



Original Article

Correlation between Robinson's cytological grading with Modified Bloom-Richardson histopathological grading for Breast carcinoma

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ABSTRACT

Background: Invasive breast carcinoma is the most common carcinoma in women. Fine needle aspiration cytology is the important part of triple assessment of the palpable breast lump. Robinson's cytological grading can be done in fine needle cytology smears of breast lump. The aim of this study is to grade breast tumor on cytology according to Robinson cytological grading and to correlate with histopathological grading.

Materials and Methods: This prospective study was done in Department of pathology, Bhaktapur Cancer Hospital from 1st April 2014 to 30th July 2015. A total of 58 cases of cytologically confirmed breast carcinoma were included in this study and correlated with histopathological findings. Robinson's cytological grading was done on cytology smears and modified Bloom-Richardson grading was done on histological sections. Correlation between these two grading was done by using spearman's correlation coefficient.

Results: Cytologically, most common tumor was grade II (62.1%) followed by grade I (19%) and grade III (19%). Histologically, most common tumor was grade II (63.8%) followed by grade III (22.4%) and grade I (13.8%). High concordance rate was found in grade II tumor (80%) with absolute concordance being 65.9%. On cytology, cell dissociation and nuclear margin were determining features ($p < 0.005$). Correlation between cytological grading and histological grading was statistically significant ($p < 0.005$).

Conclusion: Robinson's cytological grading is the easy grading method that can be done on cytology smears of the breast carcinoma to achieve relevant information regarding aggressiveness of the tumor and to plan for neoadjuvant chemotherapy preoperatively.

INTRODUCTION

Invasive breast carcinoma is a most common carcinoma in women. It accounts for 23% of female, 27% in affluent

countries, which is more than twice the occurrence of cancer in women at any other site.¹ From the hospital based cancer registry of Nepal, which contains pooled data from seven major hospitals where cancer is diagnosed and treated, there were a total of 2910 cancer patients in the year 2009, 211 of these were breast cancers, which represent 7.2% of all malignancies, and is the second most commonly diagnosed cancer in Nepal.² Cancer education, screening and early detection are the key elements to influence the diagnosis,

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Table 1: Cytological grading according to Robinson's Grading system

| Score | 1 | 2 | 3 |
|-------------------|--------------------------|--------------------------------------|--------------------------|
| Cell dissociation | Cells mostly in clusters | Mixture of single cells and clusters | Mostly single cells |
| Cell size | 1-2 times size of RBC | 3-4 times size of RBC | >=5 times size of RBC |
| Cell uniformity | Monomorphic | Mildly Pleomorphic | Pleomorphic |
| Nucleoli | Indistinct | Noticeable | Prominent or pleomorphic |
| Nuclear margin | Smooth | Slightly irregular/folds and grooves | Buds and clefts |
| Chromatin | Vesicular | Granular | Clumped and cleaved |

treatment and prognosis of breast cancer in Nepal.³

Fine needle aspiration cytology (FNAC) is an important of the triple assessment of palpable breast lump. It has been shown that the FNAC can reduce the number of open biopsies.⁴ The standard prognostic factors, recognized by the National Cancer Institute in 1990, include lymph node status, tumor size, nuclear grade, steroid receptor content, tumor type, and cellular proliferation rate and recommend that for patients who undergo preoperative chemotherapy or radiotherapy, breast fine-needle aspirates

Table 2: Histological grading of breast carcinoma (modified Bloom-Richardson grading)

| Score | 1 | 2 | 3 |
|---|---------------|-----------------|---------------|
| Tubule formation | >75% of tumor | 10-75% of tumor | <10% of tumor |
| Nuclear pleomorphism | Minimal | Moderate | Marked |
| Mitotic count (per 10 high power field) | 0-9 | 10-19 | >20 |

