COMPARATIVE STUDY OF FINE NEEDLE ASPIRATION CYTOLOGY AND HISTOPATHOLOGY IN GRADING BREAST CARCINOMA

by

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Under the guidance of

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LIST OF ABBREVIATIONS USED

1.	DNA	--	Deoxyribonucleic Acid
2.	et al.	--	And Others
3.	ER	--	Oestrogen Receptor
4.	FNA	--	Fine Needle Aspiration
5.	FNAC	--	Fine Needle Aspiration Cytology
6.	H & E	--	Haematoxylin and Eosin
7.	HPF	--	High Power Field
8.	IHC	--	Immunohistochemistry
9.	N/C ratio	--	Nuclear cytoplasmic ratio
10.	NOS	--	Not Otherwise Specified
11.	PR	--	Progesterone Receptor
12.	RBC	--	Red Blood Cell
13.	SBR	--	Scarff Bloom Richardson
14.	TGS	--	Taniguchi Grading System
15.	Yrs	--	Years

ABSTRACT

Background :

Grading of tumors in Fine Needle Aspiration Cytology (FNAC) would be useful particularly for patients with advanced stages of the disease who may receive primary chemotherapy or radiotherapy followed possibly by surgery.

Objectives :

1) To assess the cytological grade in fine needle aspiration cytology smears of breast carcinomas.
2) To compare the cytological grade with histological grade in surgical specimens and biopsies of axillary lymph nodes.

Methods :

The study included 38 female patients of invasive ductal carcinoma diagnosed cytologically. Cytological grading of smears was done by Taniguchi grading system. Respective surgical specimens were processed and histological grading was done by Scarff Bloom Richardson grading method. The lymph nodes were also studied for detection of metastasis.

Results :

Based on Taniguchi grading method the cases were classified into grade I (5.26%), grade II (42.11%) and grade III (52.63%). Based on Scarff Bloom Richardson grading method the cases were classified into grade I (2.63%), grade II (52.63%) and grade III (44.74%). Total Concordance between the two grading systems was seen in 78.95% of cases. Positive correlation was seen between the two grading systems. Lymph node metastasis was seen in 52.63% of cases which was maximum in grade III cases.

Conclusions :

Cytological grading allows prognostic evaluation of breast carcinoma along with diagnosis without additional morbidity or expense to the patient. Taniguchi's grading system is simple, takes little time, is reproducible and correlates precisely with the histological grade. Hence cytologic grade should appear in FNAC reports of ductal breast carcinoma for proper management.

Keywords :

Fine needle aspiration cytology; Invasive ductal carcinoma; Taniguchi grading system; Scarff Bloom Richardson grading system.

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INTRODUCTION

Breast cancer is the second most common cancer among Indian females. The cumulative incidence in females until 64 years of age is 1-2%.[1]

Carcinoma breast comprises a heterogeneous group of patients. Many factors other than clinical stage like tumor type, histological grading, hormone receptor status, DNA ploidy, cell proliferation markers and expression of different oncogenes determine prognosis in a given patient.[2]

Nottingham method described by Elston and Ellis for histological grading of breast carcinoma is a widely accepted tumor grading system and has been found to have good prognostic correlation.[2]

The use of Fine Needle Aspiration is frequently limited to establishing the benign or malignant character of a given lesion, although it is a proven diagnostic technique in clinical practice. It has been shown that FNA can provide additional information about intrinsic features of the tumour as well as its prognosis.[3]

FNA is safe, reliable and time saving out door procedure with little discomfort to the patient. It is helpful not only in diagnosis and planning of treatment, but also helpful in prognostication of the tumor factors like nuclear grading, mitotic index, hormone receptor status and DNA contents.[4]

Neoadjuvant therapy including preoperative chemotherapy and tamoxifen is becoming increasingly common for early breast cancer. Hence it is desirable to grade tumors before surgery so that most appropriate medical regimen can be selected. Hence much attraction is focused on grading tumors on FNAC. Such grading would

allow assessment of the tumor in situ, and the morbidity associated with over treatment of low grade tumors could be avoided.[5]

Nuclear grading on Fine Needle Aspiration is feasible, reproducible and may assume prime importance for patients who may receive chemotherapy prior to the resections of the tumors, and in those who present with metastases.[1]

Two of the most important microscopically derived morphologic prognostic factors for breast carcinoma patients are histologic type of tumor and nuclear grade[6].

Cytologic grading has shown a positive correlation with histologic grade, therefore cytograde is useful in predicting histograde preoperatively. Cytologic grade would thus provide relevant information on the tumor biologic behaviour and could be a useful parameter to take into consideration when selecting neoadjuvant therapy.[3]

Fine needle aspiration cytology is increasingly being used for preoperative diagnosis of breast cancer in order to determine various prognostic parameters and the best therapy that can be offered to the patients.[2]

Nuclear grading of breast carcinoma is easy to perform. It has been found that it correlates well with tissue nuclear grade. Hence nuclear grading should be included as a fundamental cytologic parameter in the FNAC report whenever possible.[7,8]

The National cancer Institute Bethesda has recommended that tumor grading on FNA material should be incorporated in FNA reports for prognostification. Also importance was laid on the cytological grading system which would correspond closely to the grading system used in histological material but the most reliable method for cytological grading that closely reflects the most widely used histological grading system is yet to be determined.[2]

AIMS AND OBJECTIVES

1) To assess the cytological grade in fine needle aspiration cytology smears of breast carcinomas.
2) To compare the cytological grade with histological grade in surgical specimens and biopsies of axillary lymph nodes.

REVIEW OF LITERATURE

HISTORICAL ASPECTS

Needle aspiration cytology as we know today dates back to around 1950. However, the idea to obtain cells and tissue fragments through a needle introduced into the abnormal tissue was by no means new. Already in the midninteenth century, Kun, Lebert, and Menetrier employed needles to obtain cells and tissue fragments to diagnose cancer.[9]

Cytology took its momentum in the nineteenth century due to work done by Thiersch and Waldeyer. Their contributions are considered more than any one else, for development of human cytology. In 1865 and 1867 they proposed the epithelial origin of carcinoma of skin and breast respectively. These critical observations were important for the development of diagnostic cytology as they formed the basis for recognition of precancerous epithelial abnormalities. This made cytological technique as an acceptable diagnostic tool.[10]

The introduction of aspiration cytology in twentieth century is attributed to young surgeon Hayer Martin and Edward Ellis, Ewing technicians at memorial hospital for cancer and allied Diseases, New york USA . They in 1926 studied 1400 cases and advocated aspiration by using needle of thicker caliber (18 Gauge.). Professor Dudgeon and Patrick from Great Britain in 1927 proposed the needling of tumor as a means of rapid microscopic diagnosis.[9,10]

A break through reason for cytology to become popular and relevant is due to the pioneering work in exfoliative cytology by Dr George Papanicolaou and Traut. They published their paper in 1941 under the title ‘New cancer Diagnosis’ and described the use of vaginal smears in diagnosis of uterine cancers.[10]

It was in Europe and particularly in Scandinavia that the fine needle aspiration as a technique began to flourish in 1950s and 1960s. Soderstrom and Franzen in Sweden, Lopes Cardozo in Holland, Zajdela in France and others became major proponents studying thousands of cases each year.[9]

Zajicek , first pathologist in collaboration with Franzen at the Karolinska Hospital, Sweden applied the requisite scientific rigor to define precise diagnostic criteria and to determine diagnostic accuracy in a variety of conditions.[9]

The earliest F.N.A.C of breast is attributed to Skey. In 1850 he punctured the breast by a needle which turned out to be a cyst. No microscopy was undertaken at that time. It was Sir James Paget and Erichsen who in 1853 examined the tissue aspirated from the breast under the microscope. Sir Paget described the appearance of single cancer cell under the microscope.[11]

Erichsen from University College Hospital London reported seven examples of mastectomy performed for chronic abscess diagnosed by needle aspiration which simulated a scirrhous carcinoma . Grooved needles were used to assess breast lumps by Augustin and Prichard who in 1863 described the cytology of fat necrosis.[11]

In twentieth century F.N.A.C. of the breast was performed by Ward in 1912 followed by Dudgeon and Patrick in 1920. The usefulness and accuracy of F.N.A.C in breast lesions was described by Martin and Ellis in 1930 at Memorial Hospital, New York . Hadwin and Adali followed the F.N.A.C of breast in late 1940 and early 1950. Other workers in past were Dudgeons and Patrick 1927, Dudgeon and Barett 1954, Webb 1970, Zajicek 1979 and Gardecki in 1980s.[9]

REVIEW OF CYTOLOGICAL GRADING

The features for cytological grading of breast cancer are not well established. There have been only a few retrospective studies with differing conclusions. Two studies found cell clustering or dissociation useful.[12]

Walgren A[13]et al and associates (1976) found that nuclear area and nucleolar prominence were of prognostic significance and related their findings to follow-up histology.

Zajdela A[14] et al and associates (1979) using optical micrometry on air-dried Giemsa stained smears subclassified their cancers into larger nuclear type and smaller nuclear type using 12 μm nuclear diameter as the dividing point and showed significant prognostic differences between the two groups.

Moosler JA[15] et al and associates using a morphometric technique, counting 100 cells per case and measuring nuclear diameter with an optical micrometer showed a correlation between nuclear area, estrogen receptor status and histological grade.

Moriquand J[16] et al (1986) classified the breast aspirates into three grades based upon cell arrangement, cell size, nuclear morphology, staining and mitotic activity.

Hunt CM[17] et al (1990) devised a scoring system using nuclear size, nuclear pleomorphism and the presence or absence of multiple nucleoli.

