

A study of Transferrin expression in comparison with Modified Scarff-Bloom Richardson's grading system in Breast carcinoma

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Abstract

Background & Objectives: Breast carcinoma is the most common malignancy in women worldwide and extensive researches have been made with development of many prognostic, predictive and theranostic markers^[1]. One of the powerful prognostic predictor in histology is Modified Scarff- Richardson and Bloom grading system[MSBR] also known as Nottingham grading system by which tumours are classified as grade 1, 2 and 3^[2,3]. Transferrin is a potential marker for identification of dividing cells detected by routine immunohistochemistry. Its expression correlates with the cellular proliferation, being highest in rapidly dividing cells and much lower in resting and terminally differentiated cells^[4]. The aim of the study was to assess the proliferative status of the breast carcinomas with transferrin immunostaining and to compare and analyze the association with MSBR grading system for the assessment of prognosis.

Materials & Methods: A total number of 40 randomly selected patients undergoing mastectomy were investigated. The tumours were categorized as Grade 1, 2 & 3 according to the MSBR grading system. The sections were also immunostained with monoclonal antibodies against transferrin and its intensity of expression was studied. The obtained results were statistically analyzed.

Results & Interpretation: A strong statistically significant correlation was found between MSBR grading and transferrin expression.

Conclusion: Staining pattern of transferrin is more of prognostic significance in breast tumors than diagnostic value and may facilitate the decision making process in the treatment of individual cases of carcinoma breast.

Keywords: Breast cancer, Transferrin, Modified Scarff- Richardson and Bloom grading system, MSBR

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Introduction

The incidence of breast cancer is increasing and it affects approximately 1 in 8 women. Breast cancer is commonly treated by various combinations of surgery, radiation therapy, chemotherapy and hormone therapy with prognosis and selection of therapy influenced by clinical staging and pathological grading^[7]. The most popular pathological grading system in breast cancer is MSBR. In recent years, there has been a significant improvement in the understanding of molecular events and critical pathways involved in breast cancer.

Transferrin is a high abundant serum protein and is the major iron-binding protein, having a vital role in iron transport. Additionally, Transferrin has many other uses including antimicrobial functions and growth factor effects on mammalian cell proliferation and differentiation. Transferrin is a potential marker for identification of dividing cells detected by routine immunohistochemistry. Its expression correlates with

the cellular proliferation, being highest in rapidly dividing cells and much lower in resting and terminally differentiated cells^[8]. Few studies have shown that significant correlation exists between transferrin expression and MSBR grading system.

The present study was undertaken to analyze the expression of transferrin in breast carcinomas and to categorize them in to low grade and high grade tumours according to their proliferative status, in comparison with MSBR.

Materials & Methods

This study is a prospective study of 3 years which included 40 cases of infiltrating ductal carcinoma breast, received in the department of Pathology.

The mastectomy specimen, after fixation with 10% neutral buffered formalin, were completely embedded in paraffin. The sections were cut and stained with hematoxylin-eosin stains for histopathological examination. All the cases were graded with Modified Scarff Richardson and Bloom grading system as Grade 1, 2 or 3. [Figure 1A,B]. The sections were also immunostained, with monoclonal antibodies against transferrin and immunolocalization of transferrin was performed.

Positive reaction was in the form of fine granules in cytoplasmic membrane as well as in the cytoplasm.

[Figure 1C,D]. The extent of staining reaction was determined by semiquantitative grading as consistent, moderate and weak [4,5].

1. Consistent: Lesions with majority of cells (>50%) showing positive staining.

2. Moderate: Lesions in which approximately 50% of cells were positive for transferrin (>10% & <50%)

3. Weak Only: Few cells were reactive(<10%)

Statistical analysis of the data was performed using Chi-square test with Yates correction. 'P' value of <0.05 was taken as statistically significant.

Inclusion criteria: All cases of infiltrating ductal carcinoma breast, NOS were included.

Exclusion criteria: All non neoplastic, non malignant breast lesions, non ductal and special types of breast carcinoma were excluded

Results

The total number of biopsies selected for the test was 40. The age group of patients diagnosed with infiltrating ductal carcinoma of breast was 32 – 67

years. Majority of the patients were in the age group of 50-60 years.

