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Prognostic Utility of Grading in Cytological Smears of Breast Carcinoma

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Research Article

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ABSTRACT

Introduction: Invasive breast cancer is the most common carcinoma in women, accounting for 23% of all cancers in women globally and it is the leading cause of death due to cancer in urban women. Grading of breast carcinoma on fine needle aspiration smears, while the tumour is still in vivo would be most ideal and desirable, as it would help in selection of appropriate therapy for patients. Present study is taken up to grade the breast malignancy aspirate cytology according to Robinson's grading system, grade the breast carcinoma biopsies according to Nottingham histological Score and to observe the relation between the two.

Aim and objective: To grade the breast carcinoma aspirate cytology according to Robinson's grading system and to correlate the cytological grading with Nottingham histological grade.

Materials and methods: All the diagnosed cases of carcinoma breast from January 2014 to December 2015 whose FNAC and biopsy were performed at our institute were studied. Cytological grading was carried out according to Robinson's grading system and was compared with Nottingham histological grading system. Concordance and discordance was accordingly noted.

Results: A total of 30 cases were studied, age ranged from 32-80 years. By Robinson's grading system 50%, 36.67% & 13.33% aspirate were graded as I, II and III respectively. Cytological grading was correlated well with histopathological grading with diagnostic accuracy of 80%. Sensitivity and specificity of Robinson's cytological grading was 81.25% and 100% respectively.

Conclusion: Comprehensive cytological grading of breast carcinoma was possible using Robinson's cytological grading system with a good correlation with the histological grading. Cytological grading must be routinely followed as a part of all FNAC reports.

INTRODUCTION

Invasive breast cancer is the most common carcinoma in women, accounting for 23% of all cancers in women globally [1] and it is the leading cause of death in women [2] In India, data from various population based cancer registry suggest that there is gradual increase in the incidence of carcinoma breast [3].

Early diagnosis can be achieved by fine needle aspiration cytology (FNAC) which is an ideal method to diagnose cancer in a palpable breast mass as it has the advantage of being a simple outpatient procedure, rapid, relatively painless and cost effective [4]. Grading of breast carcinoma, while the tumor is still *in vivo*, would be most ideal and desirable, as it would be helpful in the selection of patients for appropriate therapy [5]. National Cancer Institute Bethesda also recommends that the tumour grading be incorporated in cytology reports. Many studies have shown that breast carcinoma of grade I and II are considered prognostically favourable and grade III unfavourable. Present study is been taken up to grade the breast malignancy aspirate cytology according to Robinson's grading system, grade the breast carcinoma biopsies according to Nottingham Histologic Score and to observe the relation between the two systems [6,7].

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MATERIALS AND METHODS

A total of 30 cases of Breast carcinoma in female patients were studied from January 2014 to December 2015 whose FNAC and subsequent biopsy were performed in our institute. Patients who underwent surgery post neoadjuvant chemotherapy were not included in the study as chemotherapy alters the morphology of cells and the cell yield is poor.

FNAC was performed using a 10 ml syringe and 23-gauge needle and with the help of Cameco syringe holder. The aspirates were smeared on slides. Alcohol fixed slides were stained with H&E and Papanicolaou stain, air dried smears were stained with Leismhan's stain. In Robinson cytological grading system, 6 different parameters namely cell dissociation, cell size, cell uniformity; nucleolus, nuclear margin and nuclear chromatin are used to grade the tumour. A score of 1-3 is given to each parameter and tumour graded by adding the scores. Tumours that are scored in the range of 6-11 are graded I, score of 12-14 graded II and grade III given for a score ranging from 15-18 **(Table 1)**.

Table 1. Robinson's cytological grading system [8].

	Score-1	Score-2	Score-3
Cell Dissociation	Mostly in clusters	Mixture of singles and clusters	Mostly in singles
Cell size	1-2x RBC size	3-4x RBC size	≥ 5x RBC size
Cell uniformity	Monomorphic	Mildly pleomorphic	Pleomorphic
Nucleoli	Indistinct	Noticeable	Prominent/pleomorphic
Nuclear margin	Smooth	Folds	Buds or clefts
Chromatin	Vesicular	Granular	Clumped and cleared
Score 6-11- Grade I, Score 12-14–Grade II, Score 15-18-Grade III			