Simplified Black and Modified Black grading systems use the presence of nucleoli in their histological nuclear grading system which includes the regularity of nuclear outline, the delicacy of chromatin strands, presence or absence of nucleoli and the number of mitotic figures.[7]

Fisher's grading method used 3 grades, with grade 1 representing the highest level of differentiation and grade 3 equivalent to anaplasia. With Fisher's modification, 2 nuclear grades were recognized. Grade 1 and 2 were clumped together as favourable nuclear grade because of prognostic similarity, while grade 3 was considered an "unfavourable nuclear grade" and had poor prognosis.[18]

Cajulis[19] et al studied the utility of Modified Black System[11] (M.B) a three–tier system against what was coined Simplified Black[12] (S.B) which was a two–tier system of grading. It was inferred that Modified Black system which is based purely on cytological features has a very high correlation with histology, but it was the Simplified Black System which was more convenient to use and had greater interobserver agreement.

New NE [20] et al (1994) described a cytological grading system that included measuring the size of the nuclei and comparing them with the diameter of the RBC as well as assessing nuclear pleomorphism and presence of nucleoli.

Robinson et al (1995)[3] graded breast carcinomas based on cell size, cell dissociation, cell uniformity, nucleoli, nuclear margin, each being scored separately as 1-3.They were than summed to give final cytological score.

K Moroz[21] et al and associates (1997) concluded that there was a strong correlation between cytological nuclear grading and modified histological grade.

A semiquantitative scoring system was established by Taniguchi et al in 2000 for cytological grading of invasive ductal carcinoma. It was composed of seven parameters namely necrosis, cellular size, nuclear cytoplasmic ratio, nuclear pleomorphism, nucleoli, chromatin granularity and density of chromatin. Each was assessed in accordance with 1-3 points except necrosis which was assigned 0 or 1

point. The scores for each of the seven cytologic features were summed to arrive at a total score for a given case.[12]

TANIGUCHI GRADING SYSTEM[12]

1.Necrosis : Absent – 0

Present – 1

2.Cellular size : <3x RBC size – 1

3-4x RBC size – 2

>4x RBC size - 3

3.Nuclear/Cytoplasmic ratio : <50% - 1

50 – 80% - 2

>80% - 3

4.Nuclear pleomorphism : Uniform – 1

Mildly pleomorphic – 2

Markedly pleomorphic – 3

5.Nucleoli : Indistinct – 1

Noticeable – 2

Prominent – 3

6.Chromatin granularity : Fine – 1

Moderately granular – 2

Coarse – 3

7.Density of chromatin : Not hyperchromatic – 1

Moderately hyperchromatic – 2

Markedly hyperchromatic - 3

Grade I - Score 06 - 09

Grade II – Score 10 - 11

Grade III – Score 12 – 19

It was found that Taniguchi grading system was suitable for routine cytologic grading as the criteria was simpler and easier to reproduce.[12]

OTHER CYTOLOGICAL GRADING SYSTEMS OF BREAST CANCER

1) Mouriquand et al grading system.[16]

2) Hunt's et al grading system.[17]

3) Modified Black grading system.[7]

4) Simplified Black grading system.[7]

5) Fisher's modification of Blacks nuclear grading system.[18]

6) Robinson grading system.[3]

1) MORIQUAND ET AL GRADING SYSTEM[16]

In this grading system following cytomorphological features were given scores as follows.

Cells :	Isolated	- 3
	Clusters	- 0
	Large size	- 3
	Anisokaryosis	- 2
	Naked	- 3
Nuclei :	Budding	- 2
	Hypochromasia	- 3
	Hyperchromasia	- 2
Enlarged nucleoli :	Red	- 3
	Blue	- 2

Mitosis : > 3 / slide - 1

> 6 / slide - 3

Grade I :- Score : < 5

Grade II :- Score : 5 – 9

Grade III :- Score : > 10

2) HUNTS ET AL GRADING SYSTEM[17]

In this grading system three parameters were used to grade smears as follows

a)Nuclear size :	< 2 RBC	1
	2 – 4 RBC	2
	> 4 RBC	3
b)Nuclear pleomorphism :	Mild	1
	Moderate	2
	Severe	3
c)Nucleoli :	Absent	0
	Present	1

Grade :- Low grade – score : < 4

High grade – score : > 5

3) MODIFIED BLACK GRADING SYSTEM[7]

Table – 1

Nuclear characteristics	**Nuclear grade I**	**Nuclear grade II**	**Nuclear grade III**
A) Nuclear uniformity	Uniform	Anisonucleosis	Prominent anisonucleosis
B) Chromatin clumping	Present	Intermediate	Prominent
C) Nucleoli	Absent	More prominent	Very prominent
D) Mitosis	Absent	Rare	Easy to find
E) Nuclei	Small	Large	Largest

4) SIMPLIFIED BLACK GRADING SYSTEM[7]

Table – 2

Low Grade	High Grade
(A) Nuclear uniformity	Anisonucleosis
(B) Fine chromatin	Chromatin clumping
(C) Absent nucleoli	Nucleoli easily seen
(D) < 3 mitosis/ 10 HPF	> 3 mitosis / 10 HPF
(E) Small nucleus (< 3 times size of mature lymphocyte or RBC)	Large nucleus (> 3 RBC)

5) FISHER'S MODIFICATION OF BLACKS NUCLEAR GRADING[18]

Nuclear Grade I :

Cells are uniform in size

No anisonucleosis

Cells have smooth nuclear membrane

No hyperchromatism

Nucleoli are absent or small

Nuclear Grade II :

Nuclei – 2 times normal ductal cell

Moderate anisonucleosis

Smooth to slightly irregular nuclear membrane

Uniform chromatin

Small nucleoli

Nuclear Grade III :

Nuclei > 2 times normal ductal cells

Marked anisonucleosis

Cells have irregular nuclear membranes

Hyperchromatism

Prominent nucleoli

6) ROBINSON GRADING SYSTEM[3]

In this grading system six different cytologic parameters are used to grade the tumors. A score of 1 – 3 is given to each of these parameters and the tumor graded by adding the scores.[3]

Table - 3

Robinson grading system[3]

Criteria	Score		
	1	**2**	**3**
A) cell dissociation	Mostly in clusters	Mixture of single cells & cells in clusters	Mostly single cells
B) cell size	1 – 2 x RBC size	3 – 4 x RBC size	≥ 5 x RBC size
C) Cell uniformity	Monomorphic	Mildely pleomorphic	Pleomorphic
D) Nucleoli	Indistinct	Noticeable	Prominent or pleomorphic
E) Nuclear margin	Smooth	Folds	Buds/Clefts
F) Chromatin	Vesicular	Granular	Clumped & cleared

Grade I :- Score : 06 – 11

Grade II :- Score : 12 – 14

Grade III :- Score :15 – 18

Comparison of different cytological grading systems was done by Das AK et al. to determine the most reliable method for cytological grading that closely reflects the most widely used histological grading system, so that tumor grading on FNA material could be incorporated as a routine in FNA reports for prognostication.[2]

HISTOLOGICAL GRADING – SCARFF BLOOM RICHARDSON GRADING SYSTEM

Histological grade is an important determinant of prognosis that allows risk stratification within a given tumor stage.[22]

Several histologic grading systems are in use where some consider ductoglandular differentiation or tumor secretory state and some score only nuclear and nucleolar characteristics. Some use both duct formation and nuclear abnormalities.[23]

It has been recommended that all invasive breast carcinomas should be graded and the grading system must be specified in the report. Undergrading of tumors can occur when grading is performed on limited samples obtained by needle biopsy.[22]

A constant criticism of tumor grading is that it is a subjective evaluation and hence lacks reproducibility. Reproducibility in grading breast cancers can be achieved, when a histologic grading scheme with specific guidelines is used.

Pathologists must be aware of the limits of reproducibility with appropriate guidelines being followed to help maximize their agreement.[24]

Histologic grading has been criticized because its reproducibility has been perceived to be poor, due in part to subjective evaluation. Varying degrees of reproducibility have been shown in comparative studies.[23]

Greenough introduced breast carcinoma grading in 1925. He used seven histologic characteristics to classify breast carcinomas into three grades of malignancy.

The features assessed were the amount of tubule formation, secretory activity of the cells, overall size of cells and nuclei, variation in the size of both cells and nuclei, nuclear hyperchromatism, and mitotic activity.[25]

Patey and Scarrf in 1928 selected tubule formation, variation in nuclear size, nuclear hyperchromatism as principal variables that were modified by evidence of secretion (good) and mitotic frequency (bad).[25]

Bloom and Richardson in 1957 proposed a simplified grading system which utilized only three of Greenough's variables: gland formation (tubularity), degree of variation in nuclear size and shape (pleomorphism) and 'hyperchromatic figures' as an estimate of proliferation. Despite lack of specific criteria for quantifying variables measured, the grading system was effective for prognosis. Modifications to enhance reproducibility of scores resulted in the Nottingham- Bloom- Richardson system, which has been endorsed by College of American Pathologists and the World Health Organisation.[25]

Scarff Bloom Richardson grading combines details of cell morphology (nuclear pleomorphism) with a measurement of differentiation (tubule formation) and an assessment of proliferation (mitotic frequency).

