Correlation of Various Cytological Grading Systems with Histopathological Grading in Breast Carcinoma

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ABSTRACT

Background: The nuclear grading of breast carcinoma is well established on histopathology, but not in cytology. There is no single standardised grading system yet in cytology for predicting breast carcinoma prognosis in spite of various cytological grading systems.

Aim: The study was performed with an aim of evaluating various cytological grading systems on needle aspirates of breast carcinoma and to determine the best possible cytological grading system that correlates with the Nottingham modification of Scarf Bloom Richardson(SBR) histologicalgrading.

Materials & Methods: The study consisted of 30 cases of infiltrating ductal carcinoma diagnosed on cytology for which the corresponding histopathology was available.These cases were graded cytologically by eight grading systems and histologically by SBR method.The various cytological grading systems were evaluated for concordance, association, and correlation with the histopathological grading to select best possible cytological grading system. The various grading systems were also evaluated for interobserver reproducibility

Results: A positive correlation was noted between the various grading systems on cytology with SBR method on histopathology. A strong correlation (r =0.925), maximum percent agreement (93.3%), and a substantial kappa value of agreement (k = 0.885) was noted for Robinson's grading with the Nottingham modification of SBR grading system. It also showed betterinterobserver agreement (93.3%; k = 0.889).

Conclusion: The various cytological grading systems evaluated in this study showed a positive correlation with SBR method. Among them, Robinson's grading showed best concordance, correlation with histological grade and hence, may be included in the routine cytology reports.

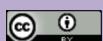*Keywords: Carcinoma Breast, Cytological Grading,Scarff Bloom Richardson Grading*

Introduction

Carcinoma of breast is one of the most common cancers in women in the world and in India it is second most common malignancy.[1] It is a leading cause of malignant death in women, with an annual incidence of more than 1,000,0000 cases. The prognosis of breast carcinoma is determined by various parameters, such as type of tumour, grade of tumour, status of hormone receptors, DNA ploidy, cell proliferation markers and expression of different oncogenes.[2]

One of the well established prognostic parameter is grading of breast carcinoma on histopathology using Elston Ellis modification of ScarffBloomRichardson (SBR) grading system.[3] In recent years, for preoperative diagnosis of breast cancer, Fine needle aspiration cytology(FNAC) is being used increasingly. Recently, attempts are also being made for determination of various prognostic parameters on FNAC for management of breast carcinoma cases.[4]The preoperative nuclear grading of breast carcinoma on cytology is becoming necessary as neoadjuvant therapy is being administered for early breast cancer treatment.[3] Prior to surgery, assessment of the tumour in situ can be performed by cytological grading of breast carcinoma, thus guiding clinician in selecting the most appropriate treatment and avoiding the morbidity associated with overtreatment of low grade tumours.[5]

Currently, for evaluation of cytological aspirates of breast carcinoma cases routinely, no single cytological grading system is being adapted in spite of many cytological grading systems being proposed by various authors.[6] Some authors have compared and correlated the outcome of these cytological grading methods with the biological behaviour of carcinoma similar to the histological grading of SBR method. But, none of the methods is considered the gold standard for nuclear grading on cytological aspirates and even among pathologists and clinicians there is no agreement to accept one of the cytological grading system as effectively as SBR grading system.[1]

In our study, we evaluated cytological aspirates of breast carcinoma using eight cytological grading systems and correlated with SBR method to determine the best cytological grading method which corresponds to the histological grading system.

Materials and Methods

Thirty cases of cytologically proven Infiltrating ductal carcinoma of breast with their respective specimens received in our department were studied over a period from 1st July 2016 to 30th June 2017.The approval for the study was obtained from the ethical committee of the institution.

Eight grading systems were employed on cytology for grading the aspirates. The aspirates were fixed, stained by Papanicolaou staining and later grading was performed by two pathologists Fisher's modification of Black's nuclear grading, Robinson's grading, Howell's grading, Taniguchi grading, Masood's grading, Dabb's grading, Khan's grading and Mouriquand's grading systems were the grading systems used by pathologists for evaluating the cytological aspirates.

