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Original Research Paper

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# CORRELATION OF NUCLEAR GRADING AND ANGIOGENESIS IN BREAST MALIGNANCY

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**ABSTRACT** Neoplasms constitute the most important lesions of the breast. Commonest are the neoplasms arising from the epithelial component, which constitute the glandular element of the breast. The most common type of carcinoma is infiltrative ductal carcinoma followed by lobular carcinoma and then other smaller groups. Many prognostic factors have been identified in breast carcinoma. The grading of breast carcinomas is one of the most useful prognostic indicators. When combined with histological tumour type and race of the patient, nuclear grade is a most powerful predictor of tumour aggressiveness. Tumour angiogenesis as a prognostic marker is a latest issue. A number of studies have found that highly vascular carcinomas have a poorer prognosis than those of low vascularity. In present study an attempt has been made to correlate nuclear grading with microvessel density due to angiogenesis so as to develop a new criterion for assessing the prognosis of different histomorphological types of carcinoma breast. Nuclear grading of the lesions was done according to the Nottingham modification of Bloom Richardson system (1991). All sections were assessed for angiogenesis by calculating MVD. The MVD increased from grade I to grade II to grade III lesions. On statistical analysis there was a positive high correlation between nuclear grade and microvessel density. Present study reveals that microvessel density correlate positively with nuclear grade.

**KEYWORDS :** breast carcinoma, nuclear grading, angiogenesis, microvessel density

## INTRODUCTION

Neoplasms constitute the most important lesions of the breast. Commonest are the neoplasms arising from the epithelial component, which constitute the glandular element of the breast.

The most common type of carcinoma is infiltrative ductal carcinoma (70%). Lobular carcinoma is the second most common histological group of breast carcinoma followed by smaller groups such as medullary, mucinous, comedo carcinoma, paget's disease, papillary, tubular and inflammatory carcinoma **(Berg and Hutter 1995[1]).**

Many prognostic factors have been identified in breast carcinoma. The most commonly used indicators for prognosis include the clinical stage of the disease, tumour size **(Galea et al 1992[2])**, lymphnode metastasis **(Pisansky et al 1993[3])**, tumour grade, estrogen or progesterone receptor status of the tumour**.**

Nuclear grade is an important prognostic factor in breast carcinoma and many studies of the nuclear grade have been performed on the histological section. Fine Needle Aspiration smears are also well suited for nuclear grading. When combined with histological tumour type and race of the patient, nuclear grade is a most powerful predictor of tumour aggressiveness **(Fisher et al 1990[4])**.

While nuclear grading has been traditionally used, tumour angiogenesis as a prognostic marker is a latest issue**.** Current studies of tumour angiogenesis rely on the concept that endothelium proliferates 30-40 times faster in tumours than in normal tissue**.** A number of studies have found that highly vascular carcinomas have a poorer prognosis than those of low vascularity**.** Angiogenesis is found to be a key event in the metastatic growth.

Angiogenesis is defined as the process of new blood vessel formation. The neovascularization may be stimulated by factor from tumour cells (autocrine) or by tumour associated inflammatory cells or by extra cellular matrix.

Tumour angiogenesis refers to the growth of new vessels towards and within the tumour.

In present study an attempt has been made to correlate nuclear grading with microvessel density due to angiogenesis so as to develop a new criteria for assessing the prognosis of different histomorphological types of carcinoma breast.

## AIMS AND OBJECTIVES:

1. To establish histomorphological type of breast lesion.
2. To carry out nuclear grading of malignant breast lesions.
3. To assess angiogenesis: quantitation of MVD (microvessel density) in malignant breast lesions.
4. To correlate the nuclear grading with angiogenesis.

## MATERIALS AND METHODS:

The tissue material for the study was obtained from the patients admitted in Nehru Chikitsalaya, BRD Medical College, Gorakhpur over a period of 14 months (September 2006 to November 2007). The tissue specimens preserved in the Department of Pathology, BRD Medical College, Gorakhpur were also utilized in the study.

51 cases of breast surgery for malignant lesions were taken and the lesions were classified according to the WHO classification.