Table 3: Evaluation of cytological features on cytological and histological grade

| Cytological score | Cytological grade | | | Histological grade | | |
|--------------------------------------|-------------------|----------|-----------|--------------------|----------|-----------|
| | Grade I | Grade II | Grade III | Grade I | Grade II | Grade III |
| Cell dissociation | | | | | | |
| Mostly clusters | 9 | 11 | 0 | 6 | 13 | 1 |
| Single cells and clusters | 2 | 22 | 7 | 2 | 20 | 9 |
| Mostly single cells | 0 | 3 | 4 | 0 | 4 | 3 |
| Cell size | | | | | | |
| 1-2 times size of RBC | 7 | 3 | 0 | 4 | 6 | 0 |
| 3-4 times size of RBC | 4 | 15 | 0 | 4 | 12 | 3 |
| >=5 times size of RBC | 0 | 18 | 19 | 0 | 19 | 10 |
| Cell Uniformity | | | | | | |
| Monomorphic | 7 | 2 | 0 | 6 | 3 | 0 |
| Mildly Pleomorphic | 4 | 29 | 5 | 2 | 29 | 7 |
| Pleomorphic | 0 | 5 | 6 | 0 | 5 | 6 |
| Nucleoli | | | | | | |
| Indistinct | 9 | 4 | 0 | 6 | 6 | 1 |
| Noticeable | 2 | 19 | 1 | 1 | 18 | 3 |
| Prominent or pleomorphic | 0 | 13 | 10 | 1 | 13 | 9 |
| Nuclear margin | | | | | | |
| Smooth | 10 | 3 | 0 | 4 | 8 | 1 |
| Slightly irregular/folds and grooves | 1 | 31 | 5 | 4 | 26 | 7 |
| Buds and clefts | 0 | 2 | 6 | 0 | 3 | 5 |
| Chromatin | | | | | | |
| Vesicular | 6 | 3 | 0 | 4 | 4 | 1 |
| Granular | 5 | 28 | 3 | 4 | 26 | 6 |
| Clumped and clear | 0 | 5 | 8 | 0 | 7 | 6 |

Table 4: Distribution of cases according to Robinson's cytological grading system

| Cytological grade | Number of cases | Percentage |
|-------------------|-----------------|------------|
| Grade I | 11 | 19% |
| Grade II | 36 | 62.1% |
| Grade III | 11 | 19% |

Table 5: Distribution of cases according to modified Bloom-Richardson grading system

| Histological grade | Number of cases | Percentage |
|--------------------|-----------------|------------|
| Grade I | 8 | 13.8% |
| Grade II | 37 | 63.8% |
| Grade III | 13 | 22.4% |

Table 6: Concordance rate of cytological grading and histological grading

| Robinson's cytological grade | Histological grade | | | Total | Concordance rate |
|------------------------------|--------------------|------------|------------|------------|------------------|
| | Grade I | Grade II | Grade III | | |
| Grade I | 5 | 5 | 1 | 11 (19%) | 45% |
| Grade II | 3 | 29 | 4 | 36 (62.1%) | 80% |
| Grade III | 0 | 3 | 8 | 11 (19%) | 72.7% |
| Total | 8 (13.8%) | 37 (63.8%) | 13 (22.4%) | 58 | 65.9% |

Table 7: Correlation of cytological features with cytological grade using Univariate analysis

| Cytological Grade | Spearman correlation coefficient | p-Value |
|-------------------|----------------------------------|---------|
| Cell dissociation | 0.569 | 0.000 |
| Cell size | 0.669 | 0.000 |
| Cell uniformity | 0.619 | 0.000 |
| Nucleoli | 0.685 | 0.000 |
| Nuclear margin | 0.755 | 0.000 |
| Chromatin | 0.641 | 0.000 |