Six parameters namely cell dissociation, cell size, uniformity, nucleoli, nuclear margin, and chromatin were included in Robinson's grading system.[7] A score of 1-3 was assigned to each parameter and the total scores were added to estimate the final grade. Total score 6-11 were assigned grade I[Figure 1], Graded II for score of 12-14 , and Graded III for score of 15-18 [Figure 2]. Four parameters were employed in Mouriquand's and Pasquier grading [8,9] consisting of cellular features, nuclear features, chromatin, and mitosis. Each parameter were scored from 0-3 and final grade obtained by adding the scores. Grade I, Grade II and Grade III tumours had a total score of <5, 6-9 and >10 respectively. Seven cytological parameters were employed in grading cytological aspirates using Taniguchi grading[10], all the parameters were assigned a score from 1 to 3 except necrosis which was scored 0 or 1. Tumours with a score of 6- 9 were Grade I, while Grade II tumours had a score of 10-11 [Figure 3] and those with a total score ranging from 12-19 were of grade III[Figure 4]

In Dabbs grading[11]of breast carcinoma cytological aspirates, shape and size of nuclei, nuclear membrane, nuclear chromatin and nucleoli were considered.

In Masoods Grading[12], six parameters such as variation in cell size, arrangement of the tumour cells, variation in nuclear size, presence of myoepithelial cells, nucleoli, clumping of nuclear chromatin were used to obtain the grades.

In Fisher's modification of Black's nuclear grading[13],breast carcinomas were graded from Grade I-III using five parameters such as size of nucleus, shape of nuclei, appearance of chromatin, presence of nucleoli and detection of mitosis. Six cytological parameters were employed in Khan et al grading [14] with each of these parameters being scored from 1-3. The tumours were of Grade I, Grade II and Grade III if the combined score was in the range of 6-10, score 11 to 14 and score 15 -18 respectively.

Howell et al grading system[15] includes three cytological parameters namely tubule formation, nuclear pleomorphism and mitotic count which are also included in histological SBR grading. The only difference between these two grading systems is the estimation of mitotic count. The final grading of these two methods is similar with Grade I tumours having a total score of 3-5, Grade II tumours with 6-7 score and Grade III tumours with 8-9 score .

In H&E stained sections of postoperative mastectomy specimens, Nottingham modification of SBR method[16] was used for histopathological grading[Figure 5,6]. Olympus CH20i microscope with HPF diameter 0.45 mm was used for counting and scoring the mitotic figures.

Recording of the results and statistical analyses of the data for determination of Chi Square test and Spearman's correlation coefficient(p) were performed by employing IBM SPSS statistics software, version 20. A p value < 0.001 was considered as statistically significant to find the association between the grading systems. Kappa(k) measurement of agreement was assessed to find the agreement/concordance among the various grading systems.

Results

The study included a total of thirty cases of invasive ductal carcinoma of breast in whom eight cytological grading systems were performed on cytological aspirates and histological grading was performed by SBR method. Age of the patients ranged 37–65 years in this study. Among the 30 cases studied, majority of the cases on both cytological grading and histopathological grading were of Grade II(18out of 30 cases). Table 1 shows the grade wise distribution of breast carcinoma cases according to the various grading systems employed in cytology and SBR grading in histopathology.

The p value in this study as determined by the Chi-square test, was found to be <0.001 and hence showed a significant association between the various cytological grading systems and the histological grading. The various cytological grading systems and histological SBR grading showed correlations and concordance as shown in Table 2.

All the eight cytological grading systems evaluated in the present study showed a positive correlation with the histological grading as determined by the Spearman's correlation coefficient(p). Among these various cytological grading systems, Robinson's grading demonstrated a concordance of 93.3% and a substantial agreement (k value 0.885) with the histological grading. The least concordance of 70% in this study was noted between Khan's grading and SBR grading, while it showed a fair agreement (k value - 0.458) with the histological grading.

To analyse the interobserver agreement among the various grading systems, assessment of the Kappa measurement of agreement was performed and Table 3 shows the agreement of various grading systems.

Assessment of the interobserver agreement among the various grading systems evaluated in the study, showed a moderate to substantial agreement.

The highest concordance of 93.3% (k = 0.889, 28 out of 30 cases,) was noted between the two pathologists for Robinson's grading, while for Taniguchi grading and Fisher's system the concordance of 90% for each was noted .Both Masoods grading and Khan's grading showed a concordance of 86.7% each. For histological SBR grading, a strong interobserver agreement was noted (k = 0.874).

Table 1: Distribution of cases according to the cytological and histological grading.

Grading	Fisher's grading	Robinson's grading	Dabbs' grading	Khan's grading	Taniguchi's grading	Mouriquand's grading	Howell's grading	Masood's grading	SBR grading
I	7	9	5	4	5	7	6	5	8
II	20	16	22	19	23	17	21	21	18
III	3	5	3	7	2	6	3	4	4
Total	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**30**

Table 2: Correlation and concordance analyses between the cytological grading systems and the histological grading.

