RESEARCH ARTICLE

CYTOLOGICAL GRADING OF MALIGNANT BREAST ASPIRATES BY SIMPLIFIED BLACK GRADING SYSTEM – EXPERIENCE AT A TERITIARY CARE HOSPITAL

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ABSTRACT

Background: Breast cancer is the second commonest malignancy of females in our country. Nuclear grading is a fundamental cytological maker. The tumor grading does not require special procedure and in-curs no additional cost, yet it is one of the most important prognostic factor determining the outcome of breast cancer patients. Hence this study was undertaken to study the cytomorphological features of breast carcinoma and grade them according to the Simplified Black Grading system.

Materials And Methods: This study was conducted on 76 cases during the period of January 2004 to December 2005. Only cases of cytologically proven duct carcinoma were included in the study. Benign lesions, suspicious cases, unsatisfactory aspirates and cases which refused the consent were excluded from the study. Clinical data was elicited and FNAC procedure performed and slides were immediately wet-fixed in alcohol for Papanicolaou's and H & E stains and few air-dried for Giemsa stain. The various cytomorphological features of the breast lesions were studied, analyzed and Graded as per the Simplified Black Grading System (Two tier grading system).

Results: Out of 76 cases diagnosed as malignant, 74 cases were females and 2 cases were males. The most common type of breast cancer was Duct carcinoma NOS type in 69 cases (90.79 %) to be followed by Mucinous carcinoma in 3 cases (3.94 %). Not even a single case of false positive was reported with a diagnostic accuracy of 100 %. Out of 76 malignant cases graded according to Simplified Black Grading System, the study revealed high grade in 45 cases (59.21 %) and low grade in 31 cases (40.79 %).

Conclusion: The tumor grading does not require special procedure and in-curs no additional cost, yet it is one of the most important prognostic factor determining the outcome of breast cancer patients.

INTRODUCTION

Breast cancer is the second commonest malignancy of females in our country and is a leading cause of mortality in the prime of their lives. Early detection & appropriate management results in a prolonged disease free interval.[1] Today is the era where the cytologists are expected to make a "Surgical Path" type of diagnosis on breast aspirates.[2] The modern trend towards the more conservative surgery and individualized treatment has increased the significance of tumor typing & grading and the importance of close correlation of mammographic, cytological & histological methods.[3]

Nuclear grading is a fundamental cytological maker.[4] The tumor grading does not require special procedure and in-curs no additional cost, yet it is one of the most important prognostic factor determining the outcome of breast cancer patients.[4] Hence this study was undertaken to study the cytomorphological features of breast carcinoma and grade them according to the Simplified Black Grading system.

MATERIALS AND METHODS

This study was undertaken in the Department of Pathology, M.R. Medical College, Gulbarga on the patients attending the OPD and IPD services and being referred for FNAC during the period of January 2004 to December 2005. The ethical clearance was obtained from the institutional ethical committee. The informed consent was taken from all the participants. The study was conducted on 76 patients. Only cases of cytologically proven duct carcinoma were included in the study. Benign lesions, suspicious cases, unsatisfactory aspirates and cases which refused the consent were excluded from the study.

The clinical data was elicited, examination of the patients performed meticulously. FNAC procedure was performed on the patients under manual guidance with aseptic precautions. The slides were immediately wet-fixed in alcohol for Papanicolaou's and H & E stains and few air-dried for Giemsa stain. The various cytomorphological features of the breast lesions were studied, analyzed and Graded as per the

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Simplified Black Grading System. Histopathological correlation was done where ever the biopsy was available.

Simplified Black Grading System

Low Grade

- Nuclear uniformity.
- Fine chromatin, no clumping.
- Absent or inconspicuous nucleoli.
- < 3 mitosis / 10 hpf.
- Small nucleus (< x3 size of mature lymphocyte / RBC.

High Grade

- Anisoneucleosis.
- Chromatin clumping.
- Nucleoli easily seen at low power objective. 3 mitosis / 10 hpf.
- Large nucleus (> x 3 size of a mature lymphocyte / RBC.

After taking all criteria in to consideration, a case is categorized as either low or high grade, if atleast three criteria in that category are present.

RESULTS

Out of 76 cases diagnosed as malignant, 74 cases were females and 2 cases were males. Maximum numbers of 59 cases (77.62 %) were noted in the age group of 31-60 years. The youngest age of the patient in the study was 27 years and eldest 79 years.

The left breast was involved in 41 cases (50.85 %) and right breast was involved in 34 cases (44.74 %). Only one case (5.57 %) had bilateral presentation.