Table - 4

Scarff Bloom Richardson Grading system[26]

Feature	Score
Tubule formation	
Majority of tumor - >75%	1
Moderate degree - 10-75%	2
Little or none - <10%	3
Nuclear pleomorphism	
Small,uniform cells	1
Moderate increase in size/variation	2
Marked variation	3
Mitotic counts-per 10 HPF(40xfields)	
0-5	1
6-10	2
>11	3

Grade I (Well differentiated) : Score 3-5

Grade II (Moderately differentiated) : Score 6-7

Grade III (Poorly differentiated) : Score 8-9

Tubule formation :

For scoring tubule formation overall appearance of tumor is taken into consideration, 1 point is given when 75 % or more of the tumor is composed of clear tubular structures exhibiting central lumina and 3 points are assigned if less than 10 % of the tumor shows tubule formation. Clefts induced by shrinkage artifact should not be mistaken for tubular structures.[23]

Nuclear pleomorphism :

The tumor areas having cells with greatest atypia should be evaluated. A score of 1 is given if cell nuclei are uniform in size and shape, relatively small, have dispersed chromatin patterns and are without prominent nucleoli. A score of 2 is given if cell nuclei are somewhat pleomorphic, have nucleoli, and are of intermediate size. If cell nuclei are relatively large, have prominent nucleoli or multiple nucleoli, coarse chromatin patterns and vary in size and shape, then a score of 3 is given.[8]

Mitotic counts :

Mitotic count is the most important constituent of histologic grade. Mitosis counting in lymph node metastasis also provides some prognostic value. Mitotic activity index is not seriously affected by fixation delay, although fixation delay does lead to worse morphology, which makes counting more difficult. Therefore it is advisable to avoid fixation delay when possible, and to keep specimens in refrigerator until fixation.[27]

Mitotic figures are to be counted only at the periphery of the tumor i.e. invasive margin of the tumor. Counting should begin in most mitotically active areas. Ten HPF are to be counted in the same area. Field should be filled with as many tumor cells as possible. Poorly preserved areas are to be avoided.[28]

Mitotic figure was defined by Van Diest et al as cells in early metaphase (clumped chromatin with coral spread), cells in Meta/Anaphase (clumped chromatin arranged in a plane), cells in telophase (separated clumped chromatin), in general absence of nuclear membrane, dark clumped chromatin with coral, linear, bipolar, tripolar or multipolar spread. Observation of atleast one chromosome, usually seen as a small hairy projection from surface of mitotic figure, preservation of amphophilic cytoplasm are in favour of mitotic figure.[28]

The presence of firecone figure, triangular or spiny nuclear projections, dark line paralleling the margin, or the presence of large dark round spots (pyknotic nuclei) were regarded as argument against a mitotic figure.[28]

Peformance of mitotic counts

Table 5

ASSIGNMENT OF POINTS FOR MITOTIC COUNTS ACCORDING TO THE FIELD AREA USING SEVERAL MICROSCOPES[26]

	Leitz Ortholux	**Nikon Labophaf**	**Leitz Diaplan**
Objective	X25	X40	X40
Field diameter(mm)	0.59	0.44	0.63
Field area(mm2)	0.274	0.152	0.372
Mitotic count*			
1 Point	0-9	0-5	0-11
2 Points	6-19	6-10	12-22
3 Points	> 20	>11	›23

*Assessed as number of mitosis per 10 fields at the tumor periphery

The clinical laboratory improvement amendments of 1988 mandated that laboratories perform cytohistological correlation, as an effort to improve anatomic pathologic quality. But the optimal methods and the value of performing correlation have not been determined .[29]

In the cytohistological correlation process, if a cytology specimen and a surgical pathology specimen are obtained from the same anatomical site, the diagnosis

are compared in order to detect possible discrepant cases. Correlation is a useful method for error detection, errors attributable either to sampling or to interpretation.[29]

Correlation also allows to determine if the grades assigned on cytological aspirate correspond well with histological grades .This information is of clinical use and of prognostic value since it enables assessment of the biological aggressiveness of the cancer without removing it. Thus by resorting to the system which closely reflects the histological grade, biologic behaviour of the tumor is assessed and systemic adjuvant treatment is instituted before surgery, either in conventional regimen or by cutaneous infusion of newer drugs.[7]

Various authors in the literature have graded the carcinomas of breast on the tissue sections and on cytological smears. There is variation in the accuracy of grading system by different authors from 53.89% to 83 %. This variability may be due to several factors such as different systems used to assign cytologic grade, its evaluation in air dried materials and different staining techniques.[30]

Robinson et al studied 377 tumors which included 286 ductal carcinomas. Grading was done by using six of the well recognized criteria of malignant disease and it was concluded that grading on FNAC was feasible and reproducible and may substitute histological grade.[3]

A study was done by Chhabra S et al in 60 cases of breast carcinomas, cytological grading of FNAC smears was done according to the grading system devised by Robinson et al. Histological grading was done according to Nottingham modification of Scraff – Bloom Richardson method. The total cytological scores were then compared with the histological grade which showed positive correlation of 84. 1% .[24]

In a study done by Zoppi JA et al on 135 breast carcinomas, nuclear grading of cytologic smears was done by Fishers modification of Blacks nuclear grading scheme. The nuclear grading was assigned to biopsies according to Scarff Bloom Richardson method. There was agreement between cytological grade and histological grade in 70. 37% of tumors.[31]

Dabbs DJ studied 20 cases of breast carcinomas in whom independent blind grading of tissue specimens and aspirates was done based on Fisher's simplification of Blacks nuclear grading scheme. It revealed concurrence of nuclear grade assignment in 95% of the cases. The study concluded that assigning a nuclear grade in breast carcinoma aspirates correlates precisely with the tissue nuclear grade.[32]

A study was done by Meena SP et al in100 cases of breast carcinoma. The cytological smears were graded by Robinson grading system and histologic grading used was modified Scarff – Bloom Richardson grading system. Sensitivity and specificity of cytological grading system were 90 .77% and 84.42% respectively.[4]

In 2008, Bhargava et al studied 30 cases of breast carcinoma. Cytological grading was done by three methods- Nottingham modification of Scarff Bloom Richardson method, Fisher's modification of nuclear grade and Robinson method. The best correlation was observed between Robinson method and histopathology method as well as ER, PR expression on smears.[1]

A study was done by Khan et al in 43 cases of breast carcinomas. Cytological grading was done according to Robinsons grading system and histological grading according to Scarff Bloom Richardson grading system. The sensitivity and specificity of cytological grading system were 89. 1% and 100% respectively.[33]

Fine needle aspirates from 104 ductal carcinomas were studied by Taniguchi et al and semiquantative scoring system was established based on seven parameters. Cytological findings were then correlated with clinicopathologic variables. The study showed that histological grade correlated positively with cytological grade and high cytological grade was associated with nodal metastasis.[12]

Dash A et al studied 93 cases of breast carcinomas in whom cytological grading was done using Taniguchis grading system. The corresponding histological grading was done according to Nottingham modification of Bloom – Richardson grading system. There was 77.4% correlation between cytological grade and histological grade.[30]

Prognostic factors in breast cancer are ranked according to previously established College of American Pathologists categorical rankings.[22]

Category I : Factors proven to be of prognostic importance and useful in clinical patient management.

Category II : Factors that had been extensively studied biologically and clinically , but whose importance remains to be validated in statistically robust studies.

Category III : All other factors not sufficiently studied to demonstrate their prognostic value.[22]

Factors ranked in Category I included TNM staging information, histologic grade, histologic type , mitotic figure counts and hormone receptor status.[22]

Category II factors include c- erb B- 2 (Her 2-neu) proliferation marker, lymphatic and vascular channel invasion ,p53,Ki67 and MIB-1.[22]

Factors in category III include DNA ploidy analysis, microvessel density, epidermal growth factor receptor, transformation growth factor-a, bcl-2, pS2 and cathepsin D.[22]

Fine needle aspiration biopsy of breast carcinoma were evaluated for 5 morphologic nuclear grade characteristics.

Nuclear characteristics evaluated and ranked as ,

(1) Nuclear pleomorphism (1-3) :	Mild .	- 1
	Moderate	- 2
	Severe	- 3
(2) Chromatin(2-4) :	Granularity : Fine	- 1
	Coarse	- 2
	Chromasia : Normochromatic	- 1
	Hyperchromatic	- 2
(3) Nucleoli(1-3) :	Inconspicuous	- 1
	Large	- 2
	Multiple	- 3
(4) Nuclear membrane(1-2) :	Smooth / regular	- 1
	Thick / irregular	- 2
(5) Mitotic Activity :	1 / 10 HPF	- 1
	> 2 / 10 HPF	- 2
Low cytonuclear grade :	Scores	- 6-9
High cytonuclear grade :	Scores	- 10-14

Corresponding histological sections were evaluated by SBR grading system. The Spearman correlation between cytonuclear grade and modified histograde calculated by linear regression analysis showed a strong correlation, r = 0.8059, $p<0.00001$. Cytologic nuclear grade from breast aspirates of invasive ductal carcinoma can be a predictor of modified histologic SBR grading system.[21]

In addition 4 morphometric standardized object measurements such as nuclear area, nuclear shape, sum optical density and average optical density were evaluated by image analysis system. The best discriminators of nuclear grade in this group of tumors are nuclear pleomorphism, nucleoli and sum optical density.[21]

Grading of breast carcinoma in isolation has prognostic value and grading has become routine in many laboratories.[22]

The other factors in combination with grading which forms a useful guide for selecting adjuvant systemic therapy are lymphnode metastasis and tumor diameter.[22]

Axillary lymphnode status has been shown to be an important predictor of disease- free survival and overall survival in breast cancer. Only 20% to 30% of node negative patients will develop recurrence within 10 years, compared with about 70% of patients with axillary node involvement. The absolute number of involved nodes is also of prognostic importance , patients with 4 or more involved nodes have a worse prognosis than those with fewer than 4 involved nodes.[22]

In a study done by Taniguchi et al, analysis of possible association of cytologic grading of breast carcinoma with histologic grading and the existence of lymph node metastasis was done. Statistical analysis showed a positive correlation between histologic and cytologic grade($r=0.337$; $p< 0.0015$) and high cytologic grade was associated with presence of nodal metastasis.[12]

The Nottingham prognostic index for breast carcinoma combines grading, nodal status and tumor diameter. This index was developed retrospectively but has since been validated over 20 years in Nottingham and other centres where diagnosis of breast carcinoma by FNAC is increasingly common. Nodal status can be scored in

the range 1 to 3 by a sampling or clearing procedure. Tumor diameter is measured by ultrasound.[7]

A high response rate to systemic adjuvant therapy was seen with the tumors rapidly decreasing in size, grade 3(faster growing) tumors are more likely to respond to chemotherapy than low-grade slow growing lesions, which are better suited to pretreatment with tamoxifen. Assessment of the biological aggressiveness of the cancer without removing it would therefore be valuable.[7]

Cytological classification of breast carcinoma based on FNA provides valuable information concerning the prognosis of patients, which is relevant to their clinical management.[16]

The combination of cytology, radiology and clinical assessment results in an accurate diagnosis in 99% of cases.