All patients of breast carcinoma diagnosed by FNAC underwent surgery constituting Lumpectomy, Modified Radical Mastectomy with or without axillary lymphnode dissection or trucut biopsy. The specimen received was processed according to standard grossing protocol, stained with Hematoxylin and Eosin and studied. Histological grading was performed using Nottingham Histological grade which included 3 criteria being tubule/acinar formation, nuclear pleomorphism and mitotic rate. If tubule formation was more than 75% of tumour area, score 1 is given, score 2 for 10-75% of tumour area and score 3 for <10% of tumour area. When the nuclei of tumour cells are small, with mild variation in size in comparison to normal breast epithelial cells, score of 1 is given. When the tumour cells are larger than normal, have open or vesicular nucleus, visible nucleoli and moderate variability in size and shape, a score of 2 is given. A score of 3 is given when tumour show bizarre cells with marked variation in size and shape. Score 1 is given for tumour with mitosis of ≤ 5/10 Hpf, score 2 for tumour with 6-12/10 Hpf and score 3 for tumour with ≥ 13/10 Hpf. Tumours with aggregate score of 3-5 is grade I, score 6-7 is grade II and score 8-9 is grade III **(Table 2)**.

The grading was performed by using Olympus CH20i microscope with field diameter of 0.152 mm^2.The cytological grade and histological grade is compared and concordance or discordance is noted accordingly.

Table 2. Nottingham histological grading system [9].

Criteria	Score–1	Score–2	Score–3
Tubule formation	>75% of tumour	10-75% of tumour	<10% of tumour
Nuclear pleomorphism	Minimal variation in size and shape of nuclei	Moderate variation in size and shape of nuclei	Marked variation in size and shape of nuclei
Mitosis	≤ 5/10 Hpf	6-12/10 Hpf	≥ 13/10 Hpf

RESULTS

A total of thirty female breast carcinoma patients studied with age ranging from 35-79 years and mean age of 51.7 years.

Out of 30 patients, majority were invasive breast carcinoma no special type (NST), constituting 25 (83.33%), followed by 2 (6.67%) each of mucinous carcinoma, apocrine carcinoma and 1 (3.33%) papillary carcinoma **(Table 3)**.

Table 3. Histological diagnosis (n=30).

Invasive breast carcinoma no special type (NST)	25 (83.33%)
Mucinous carcinoma	2 (6.67%)
Apocrine carcinoma	2 (6.67%)
Papillary carcinoma	1 (3.33%)

Out of 30 patients graded according to Robinson's system, majority 17 (56.67%) were grade I, followed by 12 (40.00%) grade II and 1 (3.33%) grade III **(Table 4)**.

Nottingham histological grade applied on 30 patients, majority 14 (46.67%) were grade I, followed by 12 (40.00%) grade II and 4 (13.33%) Grade III.

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Table 4. Comparison between Robinson's cytological grading system and Nottingham histological grading system (n=30).

		Nottingham Histological Grade			
Robinson's		**I**	**II**	**III**	**Total**
Cytological	**I**	14 (46.67%)	2 (6.67%)	1 (3.33%)	**17 (56.67%)**
Grade	**II**	0 (0.00%)	10 (33.33%)	2 (6.67%)	**12 (40.00%)**
	III	0 (0.00%)	0 (0.00%)	1 (3.33%)	**1 (3.33%)**
	Total	**14 (46.67%)**	**12 (40.00%)**	**4 (13.33%)**	**30 (100.00%)**

For convenience and statistical purposes, the three tier system of both Robinson's grading system and Nottingham histological grading system were modified into two tier system where grade I was considered as low grade and grade II and III were merged into category of high grade, similar to the study conducted by Das et al. [11] **(Table 5)** .

Table 5. Two tier grading system (n=30).

		Nottingham Histological Grade		
		Low grade	**High grade**	**Total**
Robinson's	**Low grade**	14 (46.67 %)	3 (10.00 %)	17 (56.67 %)
Cytological	**High grade**	0 (0.00%)	13 (43.33 %)	13 (43.33 %)
Grade		14 (46.67 %)	16 (54.33 %)	30 (100.00 %)

In the present study, the sensitivity was 100%, specificity was 81.2%, positive predictive value was 82.35%, negative predictive value was 100% and diagnostic accuracy was 80%. Good statistical correlation is observed between the two grading system with p value of 0.0001, obtained by Fischer exact test **(Figures 1-6)**.

