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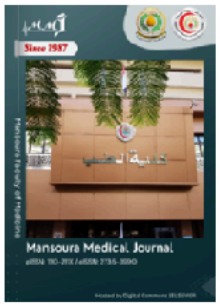

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By

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ABSTRACT

Background and aim : Carcinoma of the breast is the most prevalent cancer among Egyptian women. P53 overexpression is the most common genetic alteration found in breast cancer. The growth and proliferation of tumor cells, as well as their metastatic dissemination, have been shown to be preceded and facilitated by the formation of new blood vessels (angiogenesis). This work aimed to determine p53 expression and angiogenesis measured by microvessel density (MVD) in invasive breast carcinoma. Also, to study the relationship between p53 expression and the degree of angiogenesis.

Patients and methods : The present study was performed on the paraffin blocks from the tumors of 40 modified radical mastectomy specimens (done for invasive breast carcinoma). Immunohistochemical staining for p53 and CD34 (marking endothelial cells for MVD assessment) was done.

Results : In this study, p53 was positive in 60 % of invasive breast carcinomas. P53 positivity was highest in tumors of patients in the fifth decade, premenopausal, tumors of grade II, with a size of 2-5 cm, with positive axillary L.Ns and stage IIB. MVD was highest in tumors of pa-

tients >60 years, tumors of grade III, with a size >5 cm and stage IIIB. MVD of p53 positive tumors was higher than that of p53 negative tumors. However, all these relations were statistically non significant .

Conclusions : Microvessel density may reflect more aggressive behaviour of breast carcinoma. Also, p53 altration may promote tumor angiogenesis.

INTRODUCTION

Carcinoma of the breast is the most prevalent cancer among Egyptian women and constitutes 29 % of National Cancer Institute cases [1]. A variety of genetic alterations have been described in breast cancer.P53 overexpression is the most common genetic alteration found in breast cancer [2].

P53 is a tumor suppressor gene encoding a nuclear phosphoprotein that arrests cell cycle progress at GI phase to allow repair of damaged DNA and inhibit angiogenesis. It is frequently mutated in many human tumors [3, 4,5]. Such mutations lead to stabilization of the protein hence its accumulation. The normal wild type allel is usually rapidly metabolized while the mutated inactive P53 has a longer half life and accumulates within the tumor cells [6].

There is an increasing interest in the relationship between the presence of abnormal amounts of P53 and the clinical outcome of patients. The accumulation of P53 has been shown to occur in breast cancers indicating an aggressive, rapidly proliferating tumor with unstable genome (7,8).

The growth and proliferation of tumor cells, as well as their metastatic dissemination, have been shown to be preceded and facilitated by the formation of new blood vessels from preexisting capillaries (angiogenesis) [9]. Angiogenesis has been considered an independent prognostic factor [10], therefore, its assessment may provide additional information on the biological behaviour of the tumor, and may have applications in prognostic evaluation and as a therapeutic target in human breast carcinoma (11,12).

This work aims to determine p53 expression and angiogenesis measured by microvessel density (MVD) in invasive breast carcinoma. Also to study the relationship between p53 expression and the degree of angiogenesis.

PATIENTS AND METHODS

The present study is a retrospective study performed on the paraffin blocks from the tumors and lymph nodes for 40 modified radical mastectomy specimens (done for invasive breast carcinoma) obtained from the pathology Department in Mansoura Faculty of Medicine during the period from 2004-2006.

All available data were collected as regard age, menopausal status and tumor size. H&E stained sections for assessment of tumor type, grade [according to the Bloom and Richardson system[13] as modified by Elston and Ellis[14]] and axillary lymph node status. TNM staging (based on AJCC/ UICC TNM, 6th edition, 2003) was done.

