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ARTICLE

Is Nothingham Prognostic Index Correlated with Apoptosis and P53 Expression in Infiltrating Ductal Carcinoma of the Breast?

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The role of p53 as a prognostic factor is not clear. P53 named as "guardian of the genome" plays an important role in many intracellular regulatory systems, one of which is apoptosis, having an impact on tumor kinetics. A retrospective study was undertaken to assess the relationship of the Nothingham Prognostic Index (NPI) to p53 expression and apoptotic cell counts. To conduct the study, 160 successive cases of infiltrating ductal carcinoma of the breast were included. P53 was assessed on AP-AAP stained sections. Apoptotic cell counting (ACC) was done on the HE stained routine sections in 10 HPFs. Clinical data were derived from the hospital files. Apoptotic cell counts were higher in the p53 positive group but the difference was not significant (p=0.079). P53 positivity was found to be related to the disease-free survival (DFS) (p=0.008). NPI was significantly higher in apoptotic cell containing group (p=0.006). There was a positive linear correlation between ACC and NPI scores (p=0.004). This correlation was not present between apoptosis and disease free survival. P53 expression was found to be related with DFS but not with the NPI which is a score composed of the best prognostic indicators known today. In contrast to this, ACC was found to be closely and linearly associated to the known prognostic factors. This may suggest that the apoptotic cell counts done on routine sections may be used as a part of prognosis assessment in infiltrating ductal carcinoma. (Pathology Oncology Research Vol 9, No 2, 100–103, 2003)

Keywords: p53, apoptosis, disease free survival, prognostic index

Introduction

The prognosis in breast carcinoma has been found to be determined by many factors. Some of these factors are used to calculate prognostic indeces by using histologic grade, tumor size, axillary lymph node status, estrogen receptor status, and stage of the disease.[2,5,7] Prognostic indices give much more information than each factor used alone. One of the prognostic indices proposed by the Nothingham study group is composed of the tumor size, histologic grade and metastatic axillary lymph nodes.[5]

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Abbreviations

NPI: Nothingham Prognostic Index, AP-AAP: Alkaline Phosphatase – Anti Alkaline Phosphatase, HPF: High Power Field, HE: Hematoxylin-Eosin

Apoptosis, as a cell process under control of inducers and suppressors, seems to be one of the important determinants of cell kinetic. This role of apoptosis may offer some explanation for tumor progression and even provide information on the prognosis of some malignancies. There is some accumulation of data about the role of apoptosis as a prognostic indicator in breast cancer.[6,10,16]

P53 acts as a check point in cells regulating many intracellular events amongst which one is chemo- and/or radiotherapy induced apoptosis. Following DNA damage, p53 either stops the cell cycle for repair[9,13] or triggers the apoptotic mechanisms when the damage cannot be repaired.[14] P53 overexpression is the most common genetic alteration found in breast cancer.[13] The prognostic value of p53 overexpression in breast cancer is still a controversial issue, although most information indicates that it is related to a dismal prognosis.[8,11,15]

This study aimed to examine whether a correlation exists amongst NPI, disease-free survival, p53 overexpression and apoptosis in infiltrating ductal carcinoma of the breast.

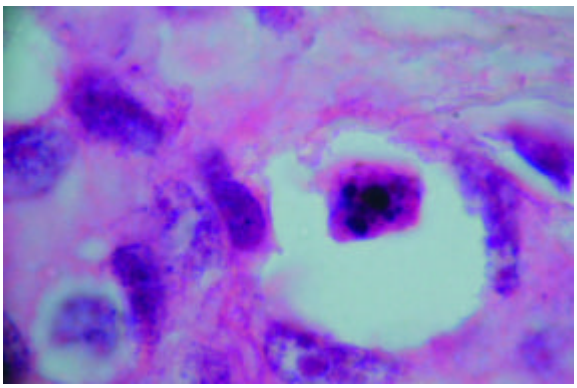

Figure 1. Appearance of apoptotic cell in infiltrating ductal carcinoma (HE x1000)

