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Research Article

Clinical Significance of Serum p53 Antibodies in Monitoring Treatment of Syrian Breast Cancer Patients

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ABSTRACT

Objective: To determine p53 antibodies in the serum of Syrain breast cancer patients and to assess the prognostic significance of these antibodies in treatment, monitoring and recurrence in those patients. **Methods:** The study was carried out at the Department of General Surgery at Al-assad university hospital and Al-bairouny oncology education tumor hospital, Damascus, Syria, between April 2008 to February 2011. Serum of 60 patients of breast cancer, 35 patients of benign masses and 35 of healthy controls were analyzed using Enzyme linked immunoadsorbent assay (ELISA). **Results:** Eight of sixty breast cancer patients were positive for p53 antibodies (13.3%), one patient of 35 benign masses patients was positive for p53 antibodies (2.9%) and p53 Abs weren't detected in control group. When we followed up patients of positive p53Abs after surgery, chemotherapy and radiotherapy, there was a swing in p53 Abs concentration during the study. **Conclusion:** Our results could support the role of p53 Abs as a prognostic biomarker for patients with breast cancer.

Keywords: p53 gene, p53 antibodies, breast cancer.

INTRODUCTION

First described in 1979, and initially believed to be an oncogene, p53 was the first tumor suppressor gene to be identified. The *p53* gene codes for a multifunctional DNA-binding protein that is involved in cell cycle arrest, DNA repair, differentiation, and apoptosis and is frequently mutated in human cancers[1]. Muations of the p53 gene are the most frequent genetic alteration associated with human malignancies being mutated in approximately 20-40% of breast cancer[2]. Apoptosis serves to eliminate damaged cells in order to prevent the propagation of deleterious genetic mutations that would otherwise be harmful for an organism. Failure to correct or remove DNA damage and accumulation of somatic mutations leads to unrestrained cell-cycle progression and, ultimately, cancer. In accord, inactivation of p53 is one of the most frequent mutations observed in human cancers and is detected in approximately 50 % of all tumors[3]. Over the past 15 years, tumor specimens from at least 9793 patients were included in 29 different studies to assess the association between *p53* gene mutation and prognosis of breast cancer: 24% of these studies found no association between *p53* status and prognosis, whereas 21% reported a significant adverse prognostic impact on univariate but not multivariate analyses; whereas the remaining 55% found prognostic significance on both univariate and multivariate analyses[4]. Because p53 accumulation is the main trigger of humoral response, it was of interest to examine the behavior of these p53 Abs during therapy to see whether there was a relationship between tumor disappearance and a decrease in p53 Abs[5]. Breast cancer is the most commonly diagnosed cancer among women and second only to lung cancer as the leading cause of cancer related deaths in women. It is estimated that 212,920 women in the United States were diagnosed with breast cancer in 2006, and that 14,970 women died of the disease[6]. In Syria, according to Syrian tumors Association, breast cancer cases discovered in 2007 about 15%, and about 11564 cases of breast cancer in Syria between 2000 until 2007 were discovered.

MATERIALS AND METHODS

Patients and tumor samples

The study was carried out at the Department of General Surgery at Al-assad university hospital and Al-bairouny oncology education tumor hospital, Damascus, Syria, between April 2008 to February 2011. Sixty patients who were diagnosed with breast cancer and treated were enrolled in this study. Pateints Sera were collected during the therapy phase preoperatively or before primary combination of radiotherapy and chemotherapy were initiated.

The following data of each patient shown in table 1 were analyzed: age, tumor size, stage of tumor, axillary lymph node metastases.

Serum samples

Blood samples were collected from 60 patients with breast cancer, 35 patients of benign masses and 35 of control. Serum samples were obtained by centrifuging Blood specimens at 3000g for 10 minutes and stored at -80 °C until analysis.

Stages of study

Study was fulfilled in three stages:

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Table 1- Data on 60 patients with breast cancer.

Patients with breast cancer	Number *(N=60)*	%
Age (years)		
<50	39	65
>50	21	35
Tumour size (cm)		
<2	10	17
>2	50	83
Clinical stage (TNM)		
I/II	32	53
III/IV	28	47
Axillary lymph node		
Negative	49	82
Positive	11	18
Metastatic disease		
Yes	9	15
No	51	85

Stage 1 (Before therapy) extended from April 2008 to February 2009 to investigate p53 Abs.

Stage 2 (After therapy, surgery or during chemotherapy or radiotherapy) extended from June, 2009 to July, 2010 to follow up patients with positive p53 Abs in stage 1.

Stage 3 (After surgery, chemotherapy and radiotherapy) extended from August, 2010 to February, 2011 to follow up and evaluate patients with positive p53 Abs in stage 2.

Analysis of p53 antibodies in the serum

We used IN VITRO p53 antibody ELISA kit (Homburg, Germany) to quantitative determination of human p53 antibodies in serum. The special feature of this ELISA is that cell extracts from human tumor cells are used containing p53. On the antigen plate, this immunoreactive and native p53 protein is fixed on a monoclonal antibody. To monitor nonspecific bonds between the patient serum and the antigen-antibody complex or the microtitre plate, we used a control antigen plate coated with a control antigen.