Immunohistochemistry

Paraffin sections were cut on poly-L lysine- coated slides for immunohistochemical staining for p53 and CD34 (marking endothelial cells for MVD assessment). Monoclonal Mouse Anti-human p53 protein, DO-7, prediluted Code No: N 1581 and Monoclonal Mouse Anti-human CD34 Class II, QBEnd 10, prediluted Code No: N 1632, DAKO Corporation, Carpinteria, CA, USA. Staining was performed using standard immunoperoxidase kits (DAKO LSAB® kits). DAB was used as chromogen.

Staining for p53 was nuclear. Nuclear staining was accepted as p53 positivity if observed in at least 10 % of the nuclei [15]. For CD34, any brown-staining endothelial cells, with or without a lumen, irrespective of size, were counted as a single vessel. Non staining vascular profiles were not included in the count. Areas of fibrosis, necrosis and inflammation, as well as vessels with a muscle wall, were excluded from the counting [16]. The microvessel density (MVD) is defined as the number of manually counted vessels per mm^2. The procedure and criteria followed were those

suggested by Weidner et al [17]. All available tumor slides were examined at low-power magnification (x40) to identify the areas with the highest number of vessels within the tumor (hot spots). The areas with the highest vascularity were most frequently seen at the margins of the carcinoma. Three separate, non-overlaping fields were selected from those areas and all CD34 stained microvessels were counted in each field. Counts were performed with a Leica microscope using a x10 ocular and a x25 objective lenses. Results were expressed as the highest number of microvessels identified and counted within any single x250 HPF of the three fields counted. P53 and MVD were correlated with the various clinicopathologic prognostic parameters including age, menopausal status, tumor grade, lymph node status, tumor size and tumor stage.

Statistical analysis :

Statistical analysis was done by using SPSS statistical package for social science program version 10, 1999. Significance was considered when p value ≤ 0.05

RESULTS

In the present study, all patients were females. The age ranged from 25 to 63 years with a mean 45.92±8.74, most patients were in fifth decade (47.5 %) followed by sixth decade (25 %), premenopausal (70 %). Most of the tumors (77.5 %) were GII followed by GIII (17.5 %), with a size ranged from 2-5 cm (80 %) followed by those of a size >5cm (15 %), had axillary lymph node metastases (67.5 %) and stage IIB (47.5 %) followed by stage IIA (30 %) (table 1) .

p53 immunostaining results

Of the 40 breast carcinomas included in the current study, 24(60 %) were positive for p53 immunostaining (Fig.1). The highest percentage of p53 positivity was in tumors of patients in the fifth decade (50 %) followed by sixth decade (29.2 %), premenopausal (70.83 %), tumors of grade II (75 %) followed by grade III (25 %), with a size of 2-5 cm (83.3 %) followed by those of a size >5cm (16.7 %), with positive axillary L.Ns (70.83 %) and stage IIB (54.2 %) followed by stage IIA (29.1%). No statistically significant relation was found

between p53 positivity and any of those variables (table 2).

Microvessel density (MVD) (Figs.2&3)

Mean MVD was highest in tumors of patients >60 years old (44±1.41) followed by patients in the fifth decade (32.57±18.67), GIII tumors (32.57±21.09)followed by GII tumors (28.96±18.10) tumors with a size >5cm (37.16±19.91) followed by tumors 2-5 cm in size (28.21±18.21), stage IIIB tumors (43 one case) followed by stage IIIA tumors (33.83±24.38). There was minimal difference in MVD as regard menopausal and L.N. status. The relation of MVD to all these variables was statistically non-significant (Table 3).

MVD was higher in p53 positive tumors (30.95+18.93) than p53 negative tumors (25.56+17.7) and the difference was not statistically significant (P=0.37) (table 4).