	Fisher's grading	Robinson's grading	Dabbs' grading	Khan's grading	Taniguchi's grading	Mouriquand's grading	Howell's grading	Masood's grading
Correlation (spearman ρ)	0.727	0.925	0.602	0.665	0.755	0.647	0.776	0.776
Concordance	80	93.3	73.3	70	83.3	80	83.3	83.3
Agreement (kappa k)	0.619	0.885	0.469	0.458	0.658	0.652	0.675	0.675

Table 3:Analysis of inter-observer agreement for cytological grading systems.

	Fisher's grading	Robinson's grading	Dabbs' grading	Khan's grading	Taniguchi's grading	Mouriquand's grading	Howell's grading	Masood's grading	SBR grading
Interobserver agreement	27/30	28/30	25/30	26/30	27/30	21/30	24/30	26/30	28/30
Percentage	90	93.3	83.3	86.7	90	70	83.3	86.7	93.3
kappa κ	0.778	0.889	0.593	0.759	0.778	0.469	0.621	0.733	0.874

Table 4: Correlation, concordance and Interobserver agreement of various cytological grading systems with other studies.

| Author	Analysis	Robinson's grading	Moriquand's grading	Fisher's grading	Taniguchi's grading	Khan's grading	Howell's grading	Dabbs grading
Saha et al	Correlation	0.799	0.715	0.535	0.686	0.744	0.674	--
	Concordance	77.19	77.19	70.18	75.44	66.67	63.16	--
	Interobserver agreement	0.48	0.57	0.48	0.46	0.46	0.4	--
Einsten et al	Correlation	0.738	0.613	0.654	0.615	0.696	0.614	0.604
	Concordance	77.7	68	76.3	66.6	72.2	69.4	72.20%
	Interobserver agreement	0.61	0.418	0.526	0.401	0.515	0.436	0.459
Present study	Correlation	0.925	0.647	0.727	0.755	0.665	0.776	0.602
	Concordance	93.30%	80%	80%	83.30%	70%	83.30%	73.3
	Interobserver agreement	0.885	0.65	0.619	0.658	0.458	0.675	0.469
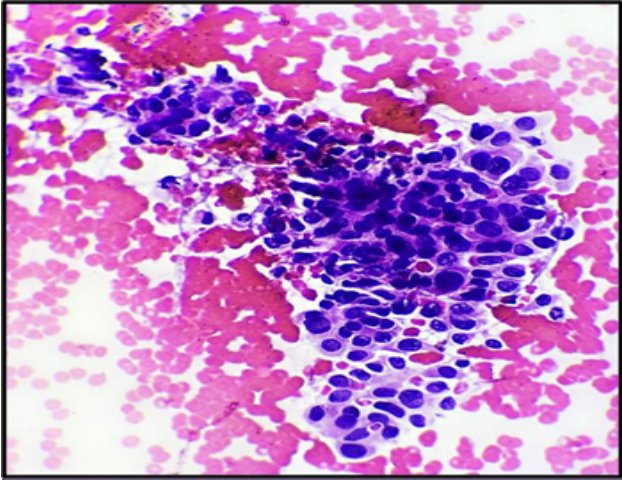
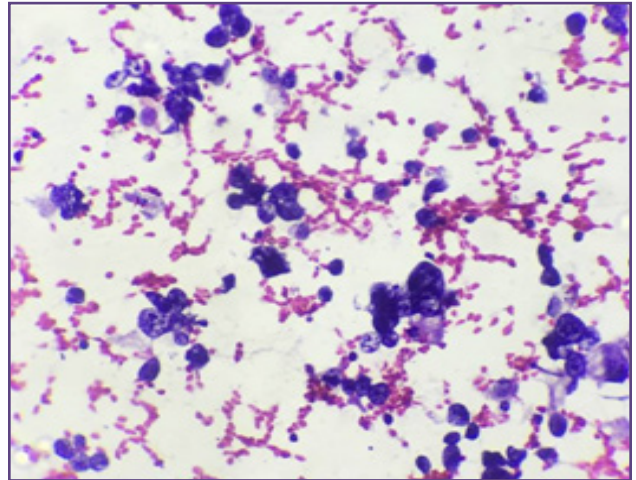**Fig. 1: Robinson's Grade I with monomorphic cells in clusters (Pap, 100X).**