Table 8: Multiple regression analysis of cytological grade

| | Regression coefficient | p-Value |
|-------------------|------------------------|---------|
| (Constant) | -.320 | .063 |
| Cell dissociation | .263 | .000 |
| Cell size | .099 | .176 |
| Cell uniformity | .072 | .417 |
| Nucleoli | .104 | .154 |
| Nuclear margin | .415 | .000 |
| Chromatin | .221 | .013 |

can be used to provide prognostic information.⁵ Cytological grading of breast carcinoma, which is a simple, feasible and reproducible method, can be used for selection of neoadjuvant therapy and would allow the assessment of the tumors without any surgical intervention so that the morbidity associated with overtreatment of low-grade tumors can be avoided.⁶ The concept of nuclear grading in breast carcinoma was introduced by Black et al⁷ in 1955. It was applied in cytological smears after modification by Fisher et al^{8,9} or after simplification by Cajulis et al.¹⁰

Histopathological grading of breast carcinoma using modified Bloom-Richardson grading system is a widely accepted tumor grading system and has been found to have good prognostic correlation. Based on the cytological features, various grading systems have evolved. Of the different cytological grading methods corresponding to modified Bloom-Richardson grading, the Robinson's cytological grading was found to be useful in grading breast carcinoma in FNAC.¹¹

The purpose of the present study was to compare cytological grading using Robinson's method with histological grading using modified Bloom-Richardson grading method.

MATERIALS AND METHODS

This was a prospective study done in department of pathology, Bhaktapur cancer hospital, Nepal from 1st April 2014 to 30th July 2015. 58 cases with cytologically confirmed breast carcinoma were included in this study and correlated with histopathological findings. FNAC of breast lump was done with 22 gauge needle which were attached to 10ml syringes. Smears made were stained with Giemsa as well as Hematoxylin and Eosin stain (H&E). 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 (Table 1). The lumpectomy and mastectomy specimens of the corresponding cases were fixed in 10% formalin, routinely processed and stained with H&E stain. Histopathological grading was done according to modified Bloom-Richardson grading method in which tubule formation, nuclear pleomorphism and mitotic count were evaluated (Table 2).

Correlation between Robinson's cytological and modified Bloom-Richardson histopathological grading was done. Data was analysed using the statistical package for social science (SPSS) and final correlation was established using

Table 9: Correlation of histological features with histological grade using Univariate analysis

| Histological features | Spearman's correlation coefficient | |
|-----------------------|------------------------------------|---------|
| | r-value | p-value |
| Tubule | .575 | .000 |
| Pleomorphism | .584 | .000 |
| Mitosis | .641 | .000 |

Table 11: Correlation of cytological grading with Histological grading

| | Spearman's correlation coefficient | |
|--|------------------------------------|---------|
| | r-value | p-value |
| Cytological Grade and Histological Grade | .575 | .000 |

Spearman's correlation coefficient.

RESULTS

A total number of 58 cases were included in this study, aged between 27 years and 80 years. Mean age was 46.12 years. Cytological scores were analyzed using cytological grade and histological grade and the details are shown in Table 3, Figure 1, 2 and 3.

In this study, using Robinson's cytological grading, maximum number of carcinoma were grade II (62.1%), followed by grade I (19%) and grade III (19%) as shown in Table 4. Using histopathological grading, maximum numbers of cases were grade II (63.8%), followed by Grade III (22.4%) and Grade I (13.8%) as shown in Table 5.

The absolute concordance rate was 65.9% (Table 6). The highest concordance rate of 80% was found in Grade II tumor.

DISCUSSION

FNAC may be performed as the first-line investigation, especially in symptomatic and screening populations. The main purpose of FNAC of breast lumps is to confirm cancer preoperatively and to avoid unnecessary surgery.¹² Among the various cytological grading systems, Robinson's system is simple, more objective, takes little time and effort, is reproducible and correlates precisely with modified Bloom-Richardson histological grade.⁶

Cytological grade and histological grade was evaluated according to the Robinson's cytological features. In the current study, the majority of the cases were Grade II (62.1%) followed by Grade I (19%) and Grade III (19%) cytologically. Histologically, commonest tumor was Grade II (63.8%) followed by Grade III (22.4%) and Grade I (13.8%). Sood N et al⁵, Gore CR et al¹³ and Phukan JP et