Sections from the lesions were taken and then stain with H & E stain & examined microscopically

### Nuclear grading of breast carcinomas:

The nuclear grading was done on tissue sections. For more objectivity four criteria were selected – nuclear size, nuclear margin, chromatin pattern and nucleolar characteristics and to each criteria scores were allotted and these scores were finally summed up to assign grade.

| Criteria | Score | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Nuclear size | 1-2 times the size of RBC | 3-4 times the size of RBC | >5 times the size of RBC |
| Nuclear margin | Smooth | Slightly irregular/ folds or grooves | Buds and clefts |
| Chromatin pattern | Vesicular/ reticular | Granular | Clumping/ clearing |
| Nucleoli | Indistinct | Noticeable | Abnormal |

Score 4-6 Grade 1
Score 7- 9 Grade 2
Score 10-12 Grade 3

**Counting procedure for vessels:**
In all cases of breast malignancy most discrete blood vessels which appeared as lumen lined by endothelial cells were counted in high power lens using 40X objective and 10X eyepiece in 200 fields in H & E stained sections. In small bits, only maximum possible fields were seen. Simultaneously the morphology of microvessels in the form of size, shape and thickening of vessel wall was observed. To avoid bias, counting was done by two different observers.

The microvessel density for each group of lesions was correlated with the nuclear grade.

**RESULTS:**
In the present study fifty one formalin fixed and paraffin embedded surgical specimens of various malignant disease of breast were studied to grade the lesions by Bloom Richardson's nuclear grading system (1991) along with density, morphology and localization of blood vessels. For this routine staining method (H&E) was employed.

In H&E stained sections the lesions were graded according to the Nottingham modification of Bloom and Richardson system (1991), along with nuclear grading. For microvessel counting only well formed vessels were identified in which endothelial cells were also present. Capillaries were identified with the help of contained Red Blood cells within the lumen.

A statistical analysis has been done between microvessel density and nuclear grading to establish a relationship between the above two.

The study group consisted of 51 malignant breast lesions which were categorized according to WHO classification.

Out of total 51 cases studied maximum 50(98.04%) cases were invasive carcinoma, in which maximum 42(82.35%) cases were Invasive ductal carcinoma (NOS), followed by 7(13.73%) cases of Invasive lobular carcinoma and 1(1.96%) case of IDC(NOS) Scirrhous type. Only 1(1.96%) case was non invasive tumour, which was papillary carcinoma in situ Among the 51 cases studied, maximum patients were in age group 40-50 years (41.17%) followed by 35.3% patients from 50-60 years.17.64% patients were from above 60 years and 5.89% patients from 30-40 years of age group.

Only one case of ductal carcinoma in situ, papillary type showed nuclear grade I, with nuclear size being thrice of a red blood cell, and smooth nuclear margin with granular chromatin and indistinct nucleoli.

Maximum 20 (46.5%) cases of present study belong to nuclear grade II with score 7-9 followed by grade III 15(34.9%) cases with score 10-12. Only 8 cases (18.6%) were of grade I with score 4-6.

Out of the four criteria employed for nuclear grading the assessment of nucleoli was difficult in the histological sections specially if the tumour cells were crowded and overlapped, however in these cases the slides were carefully scanned for the thin areas and the nucleoli assessed.

All 7 cases of lobular carcinoma studied showed nuclear grade I with nuclear size thrice a red blood cell size having granular chromatin, smooth margins and inconspicuous nucleoli with scores 4-6, belonging to Grade-I.

On analysis of nuclear grading scores of all the 51 cases, Nuclear Grade-II is the commonest grade in IDC where as grade I in lobular carcinoma.

Maximum 20(39.2%) cases of present study belong to nuclear Grade-II followed by grade-I 16(31.4%) cases & 15 cases (29.4%) were of Grade-III.

MVD in invasive lesions with nuclear grade-I was varied from 1-25 with an average of 3.66.

The MVD in invasive lesions with nuclear grade-II varied from 1-25 and the mean was 4.26. In most cases of IDC the blood vessels were identified in the fibrous septa. In cases with extensive desmoplasia identification of vessels was difficult.

In invasive lesions with nuclear grade-III the MVD ranged from 1-30 with a mean of 5.47. It was noticed that extensive desmoplasia and necrosis have altered the blood vessel count.

