CORRELATIONSHIP OF CATHEPSIN D AND TOPOISOMERASE II ALPHA WITH NUCLEAR GRADING IN BREAST CANCERS

Maj PRABAL DEB*, Lt Col GU DESHPANDE (Retd)*

ABSTRACT

56 cases of infiltrating duct carcinoma of the breast were studied for the expression of cathepsin D and topoisomerase II alpha. The results were correlated with the morphological differentiation, as determined by the Nottingham's modification of the Bloom-Richardson system. Cathepsin D positivity in tumour cells and stromal cells was seen in 44.6% and 55.4% cases respectively, whereas topoisomerase II alpha positivity was seen in 33.9% cases. In grade II tumours cathepsin D in tumour cells and stromal cells was 44.4% and 47.2% respectively, as compared to 27.8% positivity for topoisomerase II alpha. The corresponding figures for grade III tumours were 50.0%, 79.6% and 64.3% respectively. As grade I comprised only 3 cases no statistical correlation could be observed. It is evident that with increase in tumour grade there is a statistical increase in expression of cathepsin D, a lysosomal acidic protease-implicated in the process of tumour invasion and metastasis, and of topoisomerase II alpha, a marker of rapid cell proliferation and aneuploidy.

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KEY WORDS :Breast carcinoma; Cathepsin D; Grading; Prognosis; Topoisomerase II alpha.

Introduction

Modified Scarff Bloom Richardson (SBR) system of nuclear grading [1], is assuming more and more importance to prognosticate breast cancer. The treatment schedule is modified many times and is based on nuclear grade of the tumour. Two new parameters have been recently added to the battery of parameters to prognosticate breast cancers. These parameters are, cathepsin D and topoisomerase II alpha. We wanted to find the usefulness of these markers as prognostic indicators for breast cancer. Comparison of these two parameters was carried out with the nuclear grading of breast cancer.

Cathepsin D is a tissue protease that is presumed to play a role in tumour invasion and it has attracted considerable attention in recent years as a potential prognosticator in breast cancer [2]. Although it was originally proposed as an indicator of poor prognosis, additional studies have reported inconsistent correlation between cathepsin D and prognosis in mammary carcinoma.

Topoisomerase enzymes are nuclear enzymes which play an important role in DNA replication, formation of chromosome scaffolds, chromatin organisation and may be involved in DNA transcriptase and repair [3,4]. Topoisomerase II has alpha and beta isoforms. Topoisomerase II alpha is not detectable in G0 phase of cell cycle but its activity increases dramatically during S phase, peaks in G2-M phase and then

declines. Topoisomerase beta is thought to represent structural element of the nucleolar remnant and play a role in regulation of ribosomal gene transcription [5]. Limited information is available on activity of topoisomerase II alpha in human neoplasms but increased expression of it has been associated with most aggressive and highly proliferative neoplasms [3].

Material and Methods

Patients : 56 cases of carcinoma breast which were diagnosed and treated in Malignant Disease Treatment Centre of a large referral hospital were included in the study. Table 1 summarises the clinical character of these 56 patients.

It can be observed that all patients were females and median age was 48.7 years. The youngest patient was 25 years old and eldest patient was of 75 years. Out of 56 cases, 16 patients were premenopausal and 40 were post menopausal. Maximum number of cases were in stage II (22-39.3%) and stage III (26-46.4%). Lymph node status vis-a-vis metastasis seen on histological examination showed that 17 patients (30.4%) were negative, however 39(69.6%) had metastasis in lymph nodes. Histological typing showed that 53 cases (94.6%) were infiltrating duct carcinoma- no specific type and 1 case (1.8%) each, were found to be medullary, colloid and infiltrating lobular carcinoma.

Nuclear grading : All 56 cases were observed by three observers independently and then any difference in opinion was resolved by examining the slides jointly on a multihead NIKON-LABOPHOT microscope. Nuclear grading to each case was given by totalling the scores of tubule formation, nuclear pleomorphism and mitotic figure counts based on the modified SBR system [1].

Immuno-histochemistry : Four micrometer paraffin sections were melted, cleaned in xylene and hydrated in decreasing grades of ethanol. After blocking of endogenous peroxidase activity with a solution of hydrogen peroxide, slides were sequentially treated

*Graded Specialist (Pathology), IMTRAT, C/o 99 APO, *Head, Department of Pathology, Deen Dayal Memorial Hospital, Pune - 411 004.

TABLE 1
Clinical and histological characters (n=56)

Characteristic	Number of patients
Sex	
Male	0
Female*	56
Median age in years	48.7 years (range 25 - 75 years)
Menopausal status	
Pre	16
Post	38
Clinical stage	
Stage I	0 (0.0%)
II	22 (39.3%)
III	26 (46.4%)
IV	6 (10.7%)
Tx	2 (3.6%)
Lymph node status	
Negative	17
Positive	39
Histological typing	
IDC (NST)	53 (94.6%)
Medullary	1 (1.8%)
Colloid	1 (1.8%)
Invasive lobular	1 (1.8%)

Tx - tumour size was known as primary treatment was done earlier

IDC (NST) - invasive ductal carcinoma - no special type

with primary rabbit anticathepsin D antibody, followed by biotinylated anti-rabbit immunoglobulin and avidin-biotin peroxidase complex. Diaminobenzidine was used as a chromogen in presence of hydrogen peroxide. Slides were then counterstained with Meyer's hematoxylin. All reactions were carried out at room temperature. Similar method was followed for topoisomerase II alpha staining by using primary rabbit antitopoisomerase II alpha antibody in place of anticathepsin D.