Table 10: Multiple regression analysis of Histological grade

| | Regression coefficient | p-value |
|--------------|------------------------|---------|
| (Constant) | -.274 | .106 |
| Tubule | .405 | .000 |
| Pleomorphism | .312 | .000 |
| Mitosis | .398 | .000 |

Table 12: Comparative table on cytological grading and histological grading in relation to the present study

| Study done by | p-value |
|----------------------|---------|
| Present study | 0.000 |
| Rajan J and Pai KP14 | 0.000 |
| Lingegowda et al18 | <0.001 |
| Frias et al21 | <0.0005 |

al¹¹ also found grade II as a commonest tumor on cytological grading and histological grading, whereas Rajan J and Pai KP¹⁴ in their study found grade I as a commonest tumor.

Present study showed, absolute concordance rate of 65.9% which is comparable with the study done by Sood N et al⁵, Phuken et al¹¹ and Das et al¹⁵ where they found absolute concordance rate of 68.97%, 72.2 % and 69% respectively. The highest concordance rate of 80% was found in Grade II tumor in our study which was similar to the study done by Phuken et al¹¹ where they found 83% concordance rate in grade II and grade III tumor. But in contrast to this study, Sood N et al⁵ found highest concordance rate in grade I tumor (75%).

The highest level of discordance was found in grade I tumor in our study which is similar to the study done by Phukan et al¹¹ however sood N et al⁵ found highest level of discordance in Grade III tumor. The cause of discordance may be because of subjective variation during assessment of cytological features that are not the features of histological grading. Dabbas DJ et al¹⁶ and Howell LP et al¹⁷ found difficult to assess tubule formation and mitotic index on cytology and conclude that the discordance may be due to the disparities between cytological and histological features.

In our study, using univariate analysis, all of the cytological features show strong correlation with cytological grade ($p < 0.005$) however the multiple regression analysis show cell dissociation and nuclear margin as a most important influenceable factor. All histological features shown strong correlation and influence in histological grading. Das et al¹⁵ also found strong correlation between cytological features and cytological grade with cell dissociation, nuclear margin, nucleoli and uniformity as a most influenceable factor. Similarly study done by Lingegowda et al¹⁸ found strong correlation between cytological features and cytological grade with cell dissociation, nucleoli and nuclear margin

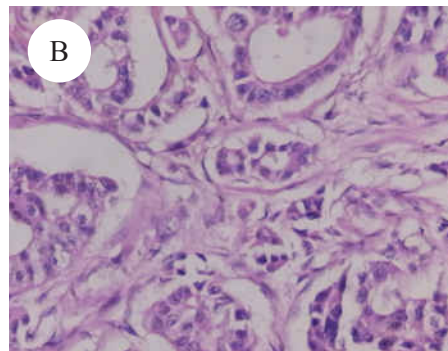
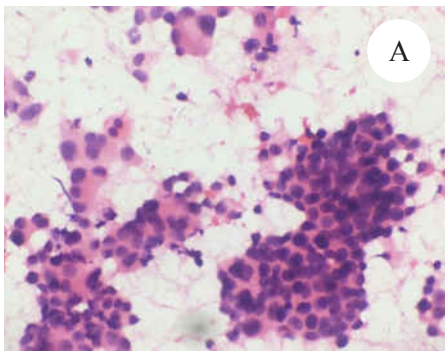


Figure 1(a): Robinson's grade I. Uniform cells with fine nuclear chromatin, smooth nuclear margin and inconspicuous nucleoli (Hand E Stain, X400). **Figure 1(b):** Modified Bloom-Richardson grade I, score 5 of the same patient showing tubules with mildly pleomorphic nuclei (H & E stain, X400).

