of 41-50 years followed by 51-60 years which is similar to this study.<sup>11,16-18</sup>

The maximum age in this study was 75 years which is similar to the study carried out by Kareem NM et al, Agarwal AA et al and Deshmukh SD et al with maximum age being 75 years, 77 years and 75 years respectively.<sup>11,19,20</sup> Similarly,

minimum age in our study was 33 years which is similar with Kaur M et al and Kareem NM et al with minimum age being 34 years and 36 years respectively.<sup>20,21</sup>

In this study, all 36 patients were female. In contrast, two male patients were included in Kathed V et al (total number of cases: 112) and one male patient in Lingegowda JB et al (total number of cases: 50).<sup>18,22</sup> Male patients were not found in this study. This could be due to a smaller number of sample size and also due to less awareness about breast carcinoma in male patients.

In this study, 63.9% (23 cases) presented with lump in right breast. This is similar to the study conducted by Sudhakar G et al and Pandey P et al with 54% and 63.33% of cases with right breast lump respectively.<sup>16,23</sup> Whereas in the study conducted by Lingegowda JB et al and Chandanwale SS et al, breast lump was seen predominantly in Left sided breast.<sup>22,24</sup>

In present study, the tumor was predominantly seen in UOQ followed by UIQ. This is in concordance with the study done by Teronpi J et al which showed maximum number of 31 out of 60 cases i.e. 51.67% having palpable lump in UOQ followed by 9 cases (15%) in UIQ.<sup>25</sup> Similarly, Pandey P et al also showed maximum of 50% of cases among total 30 cases having palpable lump in UOQ.<sup>23</sup>

In this study, maximum 75% of cases were diagnosed as invasive breast carcinoma, no special type. This finding is consistent with the fact that it is the most common type of invasive breast carcinoma.<sup>26</sup> Similarly, Agarwal AA et al, Teronpi J et al and Saha K et al also diagnosed maximum number of cases as invasive ductal carcinoma, no special type with total cases of 93.33%, 54% and 93% respectively.<sup>10,11,25</sup>

In this study, maximum numbers i.e. 16 of 36 cases (44.4%) were in RCG Grade II followed by 11 cases (30.6%) in grade III and 9 cases (25%) in grade I. This finding is similar to the studies conducted by Wani FA et al, Sudhakar G et al and Deshmukh SD et al as shown in Table 6.<sup>16,19,27</sup> Jayasree KAM et al, Kareem NM et al, Lingegowda et al and Jyothi AV et al showed maximum number of cases in Grade I having 28 (50.9%), 33 (47.14%), 30 (60%) and 22 (50%) cases respectively.<sup>17,20,22,28</sup> Jyothi AV et al has mentioned that it may be due to the early presentation of patients because of increased awareness of breast cancer in and around of study location.<sup>28</sup> Patel JPP et al showed highest number i.e. 17 of 35 cases (49%) in Grade III.<sup>29</sup>

In this study, maximum number i.e. 15 of 36 cases (41.7%) was in MBRG Grade II followed by 11 cases (30.6%) in grade III and 10 cases (27.8%) in grade I. This finding is similar to Chalise S et al, Das A.K et al, Chandanwale SS et al, Sudhakar G. et al and Phukan JP et al as shown in Table 7.<sup>9,12,16,24,30</sup>

Among 36 cases, the total of 23 cases (63.89%) showed concordance between the respective cytological and

histological grades (Table 4). Maximum concordance rate was seen in Grade I (77.8%) followed by Grade II (62.5%) and minimum concordance was seen in Grade III tumor i.e. 54.5%. Similar findings were also reported by other studies such as Kaur M. et al having concordance rate of 79%, 78% and 71% in Grade I, II and III respectively<sup>21</sup> and Sood N. et al having concordance rate of 75%, 70.67% and 60% in Grade I, II and III respectively.<sup>8</sup>

Among the 13 discordant cases, seven cases were under graded whereas remaining six cases were overgraded in MBRG. Highest discordance was observed in grade III tumors followed by grade II and grade I. Discordance may be due observer variability when assigning the grade. It is more likely observed while identifying features such as nuclear margins (smooth/ folds/ buds and clefts) and chromatin pattern (vesicular/ granular/ clumped and cleared). This discrepancy may also be attributable to tumor heterogeneity and large tumor size. When tumor size is large, the most undifferentiated areas of tumor may not be represented in routine FNAs. In contrast with histopathological examination, the entire tumor is sampled, so that it allows the evaluation of most undifferentiated areas.<sup>28</sup> The MBR histological grading system is based on extent of tubule formation, number of mitotic figures and degree of nuclear pleomorphism. But several features like nuclear margin, chromatin pattern and nucleoli that are included in cytological grading are not of much importance in diagnosing or grading by histopathology.<sup>31</sup>

The absolute concordance rate of this study was 63.89% which is similar to study carried out by Chalise S et al, Sood N et al, Phukan JP et al, Srivastava P et al, Lingegowda JB et al and Das AK et al with absolute concordance rate with 65.9%, 68.97%, 72.2%, 60%, 72.57% and 71.2% respectively.<sup>8,9,12,22,30,32</sup>

Concordance rates and absolute concordance rate in different studies are as shown in Table 8.

In this study, as lymph node metastasis was seen in all grades without any specific pattern, it can be concluded that lymph node metastasis occurred irrespective of grade of tumor. Similarly, the study conducted by Jyothi AV et al also showed lymph node metastasis irrespective of grade of tumor with 68.1%, 52.6% and 100% nodal metastasis in Grade I, II and III respectively.<sup>28</sup> Whereas, studies conducted by Agarwal AA et al, Robles- Frias A et al and Pal S et al showed increased nodal metastasis with increasing grade of tumor.<sup>11,34,35</sup> Positive correlation was present between presence of nodal metastasis and cell dissociation and cell uniformity parameters of RCG in current study. Whereas, the studies conducted by Robles- Frias A et al and Lingegowda et al showed positive correlation with cell dissociation, cell uniformity and nuclear margin as well. Similarly in Chalise S et al, cell dissociation was an important cytological feature for association between increase in cell dissociation and incidence of lymph node metastasis.



## CONCLUSION

This study concludes that it is possible to grade breast carcinoma on FNAC for assessing breast carcinoma preoperatively and it corresponds well with histological grade. This would provide prognostic information regarding aggressiveness of tumor and would guide the surgeon regarding judicious use of neo- adjuvant therapy and to avoid over treatment of low grade tumor.

However, histological grading remains the gold standard for grading breast carcinoma.

## Limitations of study

This study was carried out over a short period of time, which led to a smaller sample size. A similar study with a larger sample size over a longer duration would increase the accuracy of the results. Additionally, all patients with FNAC reports of C4: Suspicious of Malignancy and C5: Malignancy did not undergo surgery at BPKIHS, so these cases were lost to follow-up and excluded from this study.

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