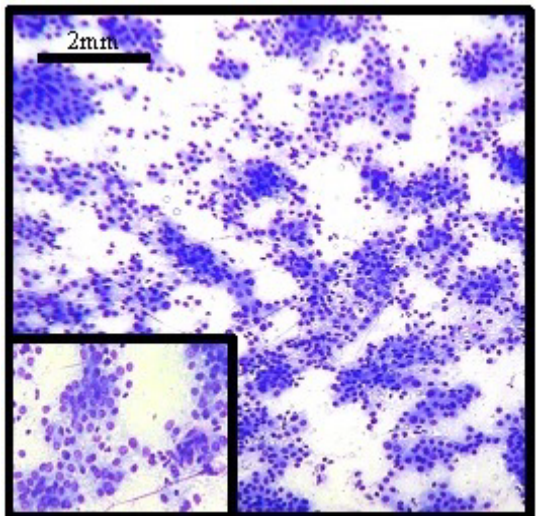

Figure 1. Microphotogragh showing tumour cells predominantly in clusters (Leishman, 4x) Inset – monomorphic tumour cells (Leishman, 10x).

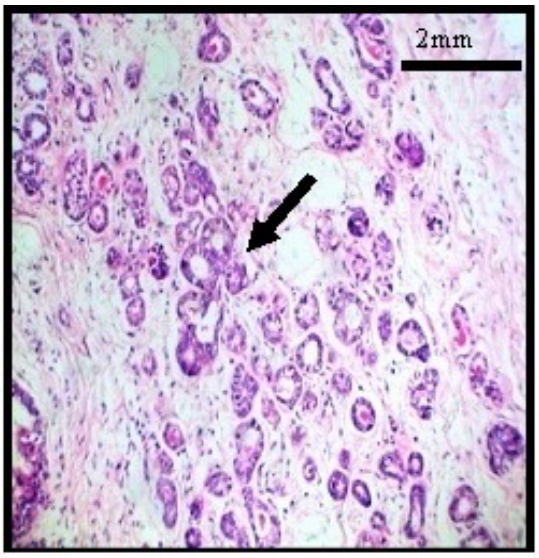

Figure 2. Microphotograph showing tumour with tubule formation in >75% of tumour area (H&E, 4x).

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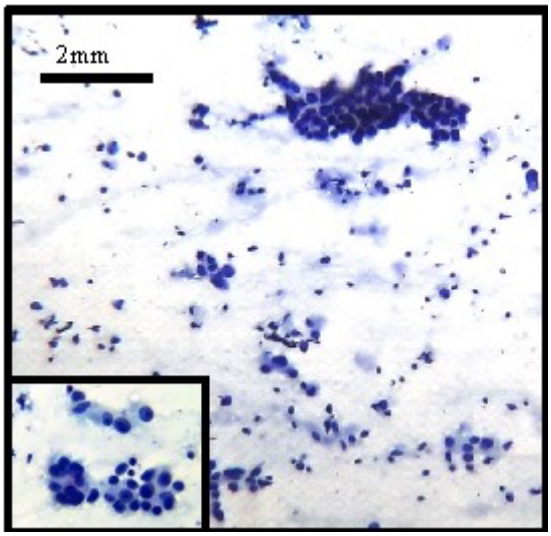

Figure 3. Microphotograph showing tumour cells in clusters and in singles (Pap, 4x)
Inset – Mild Pleomorphic tumour cells with noticeable nucleoli (Pap 10x).

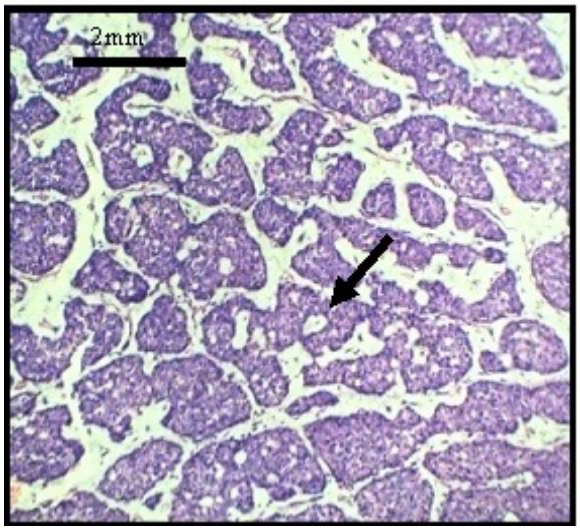

Figure 4. Microphotograph showing 10-75% tumour area with tubule formation (H&E, 4x).