The pattern of distribution of chromatin, the differential staining affinity of nucleoli, the size of nuclei, the tendency to clustering of cells lead to a prognostic classification of moriquand et al.[16] Cytological specimens were obtained by FNA or from imprints of tissue sections at the time of surgery and were scored. The smears were classified into three grades of increasing severity.

Grade 1 : Upto 5 – Good prognosis

Grade 2 : 5-9 – Intermediate

Grade 3 : > 10 – Poor prognosis with recurrence

The clinical course of the disease was correlated with cytoprognostic classification as well as other recognized prognostic factors such as the TNM classification, the histoprognostic classification of Scarff and Bloom and the steroid receptor content of the tumor.[16]

Proliferation plays an important role in the clinical behaviour of invasive breast cancer. Increased proliferation correlates strongly with poor prognosis, irrespective of the methodology used. The growth fraction can be more easily assessed by immunohistochemistry of proliferation associated antigens such as Ki67, Ki S1, topoisomerase IIα, proliferating cell nuclear antigen (PCNA), geminin or minichromosome maintenance (MCM) proteins or by DNA flow cytometric or image cytometric (two or three dimensional) assessment of the S phase fraction.[27]

Incorporation techniques (for example, with bromodeoxyuridine and tritiated thymidine) theoretically provide the gold standard of cellular proliferation. All these methods have their good and bad points from a cell biological or practical point of view. However incorporation techniques are impractical because fresh material is needed, patients need to be injected intravenously,and/or radioactivity is involved in making them unattractive in daily practice. The percentage S phase is hampered by pronounced intratumor heterogeneity. Therefore mitosis counting and the Ki67 index are the most practical methods.[27]

Mitosis counting has been shown most convincingly to provide reproducible and independent prognostic value in breast cancer. Therefore mitotic activity index(MAI) is already used in clinical practice in several countries as a single prognostic marker and is the most well established components of histological grade. Ki67 / MIB- 1 labelling and cyclin A index are promising alternatives.[27]

Das AK et al determined utility of DNA ploidy and Ki67 in preoperative management by analyzing their association with grading of breast carcinoma. DNA ploidy was determined by image analysis and Ki67 index determined by immunohistochemistry (IHC) on FNA material and their values correlated with corresponding cytologic and histologic grades. Cytologic grading showed a concordance of 71.2% with histologic grading. Combination of DNA and Ki67 indices improve the cytological grading with better correlation with histology.[34]

Her-2 amplification is associated with poor prognosis. Different techniques have been used to measure the cellular concentration of Her-2 in biopsies and surgical samples but currently the most frequently used methods are immunohistochemistry and in situ hybridization. Her-2 status could be determined in liquid based cytology samples and correlated well with the reference histological methods- IHC and in situ hybridization.[35]

Histology grade, HsMCM2 expression and cyclin A expression may reflect the proliferative activity of the tumor cells. The relation between these three parameters and their independent prognostic value in breast cancer were evaluated in a study done by Bukhlom IRK et al. It was seen that HsMCM2 expression showed a association with histological grade and showed a tendency towards poor prognosis. Cyclin A expression was highly associated with poor prognosis.[36]

METHODOLOGY

Source of data

Cytologically proven breast carcinoma cases with respective specimens received in the department of pathology, BLDEA'S SHRI B.M.Patil Medical College, Hospital and Research centre, Bijapur were studied over a period from 1st August 2007 to 31st July 2009.

Methods of collection of data

Detailed clinical history and physical examination was done in all the study patients. Informed consent for FNAC was taken from all the study patients.

FNAC was performed by 22 gauge needle attached to 10 ml plastic disposable syringe and smears were fixed in 96% alcohol and stained by Papanicolaou stain and Hematoxylin and Eosin stain. Grading of these cytologic smears was done by the method devised by Taniguchi et al.

Breast specimens were fixed in 10% buffered formalin. Gross features were recorded. Bits were given from the specimens as follows:1 bit from nipple, 3 bits from tumor for less than 5 cms and additional bits for larger tumors i.e. 1 bit per 1 cm of tumor, 2 bits from non tumor areas of uninvolved quadrants, bits from all surgical margins. Lymph nodes were detected in the specimen and bits from all the lymph nodes were given. For lymph nodes less than 5 mm, entire bit was given and larger lymph nodes were bisected and half of the tissue was taken for section. The specimens were processed and stained routinely with Hematoxylin and Eosin stain. Grading of these slides was done by the method of Nottingham modification of Bloom Richardson method. Then the cytologic grade was compared with the histologic grade and lymph node status.

Statistical analysis:

Data were analysed by using Spearman's correlation coefficient (r value) for correlation of cytological grading with histological grading. Also Chi square test was done to determine the p value to find the association between two grading systems.

Inclusion criteria:

- All cases of FNACs of infiltrating ductal carcinomas of breast not otherwise specified with respective specimens were included in the study.

Exclusion criteria:

- All cases of breast carcinomas other than infiltrating ductal carcinomas not otherwise specified were excluded.

RESULTS

The present study consisted of 38 cases of invasive breast carcinomas – NOS (Not Otherwise Specified). All of the patients were females. Age of the patients in this study ranged from 22 to 65 years. Left breast was more commonly involved than the right breast. Upper outer quadrant was the most common site. The size of tumors ranged from 2-15 cms; maximum number of cases were in the range of 2.5- 5 cms.

Table : 6

Age wise distribution of cases

Age Range (Yrs)	Total No. of cases	Percentage (%)
21 - 30	01	2.63
31 - 40	10	26.32
41 - 50	15	39.47
51 - 60	09	23.68
61 - 70	03	7.89

Maximum number of cases were seen in the age group of 41 – 50 years. Out of 38 cases studied 15 cases were seen in this age group constituting about 39.47% of tumors. The next common age group affected was of range 31-40 years where 10 cases were seen constituting about 26.32% of tumors at this age group, followed by 9 cases (23.68%) in the age range of 51-60 years. Three cases were seen in the age group of 61-70 years constituting 7.89% of cases. The age group least commonly affected was in the range of 21-30 years. About 2.63% of tumors were seen in this age group.

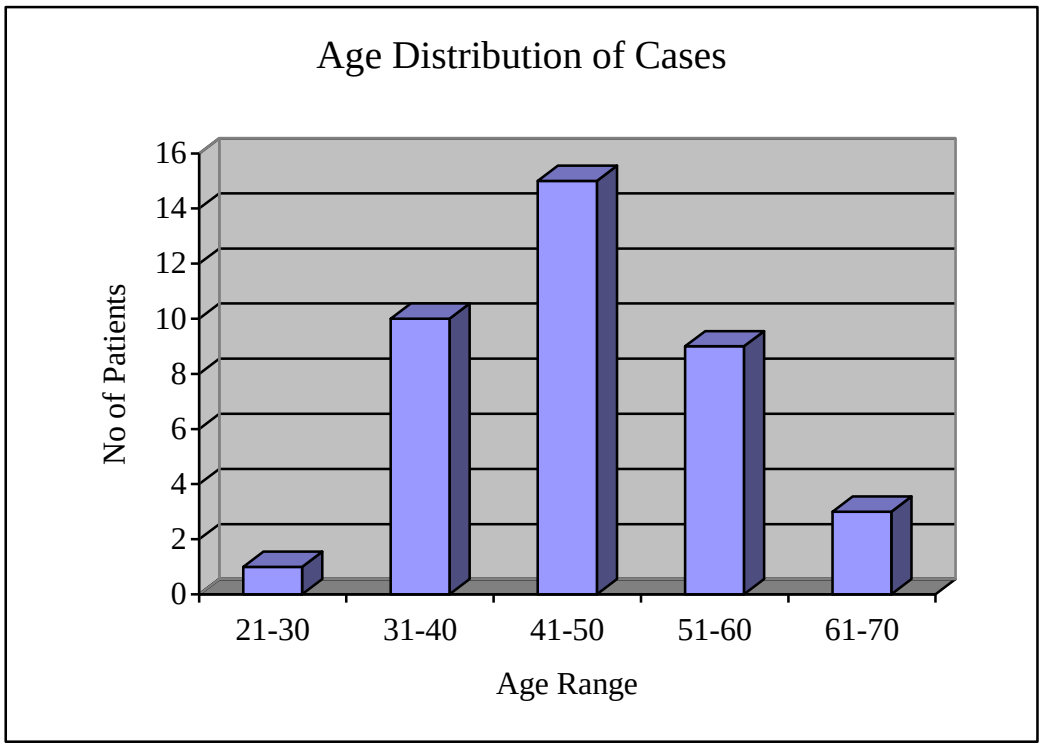

Table : 7

Distribution of cases - Taniguchi cytological grade

Grade	No. of cases	Percentage
I	02	5.26%
II	16	42.11%
III	20	52.63%
Total	38	100%

On Taniguchi's cytological grading, 2 cases out of 38 cases were of grade I which constituted about 5.26% of total cases, 16 cases were of Grade II which constituted about 42.11% of total cases and 20 cases were of Grade III which constituted about 52.63% of total cases. Total number of cases of various cytologic nuclear grades are as shown in Table :7