Evaluation of results : Positive staining was taken as minimum 10% of tumour cell positivity for cathepsin D and topoisomerase. For cathepsin D tumour cells as well as host histiocytes and fibroblasts, both intratumoral and margins of the tumour were looked for positive staining.

Results

Cathepsin D : Positive staining for cathepsin D was visualised as intracytoplasmic brown granules occupying part or all of the cytoplasmic volume of the tumour cells and stromal cells (Figs 1 & 2). Table 2 shows that out of 56 cases, 31 cases (55.4%) showed positivity for stromal cells, whereas 25 cases (44.6%) were negative. When tumour cell staining was analysed it was observed that 25 cases (44.6%) were positive in contrast to 31 cases (55.4%) which showed negative staining. The staining positivity for cathepsin D stromal cells and tumour cells were analysed as per nuclear grading of the tumour. It was observed that positivity of stromal cells was almost similar for grades I and II, (50.0%; 47.2%), but grade III however, showed positivity for 79.6% cases. The positivity for tumour cells and nuclear grading showed that there was gradual rise in percentage of positivity as the grades advanced (grade I-33.3%; grade II-44.4%, grade III-50.0%). All the results were analysed statistically and it was observed that stromal cell positivity showed statistically significant difference

Fig. 1 : IDC breast-stromal and nuclear positivity (cathepsin D x HP)

Fig. 2 : IDC breast nuclear positivity (topoisomerase II alpha x HP)

TABLE 2
Nuclear grade and cathepsin D staining

Nuclear grade	CDS +ve	CDS -ve	CDT +ve	CDT -ve
I (6)	3 (50.0%)	3 (50.0%)	2 (33.3%)	4 (66.7%)
II (36)	17 (47.2%)	19 (52.8%)	16 (44.4%)	20 (55.6%)
III (14)	11 (79.6%)	3 (20.4%)	7 (50.0%)	7 (50.0%)
Total 56	31 (55.4%)	25 (44.6%)	25 (44.6%)	31 (55.4%)

CDS - cathepsin D stromal cells, CDT - cathepsin D tumour cells

between grades I and III ($p < 0.05$) and grade II and III ($p < 0.05$) but difference between grades I and II was statistically insignificant. When statistical analysis for tumour cells and grading was carried out it was observed that although increasing percentage was seen in positivity as the grades advanced, these differences were statistically insignificant ($0.5 > p > 0.1$).

Topoisomerase II alpha : Topoisomerase II alpha staining showed (Table-3) that out of 56 cases, 19 cases (33.9%) were positive, however 37 (66.1%) cases showed no staining. When the results were analysed by comparing the positivity and nuclear

grade, it showed that not a single case of grade I showed positive staining (0.0%) but percentage of positivity increased as the nuclear grade advanced (II-27.8%, III-64.3%). Statistical analysis showed that differences between grades I and II; grades II and III and grades I and III were all statistically significant ($p < 0.05$).

TABLE 3

Nuclear grade and topoisomerase II alpha staining

Nuclear grade	TOPO +ve	TOPO -ve
I (6)	0 (0.0%)	6 (100.0%)
II (36)	10 (27.8%)	26 (72.2%)
III (14)	9 (64.3%)	5 (35.7%)
Total 56	19 (33.9%)	37 (66.1%)

Discussion

Cathepsin D is a lysosomal acidic protease that is present in all normal cells. Cathepsin D has been implicated in the process of tumour invasion and metastasis by virtue of its ability to degrade extracellular matrix and latent forms of other proteolytic enzymes [6,7]. Abnormally high levels of cathepsin D as detected by cytosol assays have been reported in various human cancers, this overexpression is believed to increase the metastatic potential of tumours [8,9]. However, few breast cancer studies showed inconsistent results as they were being done by cytosol assay [10,11]. We came across a study recently [2] where IHC technique was used and they showed a good correlation of stromal cell staining with higher nuclear grades than compared to positive staining of the tumour cells. Our results are almost matching with them and we have also seen stromal cell positivity of higher grade (grade III) to be statistically significantly higher than low grade tumours (grades I and II). Tumour cell staining also showed increasing percentage of positive staining as the grades advanced (i.e. from grades I to II and from grades II to III) however, these differences were not statistically significant.

Topoisomerase II alpha is a relatively new addition to markers of cell proliferation in breast cancers. Jarvinen et al have recently shown positivity of topoisomerase II alpha with rapid cell proliferation and aneuploidy expression in breast cancer [12]. Helleman et al have also shown higher rates of topoisomerase II alpha in high tumour grades [13]. Most important factor in all these studies of topoisomerase II alpha is that it is also related to cancer chemotherapy and positivity is associated with resistance to anticancer drugs which act as topoisomerase II alpha inhibitors [14]. Our results are quite consistent and we have also shown that negativity of topoisomerase II alpha for grade I tu-

mours and statistically significant increase in percentage of cases was seen as the nuclear grades advanced.

In conclusion, we have clearly observed in the study of 56 primary breast cancer cases that nuclear grades of breast tumours based on modified SRB system [1] correlate very well with positive staining for newly discovered topoisomerase II alpha, which is a marker of increasing cellular proliferation; and these cases also showed that higher nuclear grades are associated with increased potential for metastasis, as higher nuclear grades correlated significantly with cathepsin D staining for host stromal cells. How these presumed correlates of aggressive tumour behaviour will actually correlate with overall and disease-free survival, will however be known in this study over a period of time as we follow these 56 breast cancer cases over 5 to 10 years or so.

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