Histological Categorization of Stromal Desmoplasia in Breast Cancer and Its Diagnostic and Prognostic Utility

Mariam Shadan[1]*, Nazoora Khan[2]*, Mohammad Amanullah Khan[2]**, Hena Ansari[3]*, Sufian Zaheer[4]***

[#]*1-Senior Resident, 2- Professor, 3- Assistant Professor, 4- Associate Prefessor, * Department of Pathology, JNMC, AMU, Aligarh, ** Department of General Surgery, JNMC, AMU, Aligarh,U.P.*** Department of Pathology, Safdarjang Medical College, New Delhi, India.*

Abstract

The interplay between a tumor and its environment is exemplified by the morphological changes observed in the stroma of human breast cancer. These changes are evident as stromal desmoplastic reaction. This study evaluated the association of these stromal changes with Tumor size, Tumor grade and axillary node status. Histopathological evaluation showed marked correlation of desmoplastic reaction with higher grade and positive nodes were associated with immature desmoplastic reaction ($p<0.05$) however no association with tumor size was noted. In conclusion, stromal desmoplastic reaction can be potentially utilized to predict the grade and nodal status of patients. Therefore they can used as prognostic indicators and provide supplementary information regarding known adverse prognostic factors

Keywords - *Breast cancer, desmoplasia, prognosis, grading*

I. INTRODUCTION

Breast cancer is the second most common malignancy affecting women, with more that 1 million cases occurring worldwide annually and is the most frequent cause of cancer related deaths in women. In India, breast cancer has become the most common cancer amongst women.[1] Since Paget's "Seed and Soil" hypothesis in 1889, various studies on solid tumors have confirmed the active role of tumor stroma in the onset, growth and spread of neoplastic cells[2]. The malignant breast tissue requires complex local and systemic stromal interactions for development and progression. The interplay between a tumor and its environment is exemplified by the morphological changes observed in the stroma of human breast cancer[3]. The "tumor-associated stroma" in breast cancer includes fibroblasts, myofibroblasts, leukocytes, endothelial cells, macrophages and adipocytes, all of which contribute to the complexity of the tumor microenvironment[4]. Myofibroblasts secrete of various chemokines, cytokines, growth factors, inflammatory mediators which play an important role in oncogenesis. Myofibroblasts also produce collagen and extracellular matrix proteins, which constitutes the "desmoplastic reaction" noted in tumor stroma. They have been suggested to represent an important player in the development of the invasion process[5]. The heterogenicity of tumor cancer cells and stromal cells combined with the complex surrounding connective tissue suggests that our understanding of cancer by focusing only on tumor cell pathology is incomplete. The present study was undertaken to investigate the correlation between the known adverse prognostic factors and various stromal parameters to elucidate a relationship, if any, between tumor growth and surrounding stroma in order to discover more comprehensive and meaningful data with respect to breast cancer.

II. MATERIALS AND METHODS

114 patients with primary invasive breast cancer diagnosed in the years 2009-2014 in the Department of Pathology, JNMC (Aligarh, India) were included in the study. Patients with previous history of radiotherapy or chemotherapy were excluded from the study. In every case, hematoxylin and eosin stained sections were examined to access lymphnode metastasis, Tumor grade [6] and categorization of desmoplastic reaction.

A. Criteria for Histological Categorisation of Fibrotic Cancer Stroma [7].

Stromal assessment in each case was made at the invasive zone and was classified according to the most unfavorable stromal area. They were grouped as Mature when fine and elongated collagen fibres with fibrocytes stratified into multiple layers, Intermediate when broad bands of collagen with brightly eosinophilic hyalinisation, similar to those seen in a keloid,were intermingled with mature collagen fibres and Immature when randomly orientated keloid-like collagen bundles surrounded by loose stroma.

Statistical analysis was carried out using SPSS software (v.15.0, USA). The statistical analysis was done using Chi square to evaluate the significance of difference between association of

variables like stromal desmopalstic reaction, nodal metastasis, tumor grade and tumor size.

III.OBSERVATION

In the data set, it was observed that 51.8% of cases had an intermediate type of desmoplastic reaction, 29.8% had a mature desmoplastic reaction while only 18.4% cases demonstrated an immature stroma. (Table 1, figure 1)

Correlation between maturation of fibrotic cancer stroma and clinical parameters:-

- Axillary Status (Table 2)

The figures show that mature, intermediate and immature stromal desmoplastic reaction mature have 44.1%, 55.9%, and 85.7% of axillary metastasis respectively (P<0.05).

- Tumor size (Table 2)

On correlating tumor size with type of desmoplastic reaction, it was noted that the correlation between these two parameters was statistically insignificant (p>0.05). Furthermore no association was observed between mature desmoplastic reaction and small sized tumors (T1) or immature desmoplastic reaction and tumors greater than 5cms (T3).