Fig. 2: Robinson's Grade III showing singly scattered pleomorphic cells. (Pap, 100X).
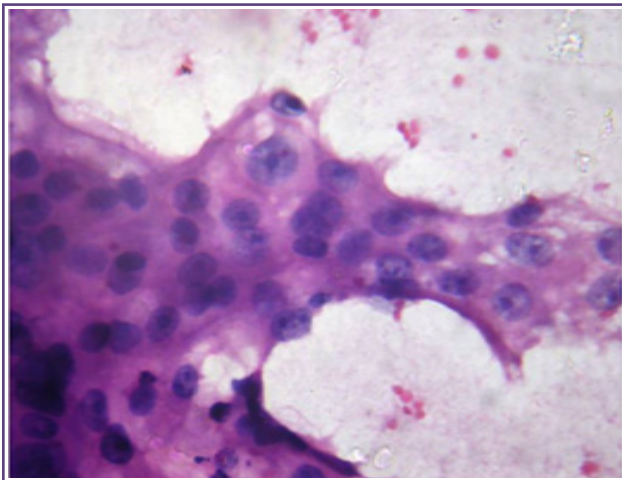

Fig. 3: Taniguchi Grade II carcinoma showing cells having prominent nucleoli (Pap, 400X).
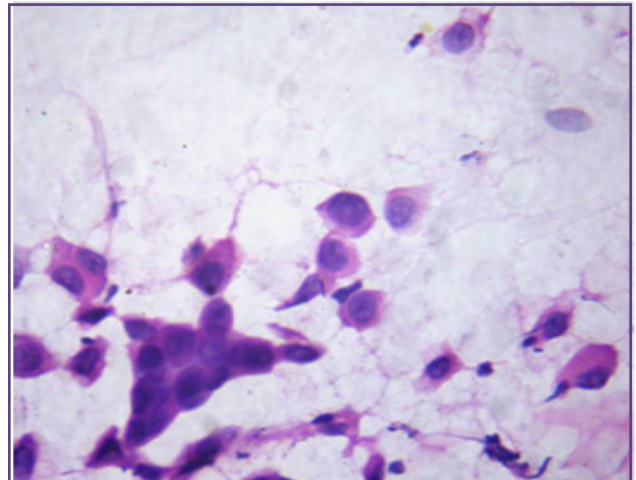

Fig. 4: Taniguchi Grade III carcinoma with high N:C ratio (more than 80%) (Pap, 400X).
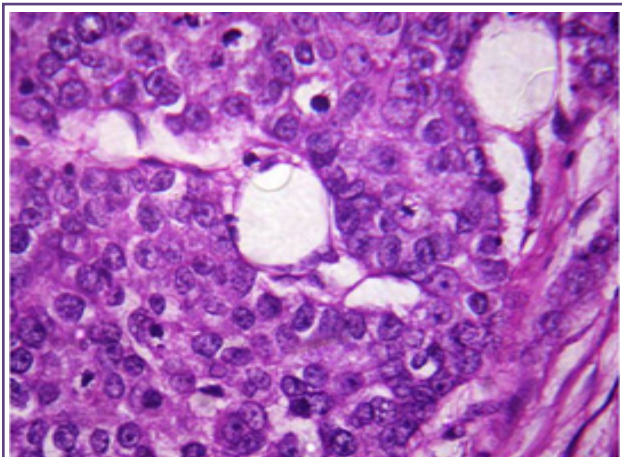

Fig. 5: SBR Grade II carcinoma showing moderate nuclear pleomorphism. (H&E, 400X).
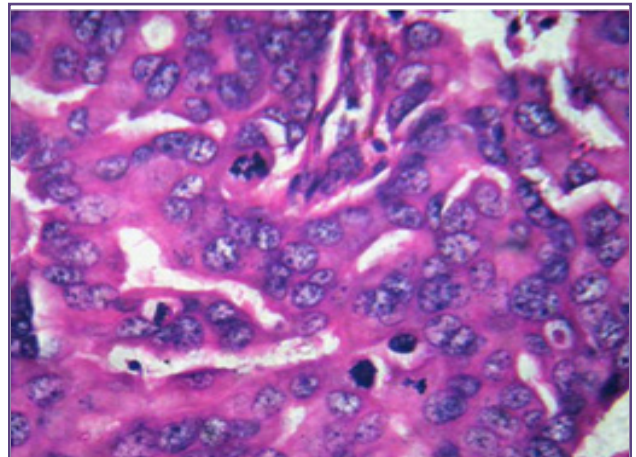

Fig. 6: SBR Grade III carcinoma showing mitosis. (H&E, 400X).