The most common type of breast cancer was Duct carcinoma NOS type in 69 cases (90.79 %) to be followed by Mucinous carcinoma in 3 cases (3.94 %). Recurrence was noted in 2 cases (2.63 %) as depicted in table 1

Table 1 Classification of Malignant Lesions.

Sl. No.	Lesion	No. of cases	Percentage
1.	Primary breast cancer		
	a) Duct carcinoma	69	90.79
	b) Mucinous carcinoma	3	3.94
	c) Lobular carcinoma	1	1.32
	d) Paget's disease	1	1.32
2.	Recurrence (chest wall)	2	2.63
	Total	**76**	**100**

Out of 76 malignant cases, 10 cases showed metastatic tumor deposits in axillary lymphnodes while cervical lymphnodes were involved in only 2 cases.

In the present study, histopathological correlation was available in 31 cases (40.79 %). Not even a single case of false positive was reported with a diagnostic accuracy of 100 % as shown in table 2.

Table –2 Cyto-Histological correlation

Cytological diagnosis	Total cases	%	Biopsy available	Positive cytology report	Accuracy	Negative cytology report
B) MALIGNANT						
Duct carcinoma	69	90.79	29	29	100	--
Mucinous carcinoma	3	3.94	1	1	100	--
Lobular carcinoma	1	1.32	--	--	--	--
Paget's disease	1	1.32	1	1	100	--
Recurrence	2	2.63	--	--	--	--
Total	**76**	**100**	**108**			

When the Nuclear features of malignancy were analyzed 63 cases (82.89 %) had a large nucleus (> 3 times the size of a mature lymphocyte / RBC), prominent anisoneucleosis was noted in 53 cases (69.74 %), coarse chromatin in 51 cases (67.11 %). Prominent nucleoli (visible even at low power) was seen in only 26 cases (34.21 %) and 51 cases (67.11 %) had < 3 mitosis / 10 hpf as depicted in table 3.

Table 3 Nuclear features in Malignant breast lesions

Sl No	Nuclear Changes	No. of cases	Percentage
1	Nuclear size		
	a. Small nucleus (< 3 times size of mature lymphocyte / RBC)	13	17.11
	b. Large nucleus (> 3 times size of mature lymphocyte / RBC)	63	82.89
2	Nucleus		
	a) Nuclear uniformity	23	30.26
	b) Anisoneucleosis	53	69.74
3	Chromatin		
	a) Fine chromatin, No clumping	25	32.89
	b) Chromatin clumping	51	67.11
4	Nucleoli		
	a) Present	50	65.79
	b) Prominent (visible even at low power)	26	34.21
5	Mitosis		
	a) < 3 mitosis / 10 hpf	51	67.11
	b) > 3 mitosis / 10 hpf	25	32.89

Out of 76 malignant cases graded according to Simplified Black Grading System, the study revealed high grade in 45 cases (59.21 %) and low grade in 31 cases (40.79 %) as depicted in table 4.

Table 4 Nuclear grading by Simplified Black Grading System

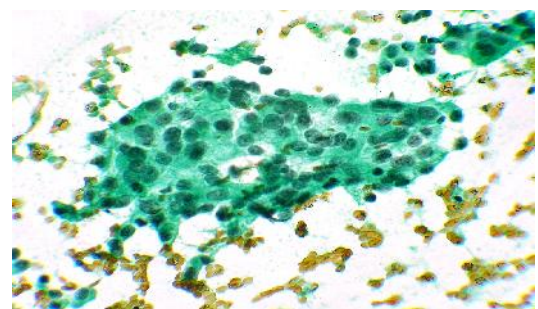| Sl No	Simplified Black Grading System	No of cases	Percentage
1	Low grade	31	40.79
2	High Grade	45	59.21
	Total	76	100

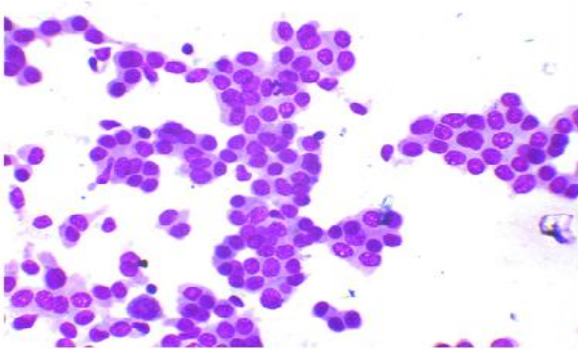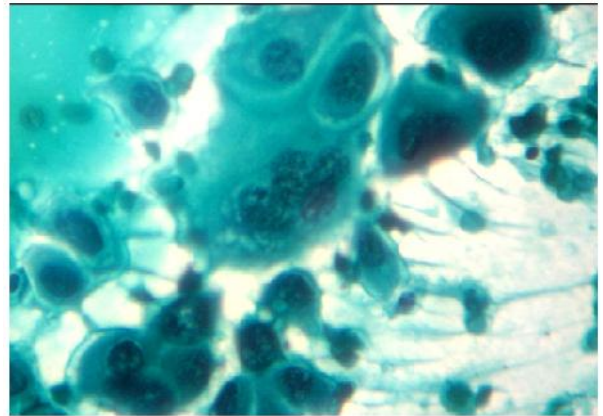**Fig. 1** Duct carcinoma - Low grade (PAPX HP)