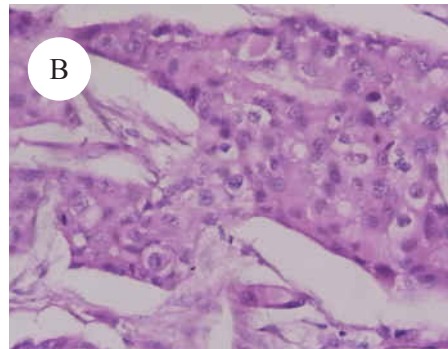
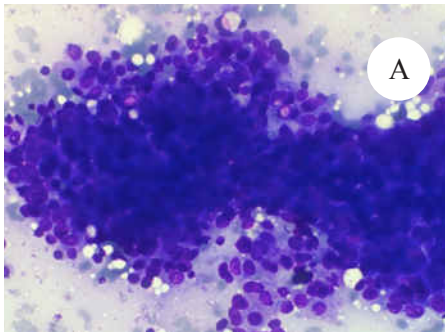


Figure 2(a): Robinson's grade II. Mildly pleomorphic cells with granular nuclear chromatin, slightly irregular nuclear margin and noticeable nucleoli in few cells (Giemsa Stain, X400). **Figure 2(b):** Modified Bloom-Richardson grade II, score 7 of the same patient showing absence of tubules with moderately pleomorphic nuclei and mitotic figure (H & E stain, X400).

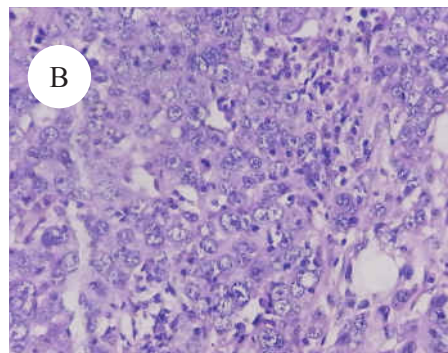
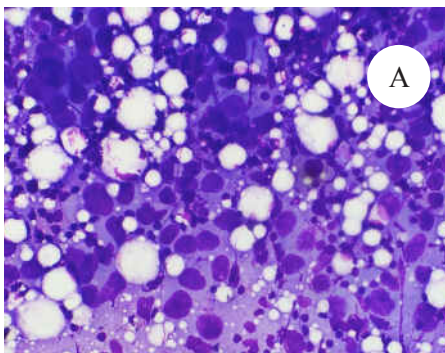


Figure 3(a): Robinson's grade III. Markedly pleomorphic cells with clumped nuclear chromatin, irregular nuclear margin and prominent nucleoli (Giemsa Stain, X400). **Figure 3(b):** Modified Bloom-Richardson grade III, score 9 of the same patient showing absence of tubules with markedly pleomorphic nuclei (H and E stain, X400).

as a most influencable factor. Rajan J and Pai KP¹⁴ in their study also found significant statistical correlation between cytological features and cytological grade however in contrary to our study they found cell size, uniformity and nucleoli as a most influencable factor.

Cell dissociation is found to be an important cytological feature as several studies showed an association between increase in cell dissociation and incidence of lymphnode metastasis as cell cohesion status and expression of

E-Cadherin are indicated by cell dissociation status thus helping in preoperative assessment of the patient.^{19,20}

This study showed statistically significant correlation ($p < 0.005$) between cytological and histological grade which was similar to the study done by Rajan J et al¹⁴, lingegowda et al¹⁸ and Frias et al²¹. Table 12 showed the cytological and histological correlation between various studies in relation to our studies.

All the cytological features are statistically significant using Univariate analysis (Table 7). Cell dissociation and nuclear margin were found to be the most influencable factors using Multiple regression analysis (Table 8). Similarly all the histological features are statistically significant using Univariate analysis and all the histological features have equal influence in histopathological grading (Table 9 and 10)

CONCLUSION

Robinson's cytological grading is a simple, feasible and easily reproducible method of assessing breast carcinoma preoperatively. This cytological grading method can be done preoperatively to determine the aggressiveness of the tumor and to plan for the neoadjuvant chemotherapy.

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