- Tumor Grade

High Tumor Grade 3 shows greater frequency of immature stromal desmoplastic reaction: 39.1%, versus 13.6% for Grade 2 and 12% for Grade 1 (P <0.05).

IV.DISCUSSION

Collagen is the major constituent of extracellular matrix (ECM). It constitutes about one-third of total proteins in human body. Both increase [8] and decrease[9] in deposition of collagen in cancer have been associated with increased invasiveness of breast cancer. In advanced stages of cancer, this collagen gets degraded by matrix metalloproteinases [10] further increasing the potential for invasion. Many malignancies, including breast, are associated with strong fibrotic/ desmoplastic reaction, which is characterized by an accumulation of fibrillar collagen [11,12]. This collagen is synthesized by myofibroblasts present in the stroma [13]

In 2004, Ueno et al.[7] histologically classified stromal desmoplastic reaction as mature, intermediate and immature depending upon the qualitative characteristics of the stromal collagen in the reactive tumor area in cases of advanced rectal cancer. They observed that immature type of stroma was mostly seen in invasive cancers and was associated with poor prognosis, worse clinical outcome and increased chances of recurrences. To the best of our knowledge in no other study such histological categorization for stromal desmoplastic reaction has been applied in breast cancer. We observed that 51.8% of cases had an intermediate type of desmoplastic reaction, 29.8% had a mature desmoplastic reaction while only 18.4% demonstrated an immature stroma.

Collagen is also essential for tumor angiogenesis which is vital for tumor growth and invasion [14]. Inhibition of collagen metabolism has been demonstrated to have anti-angiogenic effects, confirming that blood vessel formation and survival are indispensably connected with proper collagen synthesis [15]. Hence desmoplastic reaction through lymphovascular proliferation has a role in promoting nodal metastasis.

In the present study, an increase in percentage of cases with lymphnode metastasis was noted when the desmoplastic reaction transformed from mature to intermediate to immature. A statistically significant (p<0.05) association was seen between type of desmoplastic reaction and percentage of cases with lymphnode metastasis. Also a statistically significant (p<0.05) association was observed between cases with immature stroma and lymphnode metastasis. On the other hand, we did not find any association between mature stroma and absence of nodal metastasis suggesting that possibly mature stroma did not provide sufficient protection against metastasis. Wyckoff et al.(2007) [16] postulated that linear arrangement of stiff fibers promote invasion in early stages of cancer by fostering cellular migration into extra cellular matrix (ECM).Breast cancer cells migrate rapidly along these “invasion highways” composed of collagen fibers. Whereas In 2003, Wernicke et al.[17] concluded that loose and myxoid stroma was more permissible for invasion as compared to dense sclerotic stromal reaction. The findings of the present study are in concordance with those reported by Wernicke et al.(2003)[17].

The advantage of assessing stromal desmoplasia is that it can be easily done on small biopsies prior to surgery, on sections stained with Haematoxylin and Eosin alone. A fair prediction of probability of lymphnode metastasis can be made even in the absence of enlarged lymphnodes. This is important as currently micrometastasis are difficult to identify without immunohistochemical markers.

No significant (p<0.05) correlation was observed between tumor size and type of desmoplastic reaction. Also no association could be elicited between mature stroma and small sized tumors (T1) or immature stroma and large tumors (T3).

It was seen that in grade I tumors the most frequent type of desmoplastic reaction was mature type with 48% cases, while in grade II tumors, 62% cases showed intermediate type of desmoplastic reaction. The immature variant was the most frequent pattern in grade III tumors. Statistically these two parameters (i.e. stromal desmoplastic reaction and tumor grade) were significantly (p<0.05) correlated. Furthermore, a significant (p<0.05) association was noted between grade I tumors and mature

desmoplastic reaction, grade II tumors and intermediate desmoplastic reaction and grade III tumors and immature stromal desmopastic reaction.

The fibrotic or mature desmoplastic response has been associated both with increased and decreased aggressiveness in tumors [18], whereas intermediate or immature stroma has been consistently associated with invasion, poorer prognosis and increased chances of recurrence[7, 17]. However in none of these studies association of desmoplastic reaction and tumor grade have been correlated in breast cancer.

The existence of a strong association between a specific type of desmoplastic reaction and a particular grade may be helpful in future in predicting the grade of tumor by analyzing the pattern of stromal desmoplasia. This can be easily applicable to core biopsies, thus enabling a better understanding of the tumor preoperatively. This knowledge is essential for both surgeons and oncologists, and will help in planning more patient-specific personalized therapies.

Table 1: Distribution of cases according to type of stromal desmoplastic reaction

S. No.	Type of desmoplastic reaction	Number of Cases	Percentage (%)
1.	Mature	34	29.8
2.	Intermediate	59	51.8
3.	Immature	21	18.4
4.	**TOTAL**	**114**	**100**

Table 2: Correlation between stromal desmoplastic response and axillary lymphnode metastasis, tumor size and tumor grade.