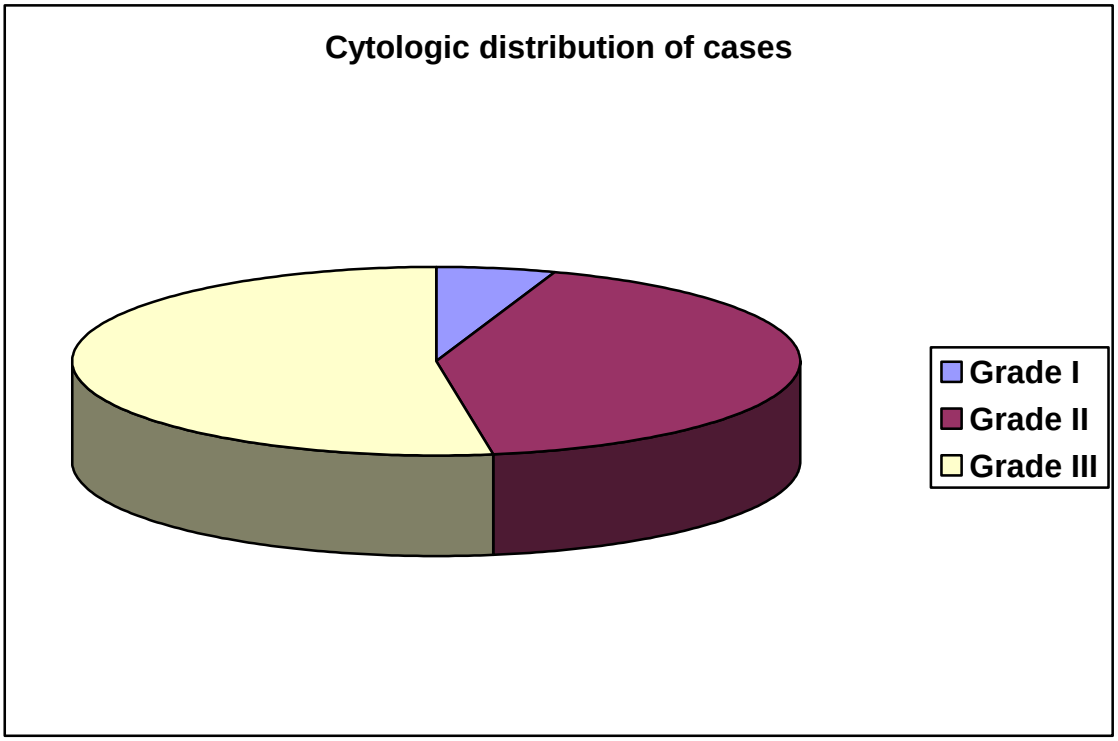

Table : 8

Distribution of cases – SBR histological grade

Grade	No. of cases	Percentage
I	01	2.63%
II	20	52.63%
III	17	44.74%
Total	38	100%

On Scarff Bloom Richardson histological grade, 1 case out of 38 cases was of grade I which constituted about 2.63% of total cases, 20 cases were of grade II which constituted about 52.63% of total cases and 17 cases were of grade III which constituted about 44.74% of total cases. Total number of cases of various histologic nuclear grades are as shown in Table : 8

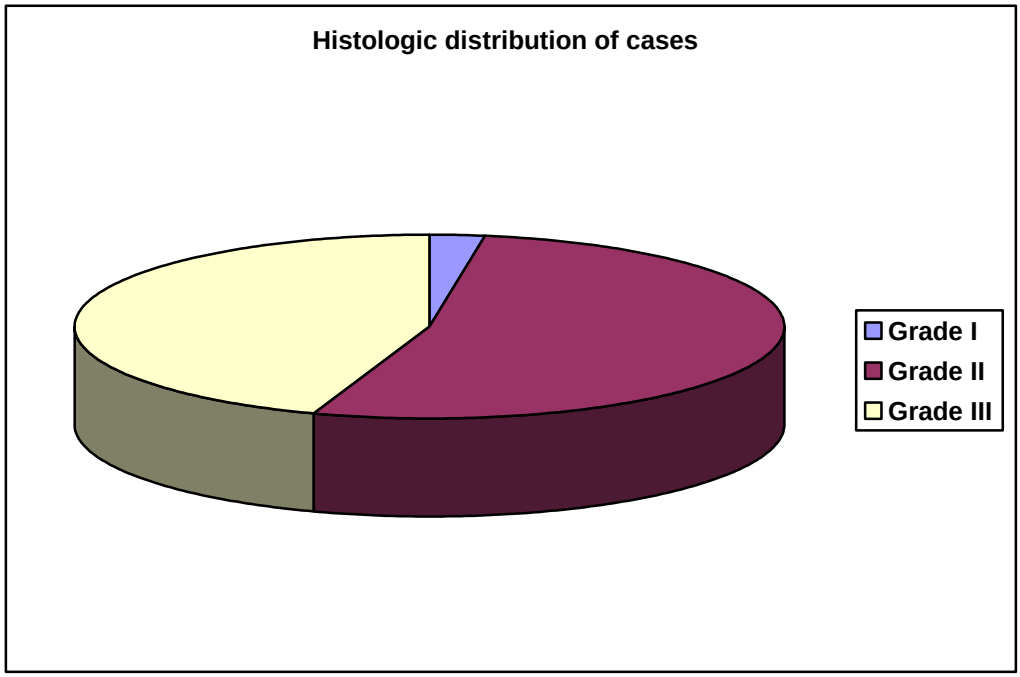

Table : 9

Comparison of cytological grade with histological grade – TGS & SBR

Cytological grade (TGS)	Histological grade (SBR)			Total
	I	II	III	
I	01	01	-	02
II	-	14	02	16
III	-	05	15	20
Total	01	20	17	38

Out of 2 cases on Taniguchi's cytological grade, 1 case was of Grade I and the other case was upgraded as grade II on SBR histological grade. Out of 16 cases on Taniguchi's cytological grade, 14 cases were of grade II and 2 cases were upgraded as grade III on SBR histological grade. Out of 20 cases on Taniguchi's cytological grade III, 15 cases were grouped as grade III and 5 cases were downgraded as grade II on SBR histological grade. Total concordance between cytologic grade and histologic grade was seen in 30 cases out of 38 cases accounting for 78.95% of total cases.

Table : 10

Comparison of cytological grade with lymph node status

Cytologic grade		Lymph node	
Grade	**No of cases**	**Positive**	**Negative**
I	02	01 (50.00%)	01(50.00%)
II	16	07 (43.75%)	09(56.25%)
III	20	12(60.00%)	08(40.00%)
Total	38	20	18

In the study, lymph node metastasis was seen in 1 out of 2 cases of grade I ,7 out of 16 cases of grade II and 12 out of 20 cases of grade III carcinomas. Totally about 20 cases among the 38 cases studied showed lymph node metastasis which accounted for 52.63% of cases. Majority of these were grade III cases which showed metastasis in 60% of grade III cases. Grade II cases showed lymph node metastasis in 43.75% of grade II cases. Grade I cases showed metastasis in 50% of grade I cases.

Table : 11

Comparison of histological grade with lymph node metastasis

Histologic grade		Lymph node	
Grade	**No of cases**	**Positive**	**Negative**
I	01	01	--
II	20	09	11
III	17	10	07
Total	38	20	18

On Scarff Bloom Richardson histological grading, lymph node metastasis was seen in 100% in grade I case ,9 out of 20 cases of grade II which accounted for 45% of grade II cases and 10 out of 17 cases of grade III carcinomas which accounted for 58.82% of grade III cases. Majority of these were grade III cases.

The Spearman's correlation coefficient was determined. The r value was 0.684 which indicates that there is a strong correlation between Taniguchi's cytological grading system and Scarff Bloom Richardson's histological grading system.

The Chi – square test was performed and the p value was determined which was less than 0.05, thus indicating a strong association between the grading by these two methods.

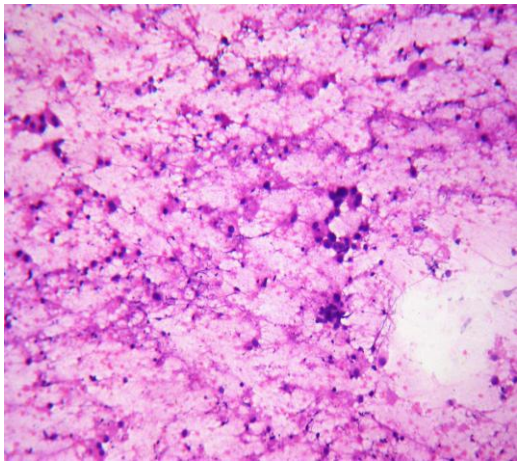

Fig 1: FNA smear showing necrosis (H&E,40x)

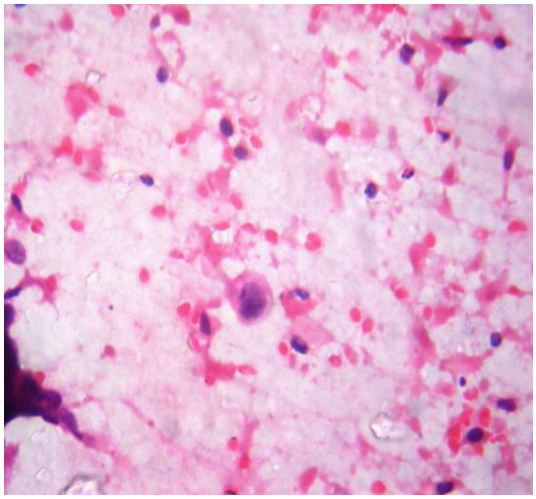

Fig 2: FNA smear showing cellular size of more than 4 times RBC size(H&E, 400x)