Materials and Methods

To conduct the study 160 cases of infiltrating ductal carcinoma of the breast, administered successively to Ankara Oncology Hospital between January 1990 and December 1991, were included. Clinical data were derived from hospital files. The histopathological examination was assessed on 10% formalin fixed paraffin-embedded HE stained 5 µm sections of surgical specimens. Apoptotic cell counting (ACC) was done on HE stained sections in 10 HPF on the same areas as mitotic counting were done for histologic grading. Criteria given by Cummings & Winterford[4] were used for apoptotic cell counting. Cells with dark basophilic staining round, oval or irregularly shaped intracellular bodies but without a nuclear membrane were accepted as apoptotic (*Figure 1*). Elston-Ellis modified Bloom-Richardson grading system was used which does not take into account the hyperchromatic pyknotic nuclei as mitotic figures.[5] Prognostic index was calculated as follows: NPI = (0.2x tumor size)+grade+axillary metastatic lymph nodes.[5]

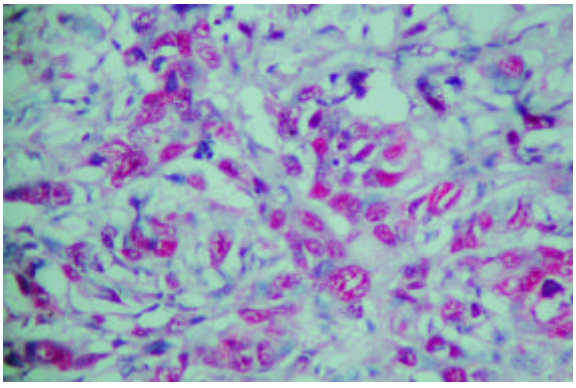

Figure 2. P53 immunhistochemical positivity in infiltrating ductal carcinoma (x200).

P53 overexpression was determined on paraffin-embedded material by using the following staining procedure: 5 µm thick sections were placed onto poly-L-lysine coated glass slides. Sections were deparaffinized in xylene and rehydrated through graded alcohol solutions. A microwave oven heating technique was used for antigen retrival. The tissue sections were placed in 0.01M sodium citrate buffer at pH6 and were heated in the microwave oven (650W) for 3 min at full power (p10), then after a 10 min pause sections were treated for another 27 min at power 3 (p3). After cooling sections were incubated with casein solution at room temperature for 10 min to eliminate non-specific staining and then incubated overnight with monoclonal anti-p53 antibody (monoclonal mouse BP53-12-1, BioGenex, USA,1:480). The staining procedure was followed by biotinylated secondary antimouse antibody and alkaline phosphatase conjugated streptavidin labeling. Following colour development with new fuchsin substrate, counterstaining was performed using Mayer's hematoxylin. Negative control staining was carried out by substituting tris solution for the primary antibody. As a positive control, a breast cancer tissue known to be p53 positive was used. Nuclear staining was accepted as p53 positivity if observed in at least 10% of the nuclei (*Figure 2*). In two cases sections were lost during antigen retrival and p53 staining was not successful even with several repetitions.

Statistical analysis was done using SPSS software. The Student's t test and one way ANOVA test were used to compare the means of the independent groups. Spearman's or Pearson's correlation coefficient test were run to test the correlations between the independent groups. Regression analysis was done to understand the type of the correlation. Confidence level was accepted as 95%.

Results

The study group included 160 patients treated either by simple mastectomy (14 cases) or by modified radical mastectomy (146 cases). The mean age was 47.4±10.9 (range 25-80) and 96.6% of patients were females. Information about the follow-up could only be obtained in 75 patients as the rest were either lost to follow-up or follow-up was done irregularly. The longest follow-up period was 96 months. The number of patients without recurrence during follow-up was 52, of which only 22 cases were followed up longer than 5 years with no recurrence. Summary of other clinical data is given in *Table 1*. The mean DFS was 41.01±28.16 months (range 8.00-96.00). NPI was calculated in modified radical mastectomy performed cases and the mean NPI was 4.02±1.32 (range 1.10-8.20).