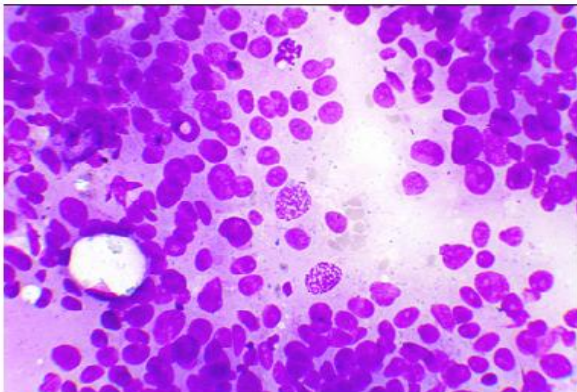**Fig.2** Duct carcinoma - Low grade (GIEMSA X HP)

Fig. 3 Duct carcinoma - High grade (GIEMSAX HP)

Fig.4 Duct carcinoma - High grade (GIEMSA X HP)
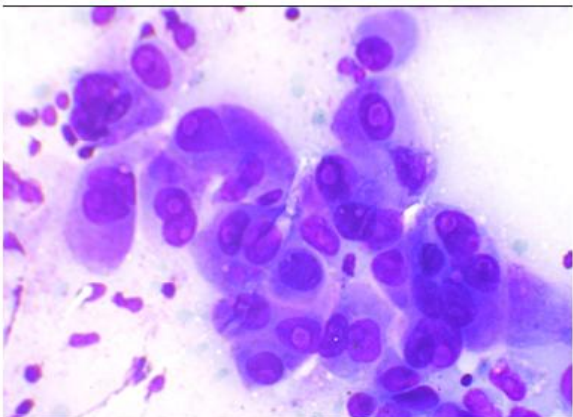

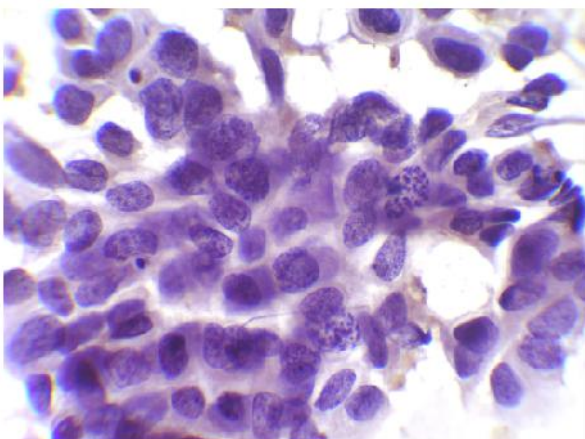**Fig. 5** Duct carcinoma - High grade (GIEMSAX HP)

Fig.06 Duct carcinoma - High grade (GIEMSA X HP)

DISCUSSION

Breast cancer is the second commonest malignancy of females in our country and is a leading cause of mortality in the prime of their lives.[1] With the advent of better radiological techniques in early detecting breast cancer, the pathologists are faced with smaller biopsy specimens which may not be adequate for other prognostic tests. Hence morphological assessment using light microscopy is still the simplest and most practical method for assessing prognostic factors.[5]

The modern trend towards the more conservative surgery and individualized treatment has increased the significance of tumor typing & grading.[3] It is the responsibility of the pathologist to give the clinician as much information as possible when rendering the diagnosis, including the tumor grade.[5,6] Grading plays a very important role in predicting the response to pre-operative chemotherapy, radiation therapy and / or in selecting alternate adjuvant therapy strategies.[7]

Neo-adjuvant chemotherapy, commonly used nowadays for treatment of breast cancer includes pre-operative chemotherapy. It has been suggested that fast growing tumors are more likely to respond to chemotherapy than low- grade, slowly growing lesions which are better suited for pre-treatment with tamoxifen.[8] So, cytological grading may provide relevant information of the aggressiveness of invasive duct carcinoma and could be a useful parameter to take in to consideration while selecting therapy and in selecting patients in whom the surgical removal of the tumor is not the best option.[9]

The modern trend in management of breast cancer point to the importance of having a tumor grading system that will allow a consistent classification of this neoplasm. A number of authors have proposed various grading systems applicable to both histological & cytological preparations. [4,7,10]