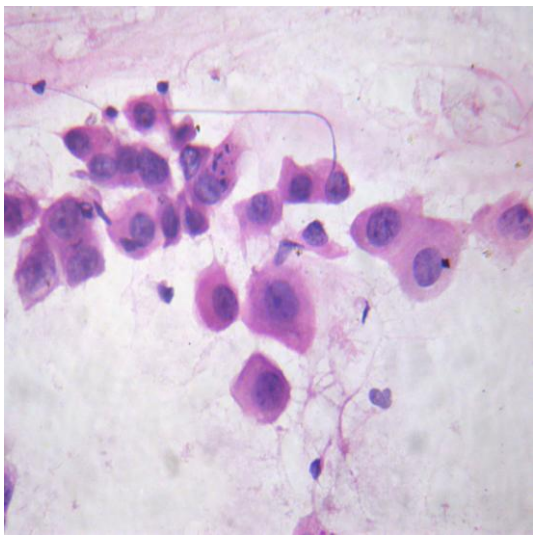

Fig 3: FNA smear showing N/C ratio of 50% -80%(H&E,400x)

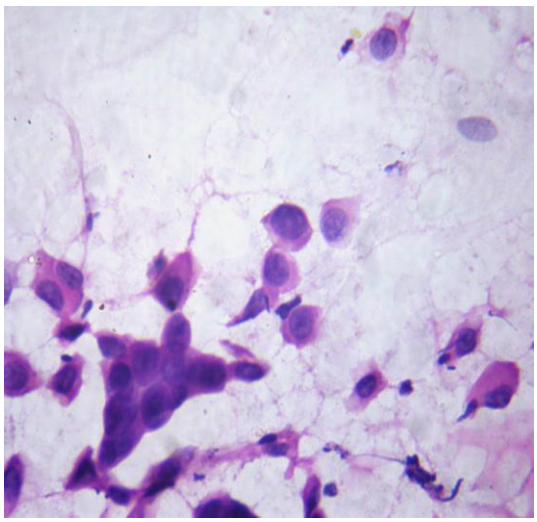

Fig 4: FNA smear showing N/C ratio of more than 80%(H&E, 400x)

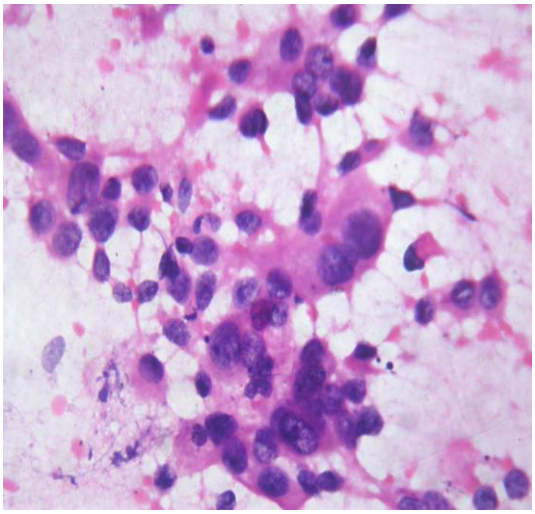

Fig 5: FNA smear showing marked nuclear pleomprphism(H&E,400x)

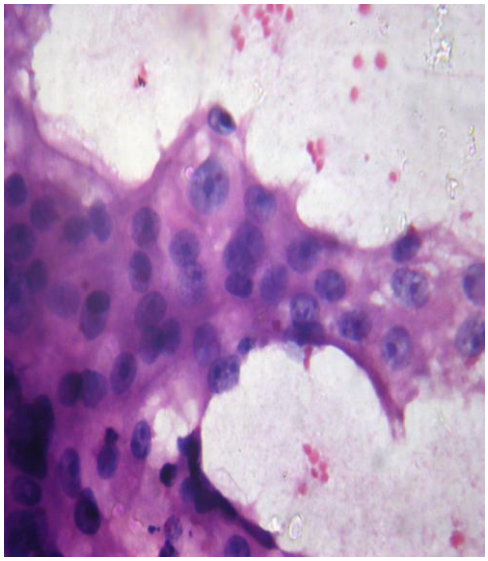

Fig 6: FNA smear showing prominent nucleoli(H&E, 400x)

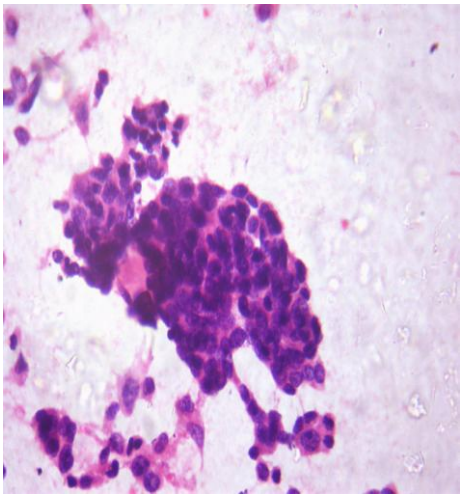

Fig 7: FNA smear showing coarse chromatin granularity(H&E, 100x)

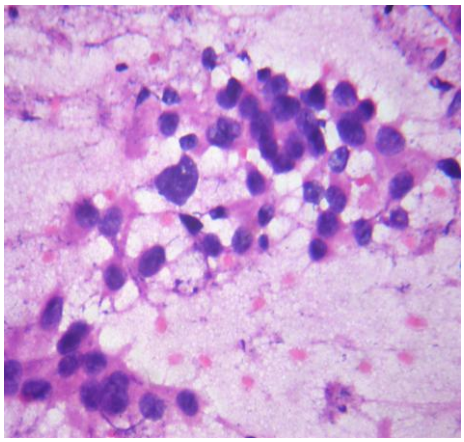

Fig 8: FNA smear showing markedly hyperchromatic chromatin(H&E, 400x)

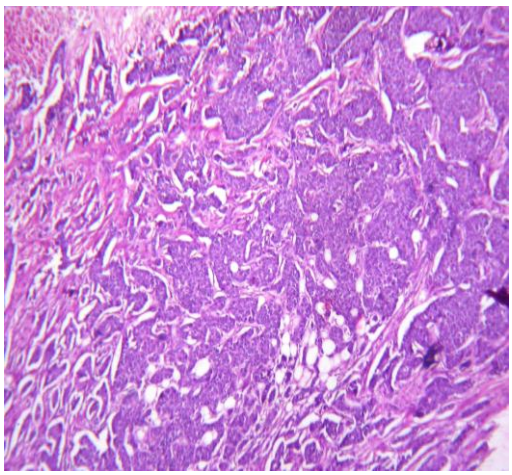

Fig 9: Grade III carcinoma with tubule formation - 3(H&E, 100x)

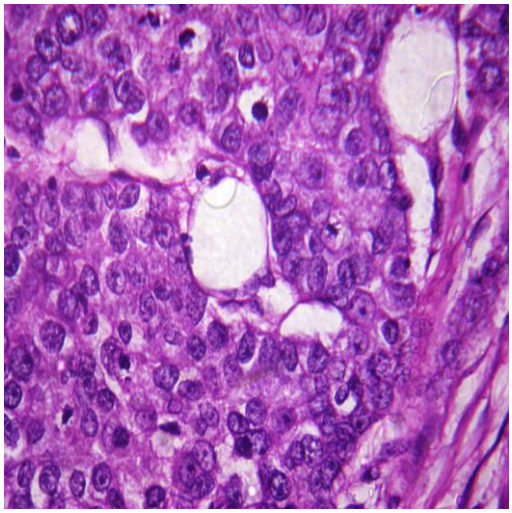

Fig 10: Grade II carcinoma with nuclear pleomorphism - 2(H&E,

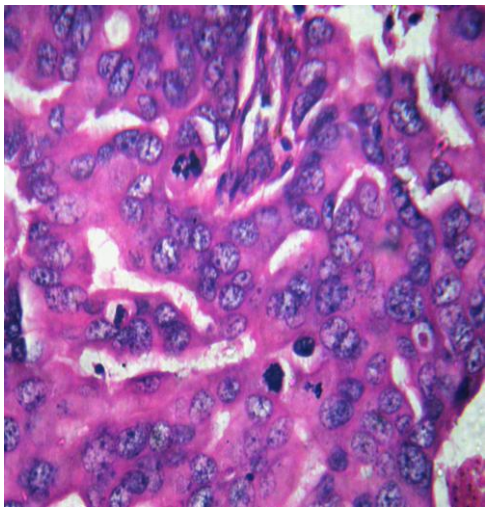

Fig 11: Grade III carcinoma with mitoses - 3(H&E, 400x)

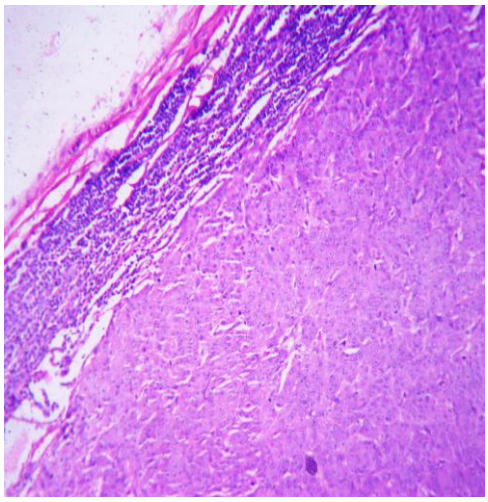

Fig 12: Lymph node metastasis of breast carcinoma(H&E, 100x)

DISCUSSION

The incidence of breast cancer in India is increasing now a days and approaching to that in the western world. The incidence of early detection of breast cancer is increasing dramatically due to public awareness and widespread use of mammography. However, no significant decrease in mortality from breast cancer has yet been noted.[28]

More reliable methods for the evaluation of factors predicting tumor progress are necessary in order to develop adequate therapy regimens for different types of breast cancers. For the establishment of prognostic markers in breast cancer, many scientific efforts have been undertaken. Tumor grade stands out as an important prognostic parameter besides the undisputed impact of tumor size and lymphnode status.[28]