The mean ACC was 13.81±18.58 (range 0-167). The mean ACC of the p53 positive group was higher thanthat of the p53 negative group, but the difference did not reach significance (*Table 2*). The mean NPI scores were not sig-

Table 1. **Clinicopathological characteristics of patients**

		n	%
Histologic grade	1	30	18.8
	2	60	37.5
	3	70	43.8
Tumor size	2 cm	32	20.0
	2-5 cm	88	55.0
	>5 cm	40	25.0
Lymph node metastases	absent	45	30.8
	1-3	30	20.5
	>3	71	48.6
Age	<35	21	13.1
	36-50	81	50.6
	>51	58	36.3
P53 expression	negative	96	61.3
	positive	72	39.7

nificantly different when p53 negative and positive groups were compared. However, the difference of DFS between the p53 negative and positive groups was statistically significant (*Table 2*).

When cases were divided according to the presence or absence of apoptotic cells, NPI scores of the group containing apoptotic cells was significantly higher than those of the other group (*Table 3*). There was a positive linear correlation between ACC and NPI (p=0.004). ACC was increasing as the NPI increased. NPI & DFS were inversely correlated (p<0.05) which meant that the DFS were shorter in cases with higher NPI scores.

Table 2. **Relationship of p53 to apoptotic cell count, prognostic index, disease free survival**

	P53 negative (n) mean ±SE	*P53 positive (n) mean ±SE*	*p*
ACC	(98) 11.95±1.50	(60) 17.32±3.00	0.079
NPI	(91) 3.94±0.13	(54) 4.20±0.19	0.257
DFS	(41) 33.80±3.57	(32) 49.72+5.56	0.015*

*SE: standard error, *statistically significant*

Table 3. **Comparison of apoptotic cells with prognostic index and disease-free survival**

	Apoptotic cells absent (n) mean ±SE	*Apoptotic cells present (n) mean±SE*	*p*
NPI	(10) 2.91±0.37	(136) 4.10±0.11	0.006*
DFS	(7) 37.43±9.70	(68) 41.38±3.46	0.726

*SE: standard error, *statistically significant*

Discussion

The best prognostic factor in breast cancer is the axillary metastatic lymph node status followed by the histologic grade and tumor size. NPI score is used to assess the prognosis using these important factors. By using NPI score one can easily assess prognosis of a single case. As expected, a higher NPI score indicated a dismal prognosis. The mean NPI score was quite high in our study group, which indicates that the most of the patients had a poor prognosis. NPI was inversely related to DFS as would be expected.

The information about DFS could only be obtained in 75 cases because most patients were not followed properly or lost to follow-up. The mean DFS of the p53 positive group is longer than the mean DFS of the p53 negative group in our study. P53 is correlated with dismal prognosis in many reports. Longer DFS in the p53 positive group was unexpected in our study. This finding is opposite to most of the data in the literature.[1,3,12] However, Lipponen *et al.* found p53 overexpression to be related with longer DFS.[10] These confusing results could be caused by the use of different anti-p53 antibodies, fixatives or tissue processing, as well as the unstandardized patient group. Complete deletion in the p53 region is another probable cause because it results in the absence of the protein. In such a case immunohistochemical detection of the p53 abnormality becomes impossible because of the absence of the p53 protein.

Our study group may contain such cases since most cases had progressive disease probably with more genetic alterations. Eighty percent of the cases had tumor size over 2 cm, 48.6% of the cases had more than 3 metastases in their axillas and 43.8% of the cases were histologic grade 3. This may have led to the finding of shorter DFS in the p53 negative group. To avoid such situations other ways of p53 determinations could be used.

ACC and NPI were closely correlated in our study. This finding strongly suggests that ACC done on HE sections using light microscopy might be of help as a prognostic parameter. Gonzalez-Campora et al. also suggested that apoptosis rather than proliferative index is an independent factor for the prognosis of survival.[6] The present results show that the mean ACC is higher in p53 positive tumors when compared the p53 negative group, although it does not reach significance. Apoptosis is the result of many processes and is a very complex cellular process under the control of many factors. Although p53 is one of the factors, it may not control the apoptotic pathway completely. In conclusion, p53 was found to be related with DFS of breast cancer while increases in ACC were found to be correlated with higher NPI scores indicating a dismal prognosis.

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