Discussion

FNAC of breast lumps performed for the diagnosis and typing of carcinoma should also evaluate the grading on aspirates.[12] The ability of FNAC to predict the accurate grade on cytological aspirates would add to its diagnostic value and this would not lead to any additional expense or morbidity to the patients.[5]

The grading on cytology for prognosis of breast carcinoma also identifies the rate of growth of tumours. It identifies Grade III carcinomas which are rapidly growing and respond well to chemotherapy and Grade I slow growing tumours which are treated with tamoxifen prior to surgery.[3]

Cytological grading has not been established yet despite histological grading having gained a strong foothold. With the advent of neoadjuvant therapy, the need for grading of malignant lesions of breast in fine needle aspirates has increased. This necessity has given rise to numerous cytological grading systems.[12]

Many authors have proposed various cytological grading systems, but none have been incorporated in routine cytology reports. The Robinson's grading system has been compared with SBR method by many authors in their studies; however, only a few studies are available regarding comparison of various cytological grading systems with histological grading. In the present study, we evaluated eight cytological grading systems and also assessed the interobserver agreement for these grading systems in cytology as well as in histopathology.

T Saha et al[17] and Einstien et al[6] found a concordance 77.19% and 68%, respectively for Mouriquand's grading in their study which was similar to our study which showed concordance of 80% for Moriquand's grading. Various studies have been performed for assessing the concordance of Fisher's modification of Black nuclear grading with histological grading by SBR method. The concordance for Fisher's grading was 80% in this study which was almost similar to other studies of Einstien et al [6] (76.3%), Saha et al [17] (70.18%), Dabbs [11] (95%), Zoppi et al.[18] (70.37%).

A concordance of 77.7% was noted between Robinson's grading and histological SBR grading in this study which was similar to numerous studies conducted by various authors as follows;A concordance of 80.76% was noted in a study by Das et al [19], 88.89% by Bhargava et al[20], 65% by Chhabra et al [21], 83% by Meena et al[22], 77.19% by Saha et al[17], 64% by Lingegowda et al[23]. Dalton et al[24] suggested that the effect of individual variation in the evaluation of a single component of Robinson's grading is reduced by analysis of the other components which is an added advantage of this system.

Saha et al[17] and Einstien et al[6] observed concordance of 75.44% and 66.6% respectively for Taniguchi's[10] grading with SBR method where as in our study it was 83.3%.

For Dabbs grading, a concordance of 73.3% with histological grading was noted in our study while in their study [11] it was 87%. For Khan's grading, we found a concordance rate of 70% in our study whereas in Khan et al [14] , Saha et al [17] and Einstien et al[6] studies, concordance rates were 97.14%, 66.67%, and 72.2%, respectively. A concordance of 83.3 % was observed in our study for Masood's grading similar to Mridha et al [25] (86%)and Rekha et al[12](86%). In our study, we also evaluated the concordance rate of Howell's grading with histological grading and it was 83.3%. In a study by Bhargava et al[20] the concordance for howells grading was 50% , while it was 57.1% in Howell et al [15] study, 82% in Lingegowda et al[23] study, 63.16% by Saha et al [17] and 69.4% in a study by Einstien et al [6].

Less number of studies have been performed for assessing the correlation of various cytological grading systems with histological grading as available in the literature. In our study, it was found that all the eight cytological grading systems showed a strong and positive correlation with histological grading. Literature search was performed to detect the studies conducted for evaluation of interobserver agreement among the various grading systems on cytology which revealed that only a few studies are available. In our study, interobserver agreement for the all cytological grading system showed substantial agreement,with k value ranging from 0.469 to 0.889 similar to studies done by Saha et al [17] and Einstien et al.[6] The concordance, correlation coefficient analysis, and interobserver agreement of this study was compared with other studies and is as shown in Table 4.

In our study, the best concordance rate of 93.3% (28/30cases), with substantial k value of agreement 0.885, and the best correlation of r = 0.925 with histological grading was observed for Robinson grading. A good interobserver agreement with k value of 0.889 (93.3%, 28/30 cases) was also observed for Robinson grading.

The major difficulties encountered in cytological grading were during scoring of cellular pattern and nuclear pleomorphism. Minor discrepancies were also noted while assigning scores to subtle nuclear pleomorphism and

cell-to-cell cohesion, as it was difficult to score them. In cytological smears, it is difficult to detect mitosis as the aspirate is scant compared to the material available in histological sections.

Conclusion

Grading on cytological aspirates of breast carcinoma is feasible and also adds valuable prognostic information regarding the aggressiveness of the tumor. It is recommended to do an effort to include information regarding grading on cytological aspirates in all FNAC reports of breast carcinomas. Cytological grading will also guide the surgeon regarding the judicious use of neoadjuvant therapy and hence overtreatment of low-grade cancers can be avoided.

A high degree of concordance was noted between various cytological grading systems and histological grading in our study. Despite various cytological grading systems, Robinson's grading is simple, easy to remember and more objective and hence can be included in routine FNAC reports of breast carcinoma.

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