Large number of surgical pathology literature citations are there on the subject of breast carcinoma nuclear grade. But only a few citations on breast carcinoma nuclear grade are in the recent cytology literature.[30]

Fine Needle Aspiration Cytology was introduced as a primary tool in the diagnosis of breast carcinoma. Additional clinically relevant information like estrogen and progesterone receptors, proliferative fraction and oncogene products can be obtained from the samples. Also information on cytologic nuclear grade can be obtained easily and reproducibly and it should be included in the cytopathology report.[30]

While FNAC is less invasive and more cost effective, it does pose an increased challenge for the pathologist. It is the responsibility of the pathologist to give the clinician as much information as possible when rendering the diagnosis including the tumor grade.[33]

Tumor grading does not require special procedures and therefore incurs no additional cost. But still it is one of the most important prognostic factors to consider in predicting outcome in breast cancer patients.[33]

With the use of conservative treatment being available to the patient, especially for patients who receive chemotherapy, radiotherapy and/or hormone therapy as primary treatment it has become of increasing interest to assess cytological nuclear grade. In such cases, if the cytologic grade are equally effective, then biopsy can be avoided and the patients can be directly subjected to surgical procedure (mastectomy, simple or modified) following preoperative radiotherapy, chemotherapy or hormone therapy.[4]

Different authors have followed different cytologic grading systems, some have even tried to compare them and evaluate the most suitable.[30]

Das A K et al studied 52 cases of breast carcinoma where cytologic grading of smears was done using Robinson's and Moriquand's grading methods and the grades were compared with SBR histological grading. Both Robinson's and Moriquand's grading systems were found to have similar concordance with histological grading, but Robinson's method was considered better because of its simplicity, and specificity.[2]

Frias et al studied 100 cases of invasive ductal carcinoma, cytologic grading was done using Robinson's method and histological grading was done using SBR method. A statistically significant association was observed between cytologic and histologic grades and between cytologic grade and presence of axillary lymph node metastasis. Similarly, cell dissociation, cell uniformity and the appearance of nuclear margins all displayed a positive correlation with regional metastasis.[3]

In a study done by Khan et al which included 43 cases of infiltrating carcinomas, cytologic grading was done by Robinson's grading method and histologic grading was done by SBR grading method. Cytologic grading was found to be fairly comparable with histologic grading, difference between the two grading methods was insignificant in all the three of the six parameters studied, cell dissociation, nucleoli and chromatin pattern were the most influencing.[33]

Chhabra et al studied 60 cases of breast carcinoma where Robinson's method was used for grading cytologic smears and SBR grading was used for histologic grading. There was agreement between cytologic grade and histologic grade in 65% of the tumors. The histologic grade correlated positively with cytologic grade.The study also showed that extent of cell dissociation and nucleoli were the most influential features.[24]

Table : 12

Comparative table on distribution of cases – Taniguchi cytological grade

Grade	Present study	Dash A et al	Taniguchi et al
I	02(5.26%)	22 (23.65%)	32(31.7%)
II	16(42.11%)	40(43.01%)	39(37.5%)
III	20(52.63%)	31(33.33%)	33(31.8%)
Total	38	93	104

In the present study 2 cases out of 38 cases (5.26%) were of grade I , 16 cases out of 38 cases (42.11%) were of grade II, 20 cases out of 38 cases (52.63%) were of grade III.

A study was done by Taniguchi et al in 104 breast carcinoma cases to estimate cytologic grade and correlate with other known prognostic factor, such as tumor differentiation, growth fraction, estrogen receptor status and nodal status. Among the 104 cases studied, 32 cases(31.7%) were of grade I, 39 cases (37.5%)were of grade II and 33 cases(31.8%) were of grade III.

Dash et al studied 93 cases of invasive breast carcinoma where cytologic grading was done according to Taniguchi's method and histologic grading was done according to SBR grading. In their study, 22 cases out of 93 cases (23.65%) were of grade I, 40 cases out of 93 cases (43.01%) were of grade II, 31 cases out of 93 cases (33.33%)were of grade III. Maximum number of cases were in grade III in the present study. In the studies done by Dash et al and Taniguchi et al, the distribution of cases differed, probably a reflection of sample size.

Table : 13

Comparative table on distribution of cases – SBR histological grade

Histo grade	Khan et al	Chhabra et al	Robinson et al	Frias et al	Dash et al	Taniguchi et al	Present study
I	14	17	09	33	24	9	01
II	17	30	29	39	45	51	20
III	12	13	14	28	24	30	17
Total	43	60	52	100	93	90	38

On SBR grading of mastectomy specimens in the present study, only 1 case (2.63%) was of grade I, 20 cases out of 38 cases (52.63%) were of grade II, 17 cases out of 38 cases (44.73%) were of grade III. Maximum cases were of grade II in the present study. In most of the studies, a large number of patients have been placed in grade II. This is one of the limitation of SBR grading in which there is unequal

distribution of patients among the three grades with over 50% of patients in grade II. Furthermore, even though there is a relatively clear prognostic separation between grade I and grade III, grade II patients often overlap with grade I or grade III.[23]

Table : 14

Comparison of Taniguchi cytological grade with SBR histological grade

Histo Grade	Chhabra et al			Meena et al			Dash et al			Present study		
	I	II	III	I	II	III	I	II	III	I	II	III
I	11	06	01	19	03	00	07	06	02	01	00	00
II	05	21	05	04	32	04	02	20	02	01	14	05
III	01	03	07	00	01	08	00	03	10	00	02	15
Total	17	30	13	23	36	12	09	29	14	02	16	20

In the present study, 2 cases were of grade I on Cytology out of which 1 case turned out to be grade II on histology, 16 cases were of grade II on cytology out of which 14 were of grade II and 2 cases turned out to be grade III on histology, 20 cases were of grade III on cytology out of which 15 cases were of grade III on histology and 5 cases turned out to be grade II on histology.In a study by Dash et al there were 9 cases of grade I on cytology out of which 7 cases were of grade I and other 2 cases turned out to be grade II on histology, 29 cases of grade II on cytology out of which 20 cases were of grade II on histology while 6 cases were downgraded to grade I and 3 cases were upgraded to grade III, 14 cases were of grade III on cytology out of which 10 cases were of grade III on histology while 2 cases were downgraded to grade II and 2 cases were downgraded to grade I on histology.

In a study by Meena et al there were 23 cases of grade I on cytology out of which 19 cases were of grade I and other 4 cases turned out to be grade II on

histology, 36 cases of grade II on cytology out of which 32 cases were of grade II on histology while 3 cases were downgraded to grade I and 1 case was upgraded to grade III, 12 cases were of grade III on cytology out of which 8 cases were of grade III on histology while 4 cases were downgraded to grade II .

A study done by Chhabra et al showed 17cases of grade I on cytology out of which 11 cases were of grade I and other 5 cases turned out to be grade II and 1 case turned out to be grade III on histology, 30 cases of grade II on cytology out of which 21 cases were of grade II on histology while 6 cases were downgraded to grade I and 3 cases were upgraded to grade III, 13 cases were of grade III on cytology out of which 7 cases were of grade III on histology while 5 cases were downgraded to grade II and 1 case was downgraded to grade I on histology .

Among the 38 cases studied , discordance between the two grading systems was seen in 8 cases accounting for 78.95% correlation between cytologic grade and histologic grade which is comparable to those reported by Dash A et al.The accuracy of grading systems by other authors varied from 53.895 to 83%. This variability may be due to several factors such as different systems used to assign cytologic grade, its evaluation in air dried materials, different staining techniques etc.The lack of correlation (21.05 %) may be due to the presence of different degrees of atypia within the same tumor and subjective nature of grading process.[30]

Table : 15

Comparative table on correlation of cytologic grading with histologic grading

Study	r value	p value
Present study	0.683	<0.05
Chhabra et al	0.774	< 0.005
Frias et al	0.774	<0.005
Taniguchi et al	0.337	<0.0015
Moroz et al	0.8059	<0.0001

In the present study Spearman's correlation coefficient (r value) was 0.684 which indicates that there is a positive correlation between Taniguchi cytologic grading and SBR histologic grading. The high value of coefficient of correlation showed a significant and marked association ($p<0.05$) between the grades assigned to cytologic and histologic specimens. Moroz et al evaluated fine needle aspiration biopsies of breast carcinoma for five morphologic nuclear grade characteristics and found a strong correlation ($r=0.8059, p<0.0001$) between cytologic nuclear grade and modified histologic grade, while Taniguchi et al observed a significant correlation($r=0.337$, $p=0.0015$) between cytologic and histologic grade. In a study done by Chhabra et al, a significant correlation($r=0.774$, $p=0.005$) was noted between cytologic grade and histologic grade. Frias et al observed a significant correlation($r=0.774$, $p=0.005$) between cytologic grade and histologic grade.

Table : 16

Comparative table of Cytological grade and nodal metastasis

Cytological grade	Present study		Frias et al (2005)		Dash et al(2005)	
	No of cases	Metastasis	No of cases	Metastasis	No of cases	Metastasis
I	02	01	36	03	22	06
II	16	07	39	25	40	14
III	20	12	25	22	31	23
Total	38	20	100	50	93	43

Lymph node status is another important prognostic factor in breast cancer. We have studied lymph nodes for regional metastasis and found that out of 2 cases of grade I, one case (50%) showed metastasis. Out of 16 cases of grade II, metastasis was seen in 7 cases (43.75%) and out of 20 cases of grade III, metastasis was seen in 12 cases (60%) . Totally out of 38 cases, metastasis was seen in 20 cases accounting for 52.63 % of lymph node positivity.

Dash et al studied lymph nodes for regional metastasis and found metastasis in 6 cases out of 22 grade I cases, 14 out of 40 grade II cases, 23 out of 31 grade III cases. Overall out of 93 cases, 43 cases showed lymph node metastasis accounting for 46.23% of lymph node positivity.