All the cases of breast carcinoma were found to be positive for transferrin, which is in accordance with a study by Gahlawat et al [4,5,6]. (Figure 2, Table 1)

Out of 7 cases of grade 1 carcinomas, 6 cases (86%) showed weak positivity and 1 showed moderate positivity for transferrin. All the 20 cases (100%) of grade 2 tumours and all the 13 cases (100%) of grade 3 tumours showed moderate and consistent positivity for transferrin, respectively. These results are close to the findings of Gahlawat et al. (Figure 3, Table 2)

The comparison between Modified Richardson and Bloom grade and transferrin expression of breast carcinomas is shown in table 2, which clearly states that there exists a linear relationship between histopathological grading and transferrin positivity as they both show an increasing trend in high grade carcinomas (grade 2 & 3) carcinomas, which is statistically significant with the p value being less than 0.05.

Table 1: Comparative study of transferrin expression in similar studies

| S No | Study | No. of malignant cases | Positive cases |
|------|----------------------------|------------------------|----------------|
| 1 | Fauk et al (1980) [14] | 22 | 16 |
| 2 | Rosiello et al (1984) [15] | 40 | 8 |
| 3 | Aggarwal et al (2003) [16] | 90 | 33 |
| 4 | Gahlawat et al (2008) [4] | 30 | 30 |

Table 2: Comparative study of intensity of staining

| S No | Study | Grade 1 | | | Grade 2 | | | Grade 3 | | | | | |
|------|----------------------------|--------------|---------------------------|---|---------|--------------|---------------------------|---------|---|--------------|---------------------------|---|----|
| | | No. of cases | Intensity of transferrin+ | | | No. of cases | Intensity of transferrin+ | | | No. of cases | Intensity of transferrin+ | | |
| | | | W | M | C | | W | M | C | | W | M | C |
| 1 | Aggarwal et al (2000) [16] | 21 | 19 | 1 | 1 | 30 | 5 | 25 | 0 | 32 | 0 | 2 | 30 |
| 2 | Gahlawat et al (2008) [4] | 12 | 2 | 6 | 4 | 11 | 1 | 10 | 0 | 7 | 0 | 0 | 7 |
| 3 | Present study | 7 | 6 | 1 | 0 | 20 | 1 | 19 | 0 | 13 | 0 | 0 | 13 |