Variable	Stromal Desmoplastic Reaction			P - value
	Mature	Intermediate	Immature	
Axillary LN positive	15	33	18	<0.05
Negative	19	26	03	
Size T1	08	08	03	>0.05
T2	21	31	08	
T3	05	20	10	
Tumor grade I	12	10	03	<0.05
II	16	41	09	
III	06	08	09	

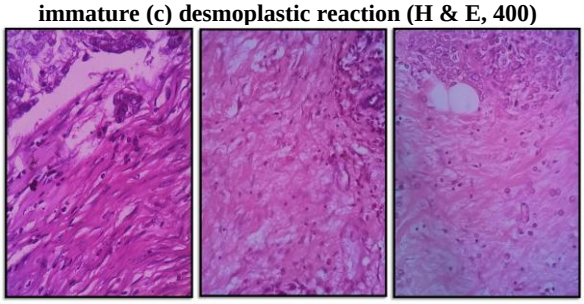
V. CONCLUSION

Hence it can be concluded that stromal desmoplastic reaction, can be potentially utilized to predict the grade and nodal status of patients. Therefore they can used as prognostic indicators and provide supplementary information regarding known adverse prognostic factors. Another advantage of assessing these stromal parameters is that they can be easily applied to core biopsies thus enabling better understanding of the tumor preoperatively. This would help immensely in deciding the line of treatment and better management of patients suffering from breast cancer.

ACKNOWLEDGMENT

Department of Pathology, JNMC, AMU ALigarh

Fig 1: IDC showing mature (a), intermediate (b) and immature (c) desmoplastic reaction (H & E, 400)



REFERENCES

[1] Globocan (2012): World Health Organisation: http://globocan.iarc.fr/Pages/fact_sheets_ cancer.aspx
[2] Mueller MM, Fusening NE. Friend or foes- bipolar effects of the tumor stroma in cancer. Nat Rev Cancer. 2004; 4(11): 839-849.
[3] Arendt LM, Rudnick JA, Keller PJ. Stroma in breast development and disease. Semin Cell Dev Biol. 2010 Feb; 21(1): 11-18.
[4] ShimodaM, Kieran T, Orimo MA.Carcinoma-associated fibroblasts are a rate-limiting determinant for tumour progression.Semin Cell Dev Biol. 2010 February; 21(1): 19–25.
[5] Halvorsen TB, Seim E. Association between invasiveness, inflammatory reaction, desmoplasia and survival in colorectal cancer. J ClinPathol. 1989; 42: 162-166.
[6] Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: Experience from a large study with long-term follow-up. Histopathology. 1991; 19: 403-410.
[7] Ueno H, JonesAM,Wilkinson KH.Histological categorisation of fibrotic cancer stroma in advanced rectal cancer.Gut. 2004;53:581–586.
[8] Levental KR, Yu H, Kass L, Lakins JN. Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell. 2009;139:891–906.
[9] Arnold SA, Rivera LB, Miller AF. Lack of host SPARC enhances vascular function and tumor spread in an orthotopic murine model of pancreatic carcinoma. Dis Model Mech. 2010; 3:57–72.
[10] Rowe RG, Weiss SJ. Breaching the basement membrane: who, when and how? Trends Cell Biol. 2008;18:560–74.
[11] Kauppila S, Stenback F, Risteli J. Aberrant type I and type III collagen gene expression in human breast cancer in vivo. J Pathol. 1998;186:262–268.
[12] Huijbers IJ, Iravani M, Popov S. A role for fibrillar collagen deposition and the collagen internalization receptor endo180

in glioma invasion. PLoS One 2010;5:e9808. [PubMed: 20339555]
[13] Lagacé R, Grimaud J-A, Schürch, Seemayer TA: Myofibroblastic stromal reaction in carcinoma of the breast: variations of collagenous matrix and structural glycoproteins. Virchows Arch.1985;408:49–59.
[14] Fang M, Yuan J, Peng C. Collagen as a double-edged sword in tumor progression. Tumor Biol. 2014; 35:2871–2882.
[15] ME Maragoudakis et al. Basement Membrane Biosynthesis as a Target for Developing Inhibitors of Angiogenesis With Anti-Tumor Properties .Kidney Int.1993; 43 (1): 147-150.
[16] Wyckoff JB,Wang Y, Lin EY. Direct visualization ofmacrophage-assisted tumor cell intravasation in mammary tumors. Cancer Res. 2007;67:2649–56.
[17] Wernicke M, Piñeiro LC, Caramutti D. Breast cancer stromal myxoid changes are associated with tumor invasion and metastasis: A central role for Hyaluronan. Mod Pathol. 2003;16(2):99–107.
[18] Liotta LA, Rao CN, Barsky SH. Tumor invasion and the extracellular matrix. Lab Invest. 1983;49:636–49.