A study was done by Frias et al in 100 cases of breast carcinoma where metastasis was seen in 3 cases out of 36 grade I cases, 25 out of 39 grade II cases and 22 out of 25 grade III cases. Overall 50% cases showed lymph node positivity.

In the present study, majority of the lymph node positive cases were of grade III which suggests that cytological grade is useful tool for predicting lymph node metastasis.

CONCLUSION

- Taniguchi's grading system is simple, takes little time, is reproducible and with rare exceptions, depending on sample limitations, correlates precisely with the histological grade.
- A positive correlation was observed between Taniguchi's cytological grading system and SBR histological grading system.
- Discordance between cytologic grading and histologic grading was seen in few cases which may be due to sampling error, the presence of different degrees of atypia within the same tumor and subjective nature of grading process.
- Maximum number of grade III cases showed lymph node metastasis which suggests that cytologic grade is a useful tool for predicting lymph node metastasis.
- Cytologic grading allows prognostic evaluation of breast carcinoma along with diagnosis without additional morbidity or expense to the patient.
- Hence it is recommended that cytological nuclear grade should appear in FNAC reports of ductal breast carcinoma for proper management.

SUMMARY

- The present study was conducted in 38 cases of cytologically diagnosed invasive ductal carcinomas – NOS.
- Cytological grading of FNAC smears was done which was later compared with histological grading.
- Cytological grading was done according to Taniguchi's grading method and histological grading was done according to SBR grading method.
- Highest incidence was seen in 4^{th} to 5^{th} decade.
- Maximum number of cases were of grade III on cytological grading while on histological grading maximum cases were of grade II
- The overall concordance on cytology and histology by Taniguchi grading system and Scarff Bloom Richardson grading system was 78.94 %.
- The Spearmans correlation coefficient, r value was determined which was 0.684 indicating that there is a positive correlation between cytological grading (TGS) and histological grading (SBR).
- Evaluation of lymph nodes showed metastasis in 52.63% of cases and majority of lymph node positive cases were of grade III.

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PROFORMA

NAME **:** OP/IP No. :

AGE **:**

SEX : D.O.A :

ADDRESS : D.O.D :

History of present illness:

Marital history:

Past history:

Family history:

Obstetrics and gynecological history:

Age at marriage:

Age at menopause:

No. of pregnancies:

General physical examination:

Systemic examination:

Per Abdomen:

Cardiovascular system

Respiratory system:

Local examination:

Location:

Site:

Size:

Adherence to skin:

Adherence to underlying structures:

Lymph node status:

CYTOLOGICAL GRADING

Taniguchi grading system:

Parameters	Score
Necrosis	
Cellular size	
Nuclear cytoplasmic ratio	
Nuclear pleomorphism	
Nucleoli	
Chromatin granularity	
Density of chromatin	
Total score:	
Grade:	

HISTOLOGICAL GRADING

Scarff Bloom Richardson grading:

Parameters	Score
Tubule formation	
Nuclear pleomorphism	
Mitotic count/10 HPF	
Total score:	
Grade:	

B.L.D.E.A'S SHRI B.M.PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTER ,BIJAPUR-586103

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT : COMPARATIVE STUDY OF FINE NEEDLE ASPIRATION CYTOLOGY AND HISTOPATHOLOGY IN GRADING BREAST CARCINOMA

PRINCIPAL INVESTIGATOR : DR. VIJAYALAXMI. S. PATIL
P.G. DEPARTMENT OF PATHOLOGY

P.G.GUIDE : DR. R. M. POTEKAR MD
PROFESSOR, DEPT OF PATHOLOGY.

PURPOSE OF RESEARCH:

I have been informed that this study is done to know the efficacy of fine needle aspiration cytology in assessing the prognosis of breast carcinomas.

PROCEDURE:

I understand that, I will undergo detailed history and clinical examination after which FNAC will be done prior to surgery and the samples will be subjected to pathological study.

RISK AND DISCOMFORTS:

I understand that, there is no risk involved in the procedures performed.

BENEFITS:

I understand that my participation in the study will help to know the prognosis of breast carcinomas.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulations of the hospital. If the data is used for publications the identity of patient will not be revealed.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more information about the study at any time.

REFUSAL FOR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw from the study at any time.

INJURY STATEMENT:

I understand that in the unlikely event of injury to me during the study I will get medical treatment but no further compensations.

I have read and fully understood this consent form. Therefore I agree to participate in the present study.

_____ _____

Participant / Guardian Date:

_____ _____

Signature of Witness Date:

I have explained the patient the purpose of the study, the procedure required and possible risk and benefit to the best of my ability in the vernacular language.

_____ _____

Investigator / P.G. Date:

_____ _____

Witness to Signature Date

MASTER CHART

TANIGUCHI GRADING SYSTEM – CYTOLOGICAL GRADE

Sl No	Age in years	Necrosis	Cellular size	N/C Ratio	Nuclear pleomorphism	Nucleoli	Chromatin granularity	Density of Chromatin	Total Score	Grade
1	60	0	2	2	2	1	2	2	11	II
2	60	0	2	2	2	1	2	1	10	II
3	50	0	2	2	1	1	3	2	11	II
4	48	0	3	2	2	1	3	3	14	III
5	40	0	2	2	2	2	2	2	12	III
6	55	0	1	2	2	3	3	2	13	III
7	65	0	3	2	2	1	3	2	13	III
8	35	0	3	1	2	2	2	2	12	III
9	56	0	2	2	3	1	2	2	12	III
10	45	0	1	2	1	3	2	1	10	II
11	60	0	3	2	3	3	1	2	14	III
12	35	0	2	2	3	3	1	1	12	III
13	40	0	3	2	2	2	2	2	13	III
14	45	0	2	2	2	1	2	1	10	II
15	55	0	2	2	2	3	2	1	12	III
16	50	0	2	1	2	2	1	1	09	I
17	48	0	3	2	3	1	1	1	11	II
18	38	1	3	3	3	3	2	2	17	III
19	45	0	2	2	3	1	2	2	12	III
20	52	0	2	2	2	2	1	1	10	II
21	65	0	1	2	2	1	2	2	10	II
22	48	0	3	2	3	3	3	2	16	III
23	38	0	2	2	3	2	2	1	12	III
24	65	0	3	2	3	2	2	2	14	III
25	40	0	3	3	3	2	2	3	16	III
26	44	1	2	2	3	2	3	3	16	III
27	35	0	3	2	2	3	2	2	14	III
28	38	0	3	2	3	3	3	2	16	III

Sl No	Age in years	Necrosis	Cellular size	N/C Ratio	Nuclear pleomorphism	Nucleoli	Chromatin granularity	Density of Chromatin	Total Score	Grade
29	50	0	3	2	2	1	2	1	11	II
30	47	0	2	2	2	1	1	1	09	I
31	60	0	2	2	2	1	1	2	10	II
32	50	0	2	2	1	1	2	2	10	II
33	60	0	2	2	2	1	2	2	11	II
34	40	0	3	2	3	1	1	1	11	II
35	24	0	2	1	2	2	2	2	11	II
36	43	1	3	3	3	3	2	2	17	III
37	45	0	2	2	2	2	1	1	10	II
38	55	0	2	3	2	1	1	1	10	II

SCARFF BLOOM RICHARDSON GRADING SYSTEM – HISTOLOGICAL GRADE

Sl No	Age in Years	Tubule Formation	Nuclear Pleomorphism	Mitosis	Total Score	Grade
1	60	3	2	1	6	II
2	60	3	2	1	6	II
3	50	2	2	2	6	II
4	48	3	3	2	8	III
5	40	3	2	3	8	III
6	55	3	3	2	8	III
7	65	3	3	3	9	III
8	35	3	2	2	7	II
9	56	2	3	1	6	II
10	45	2	2	2	6	II
11	60	3	3	3	9	III
12	35	3	3	2	8	III
13	40	3	3	1	7	II
14	45	3	2	1	6	II
15	55	3	3	2	8	III
16	50	2	2	1	5	I
17	48	3	2	1	6	II
18	38	3	3	3	9	III
19	45	3	3	3	9	III
20	52	2	2	2	6	II
21	65	2	3	1	6	II
22	48	3	3	1	7	II
23	38	3	2	1	6	II
24	65	3	3	2	8	III
25	40	3	3	2	8	III
26	44	3	3	3	9	III
27	35	3	3	3	9	III
28	38	3	3	2	8	III
29	50	3	2	1	6	II

Sl No	Age in Years	Tubule Formation	Nuclear Pleomorphism	Mitosis	Total Score	Grade
30	47	3	2	1	6	II
31	60	3	3	2	8	III
32	50	3	3	1	7	II
33	60	2	3	1	6	II
34	40	2	3	1	6	II
35	24	3	3	2	8	III
36	43	3	3	2	8	III
37	45	3	2	1	6	II
38	55	3	3	1	7	II

Master Chart

Sl No	Cytological grade	Histological grade	Lymph Node status
1	II	II	+
2	II	II	_
3	II	II	+
4	III	III	+
5	III	III	+
6	III	III	_
7	III	III	_
8	III	II	+
9	III	II	+
10	II	II	+
11	III	III	_
12	III	III	_
13	III	II	+
14	II	II	_
15	III	III	+
16	I	I	+
17	II	II	_
18	III	III	+
19	III	III	+
20	II	II	+
21	II	II	+

Sl No	Cytological grade	Histological grade	Lymph Node status
22	III	II	+
23	III	II	-
24	III	III	+
25	III	III	_
26	III	III	+
27	III	III	_
28	III	III	_
29	II	II	-
30	I	II	-
31	II	III	+
32	II	II	-
33	II	II	-
34	II	II	-
35	II	III	+
36	III	III	+
37	II	II	_
38	II	II	-