W- Weak, M- Moderate, C - Consistent

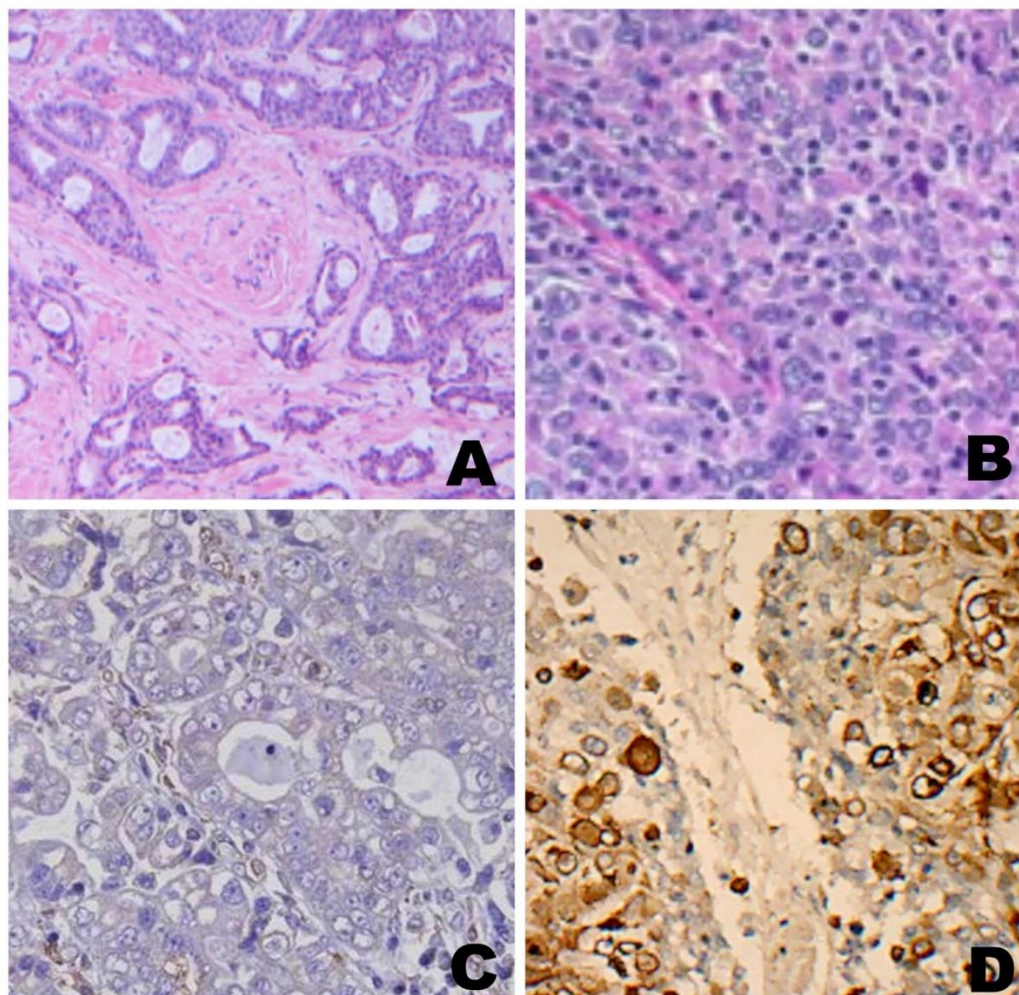


Fig. 1(A): Photomicrograph of grade1 infiltrating ductal carcinoma of breast, H&E, X100, **(B):** Photomicrograph of grade3 infiltrating ductal carcinoma of breast, H&E, X100, **(C):** Photomicrograph of IHC for transferrin in low grade breast carcinoma, X100, **(D):** Photomicrograph of IHC for transferrin in high grade breast carcinoma, X100

Discussion

Breast carcinoma is the most common malignancy in females worldwide. There are many prognosticators for breast carcinoma which includes tumour size, nodal status, tumour type, tumour grade, lymphovascular invasion, proliferation status, hormone receptor status and others. Pathologists play an important role in preoperative diagnosis and in the postoperative prognostic evaluation^[7,8]. Histological grading is a very important factor for the assessment of prognosis. Although the reproducibility is not perfect, still the Modified Scarff - Richardson and Bloom grading is the most favoured prognostic factor and highly significantly associated with survival and/or progression. Mitotic index, differentiation, and pleomorphism are scored from 1 to 3 and the scores from each category are totaled. Tumors with scores from 3 to 5 are well differentiated (grade 1), from 6 to 7 are moderately differentiated (grade 2), and 8 to 9 are poorly differentiated (grade 3)^[9,10]. A correlation between histologic grade as determined by the MSBR grading

system and 5-year disease free survival has been demonstrated in a study of 1,262 women. Patients with a score of 3 had a relative risk of recurrence of 4.4 compared with those with a score of 1^[11,12]. In addition to histological grading, assessment of proliferative status will give a clear idea regarding the prognosis of the tumour. Transferrin a glycoprotein, is a major iron transporting protein in plasma. Transferrin has many other uses including antimicrobial functions and growth factor effects on mammalian cell proliferation and differentiation. It is a growth factor required for proliferating normal and malignant cells, hence it is expressed in proliferating cells with no or low expression in resting cells. Transferrin is expressed in malignant tissues and its expression pattern correlates with the proliferative status of the tumour cells and also with histological grade. Even within the carcinomas, the transferrin positivity escalated from low grade to high grade tumours^[13] and parallels the MSBR grading system.

Conclusion

From the present study it can be concluded that Transferrin expression correlates with cellular proliferation, being highest in rapidly dividing cells. Transferrin shows an intense positive staining in malignant breast tumours with an increasing trend towards higher grade tumours. The study of transferrin is of prognostic significance in breast tumours and hence, combination of Gleason's grading system and Ki-67 immunostaining will strengthen the prognostic assessment of breast carcinoma.

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