**TABLE-1**
**CORRELATION OF MVD IN GRADE I, II AND III LESIONS**

| *S.N.* | *Grade of malignant lesions* | *MVD in H&E (Mean ±S.D.)* |
|---|---|---|
| 1. | Grade I | 3.66±0.72 |
| 2. | Grade II | 4.26±0.86 |
| 3. | Grade III | 5.47±1.42 |
| | r = | +0.98 |

A statistically significant increase in mean MVD was observed in Breast Carcinoma cases with nuclear grade I to grade II and grade II to grade III, though range may overlap.

On statistical analysis there was a positive high correlation between nuclear grade and microvessel density in H&E (r value = +0.98) (Table-1)

**DISCUSSION:**
Lesions of the breast constitute a very important chunk of the causes of morbidity and mortality in women worldwide. The large number and variety of papers published on carcinoma of the breast, the treatment, prognosis and associated factors, are an index of the complexity of the problem and indeed of the study of any tumour **(Chervallier et al 1993[5])**. Recently there has been increasing interest in the early detection of the malignant breast lesions as early therapeutic intervention improves survival.

The efficacy of any particular treatment can only be assessed if the influence of pretreatment clinical and pathological factors is known. Here in lies the importance of the search of new prognostic factors which are described relentlessly in various studies worldwide **(Murray PA et al, 1993[6])**.

One of the most fundamental aspects of oncologic pathology has been the recognition that the morphologic appearance of tumour can be correlated with their degree of malignancy. The principal methods currently used fall into two groups, the majority have followed the original concept of using multiple cellular factors while others have concentrated on nuclear characteristics.

Angiogenesis is one of the recently discovered prognostic criteria **(Magennis DP, 1998[7])**. It is a key step in breast cancer growth and metastasis. It has been found that metastasis from breast carcinoma can be initiated as early as the point at which the primary tumors becomes vascularized at a size of less than 0.125 $cm^2$.

In the present study 51 cases of malignant lesions of the breast were included.

Among the 51 cases studied, maximum patients were in age

group 40-50 years (41.17%) followed by 35.3% patients from 50-60 years.17.64% patients were from above 60 years and 5.89% patients from 30-40 years of age group. This is in accordance with study of **(Hussain M A et al 1994[8])**.

Among these 51 cases, 50 cases (98.04%) were invasive carcinomas while only one case (1.96%) was non invasive carcinoma and that was papillary carcinoma in situ. All the cases were epithelial tumour. These findings are in accordance with the study of **Hussain M A et al (1994[8])**.
Out of these 50 invasive carcinomas (epithelial), 42 (84.0%) were IDC (NOS), 1 (2.0%) IDC (NOS) scirrhous type and 7 cases (14.0%) of ILC. Similar findings were noted by **(Berg and Hutter 1995[1] and Hussain M A et al 1994[8])**.

All invasive epithelial tumours were graded according to the Nottingham modification of Bloom Richardson of the 43 cases of invasive ductal carcinomas including scirrhous type-46.5% were Grade-II, 34.9% were Grade-III while 18.6% were Grade-I. The infiltrating lobular carcinomas were also graded similarly. All the 7 cases were Grade-I. Only non invasive case papillary carcinoma in situ was grade I. Our results closely matched those of a study of **(Le Doussal et al 1989[9])**.

In Nottingham system, histologic grading of all invasive breast carcinoma is carried out regardless of the histological type. Whilst recognizing that histological grading of breast cancer will always have an underlying subjective element, the authors devised modifications to the Bloom & Richardson method in order to introduce greater objectivity. Evaluation of nuclear pleomorphism is the least satisfactory element of any tumour grading system and the only way is which differences can be identified accurately is by use of morphometry or image analysis **(C.W. Elston et al 1991[10])**.

**(I.A. Robinson et al 1994[11])** in a study graded breast carcinomas cytologically using six criteria- Dissociation, cell size, cell uniformity, nucleoli, nuclear margin & chromatin pattern. Multiple regression analysis showed that extent of cell dissociation and appearances of nucleoli were the most influential features. But nucleoli are not used in assessing histological grade unless multiple nucleoli are present.The presence of nucleoli in their histological nuclear grading system which includes the regularity of nuclear outline, the delicacy of the chromatin strands, presence or absence of nucleoli, and the number of mitotic figures. This system is as valid as the original **Bloom & Richardson method[12]**.