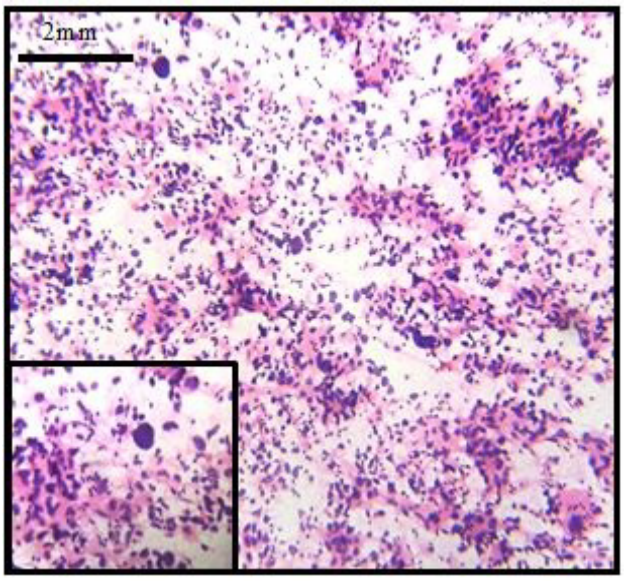

Figure 5. Microphotograph showing tumour cells predominantly in singles (H&E, 4x)
Inset – Pleomorphic tumour cells (H&E 10x).

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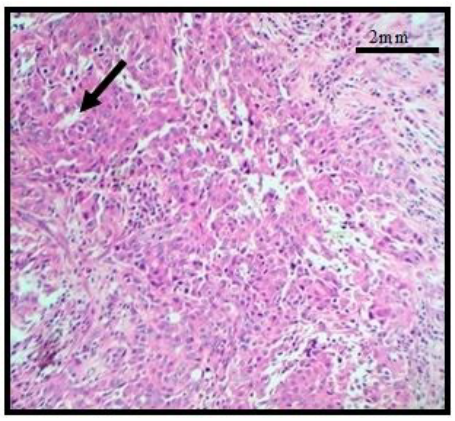

Figure 6. Microphotograph showing tumour cells with tubule formation in<10% of tumour area (H&E, 4X).

DISCUSSION

Breast cancer is the second most prevalent cancer among Indian women, the first being cervical cancer. In many urban areas, breast cancer has surpassed cervical cancer and is the most common cancer in urban Indian females [3]. FNAC smears can be used to determine various prognostic cytological parameters in patients with breast carcinoma.

The purpose of cytoprognostic grading in breast cancers is to identify fast growing tumours (grade III), which are more likely to respond to chemotherapy than slow growing tumours (grade I), which may be better suited to pre-treatment with tamoxifen. Assessment of biological aggressiveness by cytological grading without removing the tumour would, therefore, be of immense value [10].

There are six novel cytological grading system used to grade breast carcinoma, being Robinson's system, Fischer system, Mouriquand's system, Howell's system, Khan's system and Taniguchi's system **(Tables 6-9)**.

Table 6. Fischer nuclear grade of breast carcinoma.

Nuclear character	Nuclear grade		
	1	2	3
Size	Mild variation with respect to normal ductal epithelium	Twice the size of the nuclear grade 1	Larger than nuclear grade 2, oftern shows a 3 fold variation in nuclear diameter
Nuclear membrane countour	Round, smooth	Smooth	irregular
Anisonucleosis	Absent	Moderate	Marked
Chromatin	Fine	Uniform	Marked hyperchromatism, coarse, clearing may be present
Nucleoli	Absent	May or may not small nucleoli	Macro nucleoli

Table 7. Mauriquand's cytological grading system.

Feature		Score
Cells	Isolated	3
	In clusters	0
	Large cells	3
	Anisokaryosis	2
Nuclei	Naked	3
	Budding	2
	Hypochromatic	3
	Hyperchromatic	2
Enlarged nucleoli	Red	3
	Blue	2
Mitosis	>3/slide	1
	>6/slide	3
Grade 1-score < 5, Grade 2–Score 5-9, Grade 3-score ≥ 10		

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Table 8. Khan's cytological grading system.

Criteria	Score-1	Score-2	Score-3
Pleomorphism	Mild	Moderate	Severe
Nuclear size	<3x RBC	3-5x RBC	>5x RBC
Nuclear margin	Regular/smooth	Irregular/folded	Irregular/buds/clefts
Nucleoli	Absent/inconspicuous	Micro/conspicuous	Macro/multiple
Naked tumour nuclei	<3x RBC	3-5x RBC	>5x RBC
Mitotic count	Absent/minimal	Frequent	Abundant
Cellularity	Scanty	Moderate	Abundant
Cell dispersion	Mainly clusters	Cells and clusters	Mainly single cells
Lymphocytes response	Absent/mild	Moderate	Severe
Grade 1=Score 6-10, Grade 2=Score 11-14, Grade 3=Score 15-18, RBC=Red blood cell			

Table 9. Taiguchi's cytological grading system.