Table (1): Clinicopathologic characteristics 0f patients

	Number	%
Age groups		
20-30	4	10
31-40	5	12.5
41-50	19	47.5
51-60	10	25
>60	2	5
Menopausal status		
Premenopausal	28	70
postmenopausal	12	30
Histologic grade		
I	2	5
II	31	77.5
III	7	17.5
Tumor size		
<2cm	2	5
2-5cm	32	80
>5cm	6	15
Lymph node status		
positive	27	67.5
negative	13	32.5
Tumor stage		
I	2	5
II A	12	30
II B	19	47.5
III A	6	15
III B	1	2.5

Table (2): Relation between clinicopathological features and P53 immunostaining

	P53 expression				P-value
	Positive(n=24)		Negative(n=16)		
	n	%	n	%	
Age groups					
20-30	2	8.3	2	12.5	0.81
31-40	2	8.3	3	18.7	
41-50	12	50	7	43.8	
51-60	7	29.2	3	18.7	
>60	1	4.2	1	6.3	
Menopausal status					
Premenopausal	17	70.83	11	68.75	0.88
postmenopausal	7	29.17	5	31.25	
Tumor grade					
grade I	0	0.0	2	12.5	0.08
grade II	18	75	13	81.25	
grade III	6	25	1	6.25	
Tumor size					
<2 cm	0	0.0	2	12.5	0.2
2-5 cm	20	83.3	12	75	
>5 cm	4	16.7	2	12.5	
L.N status					
Positive	17	70.83	10	62.5	0.58
negative	7	29.17	6	37.5	
Tumor stage					
I	0	0.0	2	12.5	0.34
IIA	7	29.1	5	31.2	
IIB	13	54.2	6	37.5	
IIIA	3	12.5	3	18.8	
IIIB	1	4.2	0	0.0	

Table (3): Relationship of microvessel density (MVD) and clinicopathologic features

	Mean MVD	SD	Range	P-value
Age groups				
20-30	22	14.02	(14-43)	0.39
31-40	19.6	13.52	(5-33)	
41-50	32.57	18.67	(5-70)	
51-60	25.9	21.6	(3-70)	
>60	44	1.41	(43-45)	
Menopausal status				
premenopausal	28.75	17.72	(5-70)	0.97
postmenopausal	28.91	20.77	(3-70)	
Tumor grade				
I	13	11.31	(5-21)	0.42
II	28.96	18.10	(3-70)	
III	32.57	21.09	(5-70)	
Tumor size				
<2 cm	13	11.31	(5-21)	0.26
2-5 cm	28.21	18.21	(3-70)	
>5 cm	37.16	19.91	(15-66)	
L.N status				
positive	28.37	19.05	(3-70)	0.83
negative	29.69	17.73	(5-70)	
Tumor stage				
I	13	11.31	(5-21)	0.63
IIA	29.91	17.45	(7-70)	
IIB	27.42	18.06	(3-70)	
IIIA	33.83	24.38	(3-70)	
IIIB	43	-	-	

Table (4) : Relation between P53 immunostaining and MVD

P53	Total number	Mean MVD	SD	Range	P-value
Positive	24	30.95	18.93	(3-70)	0.37
Negative	16	25.56	17.7	(5-66)	

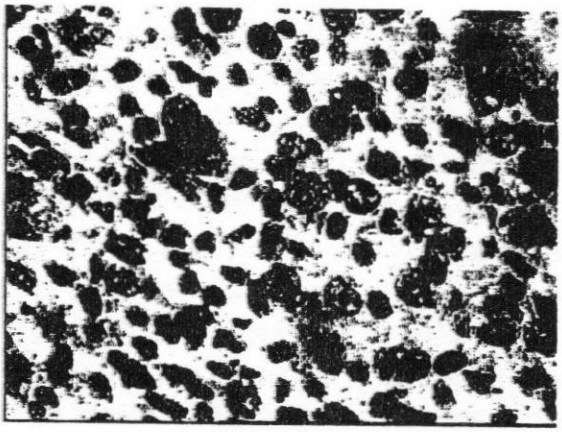

Fig. 1 G III infiltrating duct carcinoma NOS with positive nuclear p53 immunostaining.(Peroxidase X 400)