Recent interest in nuclear characteristics such as DNA content, cell cycle analysis, ploidy analysis, morphometry data and oncogenes has involved complicated and expensive procedures with specific tissue and equipment requirement. If routine histological nuclear analysis shows some of these sophisticated nuclear data, this would be an advantage for most clinical situations when the complex procedures cannot be done **(Le Doussal et al, 1989[9])**.

For each group of lesions [Non invasive and Invasive (epithelial) Grade I, II and III] angiogenesis was studied using H&E stain in all cases.

In low grade carcinomas (nuclear grade I) 1 case of papillary carcinoma in situ, 8 cases of IDC (NOS) & 7 cases of ILC were studied. The MVD varied from 1-25 with an average of 3.66.

In intermediate grade lesions (Nuclear grade II), 20 cases of IDC (NOS), The MVD varied from 1-25 and the mean was 4.26.

15 cases were assigned high grade (Nuclear Grade III). The MVD varied from 1-30 with a mean of 5.47.

The number of microvessels is higher in grade II lesions than Grade-I lesions and in Grade-III lesions than Grade-II. On statistical analysis there was a positive high correlation between nuclear grade and microvessel density (Table-1).

In a number of studies a positive correlation was found between microvessel density (MVD) and tumour grade.

**(Nakopolou et al in 1999[13])** in a study 140 cases of invasive primary breast cancer compared angiogenesis (FVIII RA & CD31 staining) with various classic clinicopathologic prognosticator (histologic type, nuclear grade, tumour size, stage, lymphnode status and steroid receptor immunoexpression) by univariate and multivariate analysis. FVIII RA immunoreactivity was significantly affected only by nuclear grade (p= 0.041) in logistic regression analysis.

Similar positive correlation were found in studies by **Gasparani et al 1994[14]; Horak et al, 1992[15] and Weidner et al, 1991[16]**.

On comparing grades obtained by various methods **Bloom & Richardson 1957[12]** found that there was a wide variability in results in their methods having predominance of Grade-II lesions, while others having majority of Grade-III lesions **(Fisher et al 1980[17], Elston et al 1991[10])**.

Different results are obtained by different researchers regarding correlation between angiogenesis and various prognostic indicators, large number of these discrepancies can be attributed to differences in the methods employed. The choice of antibody to detect endothelial cells is a compromise between the sensitivity and specificity of those available and number of different antibodies have been used with no universal agreement on which is best **(D.P. Magennis, 1998[7])**.

The number of cases in present study is small and long follow up is required to reach any definitive conclusion. Further studies with larger number of cases, with the antibodies specific to proliferating endothelium, together with the development of automated image analysis may improve the accuracy and value of measuring angiogenesis- induced microvessel density. MVD measurements may prove to be of value in assessing patients for antiangiogenic therapy, either alone or more likely in combination with chemotherapy.

The ultimate goal of each study is how it can be beneficial to the society. Any research by itself on breast carcinoma is useful because of the sheer numbers of women worldwide who suffer morbidity & mortality due to this disease. Hence there is relevance of search for new prognostic markers which may help in stratifying patients.

Also the fact that growth of tumours and their metastasis required angiogenesis brings to the fore relevant importance of antiangiogenic therapy. Ongoing research in this direction world over has shown promising results. Thus the role of angiogenesis as a prognostic marker and the role of antiangiogenic therapy both require more research & critical evaluation.

## CONCLUSIONS

The present study entitled *"Correlation of nuclear grading and angiogenesis in breast malignancy"* is a comparative study carried out for nuclear grade and angiogenesis. The MVD increased from grade I to grade II to grade III lesions. On statistical analysis there was a positive high correlation between nuclear grade and microvessel density.

Thus it was concluded from present study that microvessel density correlate positively with nuclear grade. This study was conducted with a small number of cases and definite conclusions were not possible. However results of present

study did show concurrence with those of other researchers.

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