Criteria	Score-1	Score-2	Score-3
Pleomorphism	Mild	Moderate	Severe
Nuclear size	<3x RBC	3-5x RBC	>5x RBC
Nuclear margin	Regular/smooth	Irregular/folded	Irregular/buds/clefts
Nucleoli	Absent/inconspicuous	Micro/conspicuous	Macro/multiple
Naked tumour nuclei	<3x RBC	3-5x RBC	>5x RBC
Mitotic count	Absent/minimal	Frequent	Abundant
Cellularity	Scanty	Moderate	Abundant
Cell dispersion	Mainly clusters	Cells and clusters	Mainly single cells
Lymphocytes response	Absent/mild	Moderate	Severe
Grade 1=Score 6-10, Grade 2=Score 11-14, Grade 3=Score 15-18, RBC=Red Blood cell			

Howell used the three criteria, tubule formation, nuclear pleomorphism and mitoses on cytology smears and graded breast carcinoma accordingly.

Among these six systems, the cytological grading of breast carcinoma proposed by Robinson is easy, feasible and easily reproducible [14]. This was even observed by Saha et al. [15]. The correlation between Robinson's grading system and Nottingham histological grading system has helped to grade the tumour when it is *in situ* and further guide the clinician for counselling the patient and choose the most appropriate therapy for the benefit of the patient.

In the present study, maximum numbers of patients were grade I constituting 17(56.67%).Studies conducted by Robinson et al. [8], Das et al. [11], Wani et al. [12] and Gore et al. [13] showed maximum number were grade II. For high number of grade I in our study could be the environmental factors and genetic factors in the pathogenesis of breast carcinoma and also the limited number of cases **(Table 10)**.

Table 10. Comparison of Robinson's cytological grading system in various studies.

	Present study (n=30)	Robinson et al. [8] (n=608)	Das et al. [11] (n=52)	Wani et al. [12] (n=75)	Gore et al. [13] (n=53)
Grade I	17 (56.67%)	232 (38.3%)	15 (28.8%)	28 (25.45%)	5 (10.35%)
Grade II	12 (40.00%)	234 (38.5%)	24 (46.2%)	46 (41.8%)	41 (75.86%)
Grade III	1 (3.33%)	142 (23.2%)	13 (25%)	28 (25.45%)	7 (13.79%)

The sensitivity, specificity, positive predictive value and negative predictive value of our study are comparable with the study conducted by Das et al. [11]. The negative predictive value in our study is high when compared to study conducted by Das et al. [11] as in our study; no case was graded as high grade on cytology using Robinsons grading system which turned out to be low grade on Nottingham's histological grade **(Table 11)**.

Table 11. Comparison of statistical values.

	Present study (n=30)	Das et al. [11] (n=52)
Sensitivity	81.25%	81.39%
Specificity	100%	77.77%
PPV	82.35%	94.59%
NPV	100%	44.66%
Accuracy	80%	80.76%

The diagnostic accuracy of the present study is 80% which was similar to the studies conducted by Das et al. [11], Wani

et al. [12] and Gore et al. [13] which had diagnostic accuracy of 80.76%, 90.9% and 82.76%, respectively.

Studies conducted by Sinha et al. [14] and Saha et al. [15] also show that the correlation between the Robinson's system of cytological grading and Nottingham histological grade was very well correlated.

Epigenetic mechanisms, including DNA methylation, post-translational histone modifications as well as chromatin remodeling and non-coding transcripts, play a central role in cancer initiation and progression. For instance, in breast carcinoma, histone methyl transferase G9a interacts with Snail and leads to malignancy via suppression of E-cadherin [16]. In addition, G9a also regulates the hypoxia associated genes and is involved in breast cancer survival and tumorigenesis. Epigenetically, G9a forms a heterodimer with GLP and the G9a/GLP complex functions as the main writer for depositing of H3K9me1/2 [17]. Therefore, detection the levels of either H3K9me1/2 or DNA methylation in breast cancer cells might be a novel method for monitoring the grades and evaluating the levels of patients.

CONCLUSION

Comprehensive cytological grading of breast carcinoma is possible even when the tumour is *in vivo* and without resorting to biopsy, using Robinson's cytological grading, which is reliable and an easily reproducible method to grade the cytological smears of breast carcinoma. It also has a very good correlation with the standard Nottingham histological grade. We also recommend that the cytological grading must be routinely followed as a part of all FNAC reports as it would be helpful for the clinician in the selection of appropriate therapy for the patient and also to counsel the patient regarding prognosis of the disease.

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