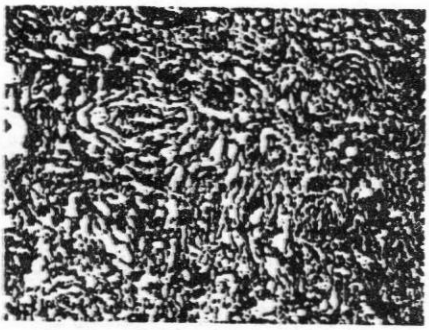

Fig. 2 An area of high microvessel density at the periphery of the tumor. (PeroxidaseX100)

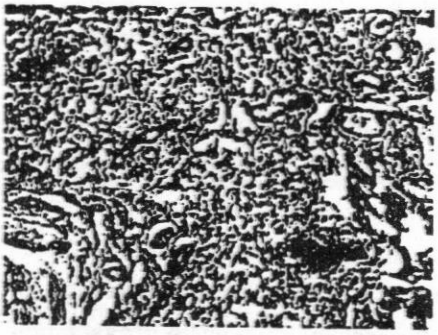

Fig.3 A case of low microvessel density between the tumor cells. (PeroxidaseX100)

DISCUSSION

In the present study, the age ranged from 25 to 63 years with a mean 45.92±8.74 years. This was in agreement with Helal et al.(18) and Cengiz-Boduroglu et al.(15)who reported that the mean age of their patients was 48 and 47.4±10.9 years respectively. Most were premenopausal, this was the same as Omar et al.(1)who found most patients were premenopausal. Most of the tumors were GII, with a size ranged from 2-5 cm, had axillary lymph node metastases and stage IIB, this coincided with ElSaghir et al.(19) who reported advanced disease remains very common in Egypt, there is no doubt that the lack of early detection program and awareness contribute to advanced presentation .

In this study, p53 positivity was found in 60 % of invasive breast carcinomas. The reported incidence of p53 positivity in invasive breast carcinomas ranged from 15 % to 82 % in studies which analysed paraffin-embedded tissue material (7,15,18,20-31). This marked discrepancy 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 (15). Indeed, not all mutations yield a stable protein and some mutations lead to a truncated protein not detected by IHC. On the other hand, wild-type p53 may accumulate in some tumors as a result of response to DNA damage or by binding to other cellular proteins, giving a positive IHC result (5)

P53 positivity was highest in tumors of patients in the fifth decade, premenopausal, tumors of grade II, with a size of 2-5 cm, with positive axillary L.Ns and stage IIB. No statistically significant relation was found between p53 positivity and any of those variables.This is in agreement with other studies who found no statistically significant relation between p53 positivity and menopausal status (18,22), tumor grade(32), tumour size and lymph node status (18,30,32,33) and indicates that the oncogeneic aberration of this tumor suppressor gene is relevant to the biological behaviour of the tumor cell per se inde-

pendent of other clinicopathologic prognostic factors.

However, some of the previous investigators and others found p53 positivity to be significantly correlated with younger age(<40), premenopausal status[33] and high histologic grade [18,28 ,31 ,33].

On studying the relation between MVD and different age groups, menopausal status, tumor grade, axillary lymph node status, tumor size and tumor stage, the P value of each relation was non significant. The non significant P value of these different groups can be explained by the relatively small number of cases. However, high tumor grade, advanced tumor stage and the large tumor size, which are considered as indicators for tumor aggressiveness, showed high MVD.

However, in other studies MVD showed a significant positive relation with tumor grade [17,34-36], tumor size [34,35,37,38], axillary lymph node status[34,38] and tumor stage[38,39].

In the present study, MVD of p53 positive tumors was higher than that of p53 negative tumors with no statistical significance. This coincides with that of Fourati et al. (36) who found the majority of p53 positive tumors to demonstrate high MVD. This is explained by that the wild-type p53 protein has been shown to inhibit angiogenesis [4,5] and mutation of this protein tilts the balance in favour of angiogenesis. However, Ivkovic-Kapicl et al.,(35) found no association between the p53 protein expression and tumor angiogenesis.

Conclusions : Microvessel density may reflect more aggressive behaviour of breast carcinoma. Also, p53 altration may promote tumor angiogenesis.

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