Based on the studies, the grading system most applicable to cytology is Modified Black Grading system (three tier grading system) originally proposed by Fischer[11] et al. The Modified Black Grading system is based solely on cytological features and has a high correlation with histology and is highly reproducible.[10] Although, Bloom Richardson Grading system is

more popular it is not applicable to cytology as compared to Modified Black Grading system.[12]

Cajulis[10] et el studied the utility of Simplified Black Grading System[13] (two tier system) versus Modified Black Grading system[6] (three tier system) concluded that Modified Black Grading System which was based purely on cytological features has a very high correlation with histology, but it was the Simplified Black Grading System which was equally effective, more convenient to use and had better inter-observer agreement.

Although Simplified Black Grading system was patterned after the Modified black Grading system, the criteria used in the latter are not as objective as the former. With the inclusion of Grade 2 in Modified Black system, the intermediate grade becomes a "waste basket" grade, since the criteria are not well established. The Simplified Black Grading System was easier, more objective and practical method of grading breast carcinomas[7]. Hence the Simplified Black Grading System was applied to grade the malignancies in our study.

The reason for high reproducibity was the more objective set of criteria used. The objectivity lies in the well defined set of criteria used namely nuclear uniformity versus anisoneucleosis, fine versus stippled chromatin, absence or presence of prominent nucleoli, specified number of mitosis / 10 hpf & nuclear size(less than three >3 times size of a mature lymphocyte or RBC.[7]

Anisoneucleosis is defined as the presence of nuclei atleast 3 X size of other nuclei. It is important, when assessing the nuclear size only the nuclei of tumor cells be considered and not those of macrophages / stromal cells.

When considering the chromatin texture, it is advisable to evaluate a number of histological and cytological preparations of breast carcinomas of different nuclear grades to set proper thresholds. Artifactual changes in the chromatin pattern may be introduced due to faulty technical preparations, especially with air drying artifacts and varying concentrations of staining solutions. Comparison with non-tumor nuclei on the same slide is suggested to differentiate between real and artifactual changes in chromatin pattern.

Generally fine chromatin is described as even distribution of chromatin resulting in a light monotonous appearance to nucleus. Hyperchromacity, in contrast results from increased clumping of chromatin giving rise to dark & light areas or if excessive a totally dark nucleus.

 Assessment of prominent nucleoli is determined by the relative ease in finding them even at low power (100X magnification). Careful attention should be given to the chromatin pattern while assessing the presence of nucleoli, since hyperchromatism may obscure its presence. Evaluation at high power however will easily reveal large prominent nucleoli.

In order to establish more objective criteria for the presence of mitosis, the presence of atleast 3 mitotic figures per 10 cellular HPF is required for a particular case to be considered in the high nuclear grade category. The strict criteria requires that only intact and not degenerating mitotic figures be considered, since a number of artifacts, especially in cytological preparations can mimic the presence of mitotic figures.

Greater objectivity is obtained when largest tumor nuclei is compared to an adjacent mature lymphocyte or RBC. If the nucleus is > 3 times the size of a mature lymphocyte or RBC, it is considered to meet the criteria of high nuclear grade as far as the nuclear size is considered.

After taking all criteria in to consideration, a case is categorized as either low or high grade, if atleast three criteria in that category are present.

So, out of 76 malignancies graded according to Simplified Black Grading system, 31 cases (40.79 %) belonged to the low grade and 45 cases (59.21 %) were of high grade category. A comparison was done between the present study and the study conducted by New[14] *et al*. as depicted in table 5.

Table 5 Nuclear Grading – Comparative Analysis.

Neuclear Grade	New[14] NE (1994)		Present Study	
	No of cases	%	No of cases	%
Low	27	54	31	40.79
High	23	46	45	59.21
Total	50	100	76	100

A variation in the nuclear grade was observed in comparison with this study. New et al reported maximum number 0f 27 cases (54.00 %) in low grade category and 23 cases (46.00 %) in high nuclear grade. In the present study maximum numbers of 45 cases (59.21 %) were present in the high grade category as compared to 31 cases (40.79 %) in low grade category. The variation in the results can be attributable to the change in demographic area with change in cultural practices, difference in staging of disease and the smaller sample size. The relationship of breast carcinoma with respect to age, typing & histopathological correlation was in accordance to other studies.[15,16,17.]

CONCLUSION

FNAC is a well accepted diagnostic technique in the management of breast lesions. Nuclear grading is a fundamental cytological maker. The tumor grading does not require special procedure and in-curs no additional cost, yet it is one of the most important prognostic factor determining the outcome of breast cancer patients.

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