With the present ELISA the first step is to bind the antibodies which are present in the sample (diluted serum) to the immunoreactive p53 antigen on the micro-titre plate. The bound antibodies from the serum are detected after the next step, which is washing with a second antibody conjugated with an anti-human IgG peroxidase. The specific bond of this antibody to human IgG molecules is definite and makes quantitative determination of the amount of IgG bound to p53 possible across a range from 0.2 to 1.5 at 450 nm. This quantitative evidence of the antigen-antibody complex is followed, after another washing step, by addition of a substrate so that a soluble product emerges. After a stopping stage, the concentration of this product can be measured photometrically at 450 nm in an ELISA reader. To avoid faulty interpretations and as a negative control, the diluted samples are incubated simultaneously in the cavities of the control antigen plate, where the p53 is replaced by a control antigen. Using this method of proof this ELISA permits direct quantitative determination of the anti-p53 antibodies in the serum in µg/ml. The cut-off value of positive p53 antibodies is > 0.4 µg/ml.

Results. Among 60 patients with breast cancer, there were 8 patients (13.3%) positive for p53 Abs and one patient of 35 patients (%2.9) of benign masses was positive for p53 Abs. By contrast, all healthy controls were negative for p53 Abs. In stage 1, there were 8 patients positive for p53 Abs. As shown in table 2. In stage 2, we could follow up five breast cancer patients, three of five (1,5,8) remain positive for p53 Abs, two of five (3, 6) were negative for p53 Abs. While, patients (2, 7) did not follow up and patient (4) died. As shown in table 2. In stage 3, we could follow up four breast cancer patients, three of four (1,3, 5) were positive for p53 Abs, patient (6) was negative for p53 Abs and patient (8) died. As shown in table 2.

DISCUSSION

Crawford et al. reported for the first time in 1982 the presence of serum p53 Abs in patients with breast cancer. In the original study of Crawford, p53 Abs were detected in 9% of breast patients tested[7]. In the study of Peyrat the frequency of positive p53 Abs was 12%[8]. In the study of Muller et al. breast and colon cancer were the tow types of cancer with the highest positivity rates of p53 Abs (34 and 31.9%)[9].

In the present study, We found that (13.3 %) of the patients with breast cancer had p53 Abs and (%2.9) patients of benign masses had positive p53 Abs. The difference of p53 Abs rate could be due to patient selection bias, different clinical stages or the use of different assays. p53 is heavily phosphorylated at the NH2 and COOH termini. Such phosphorylation can have an important influence on the reactivity of p53 Abs toward the protein, which suggests that p53 expressed in mammalian cell is a better antigen

Table 2- Clinical characteristics, follow up, and evaluation of patients positive for p53 Abs.

S. No	*Age (Years)*	*Tumor*	*Classification*		*p53 Abs concµg/ml* (The cut-off value 0.4 µg/ml)			*Evaluation*
			TNM	*Stage*	*Stage 1*	*Stage 2*	*Stage 3*	
1	45	Breast	T_2 N_3 M_+	III	6.89	3.21	4.9	Bad condition
2	50	Bread	T_2 N_0 M_0	III	1.603	-	-	Didn't follow
3	52	Breast	T_4 N_X M_+	IV	1.30	0.1	0.76	Recurrence
4	46	Breast	T_2 N_2 M_0	III	2.744	-	-	Died
5	44	Breast	T_2 N_0 M_0	II	6.090	1.6	0.92	Bab condition
6	66	Breast	T_2 N_1 M_0	II	0.840	0.2	0.09	Good condition
7	43	Breast	$T_4N_2M_0$	III	0.728	-	-	Didn·t follow
8	48	Breast	T_3 N_+ M_0	III	1.342	0.721	-	Died

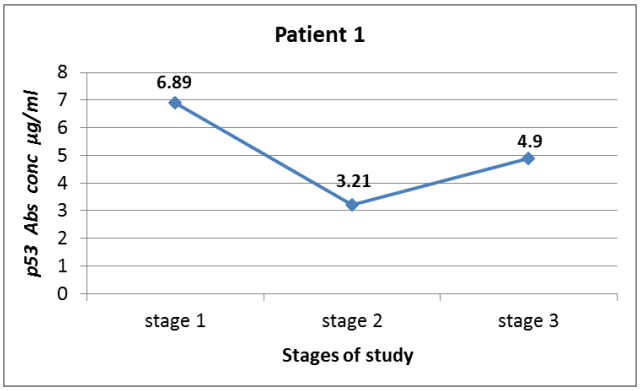

Figure 1: Monitoring of p53 antibodies in patient 1.

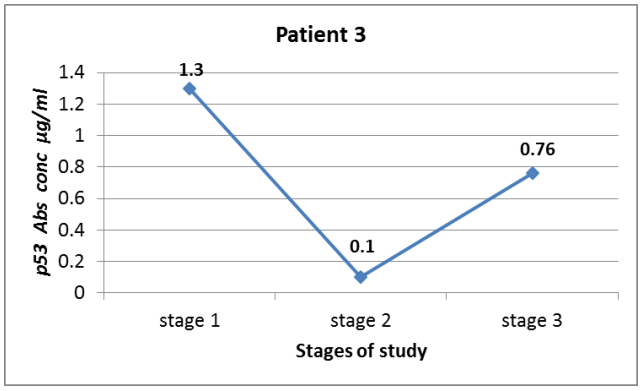

Figure 2: Monitoring of p53 antibodies in patient 3.

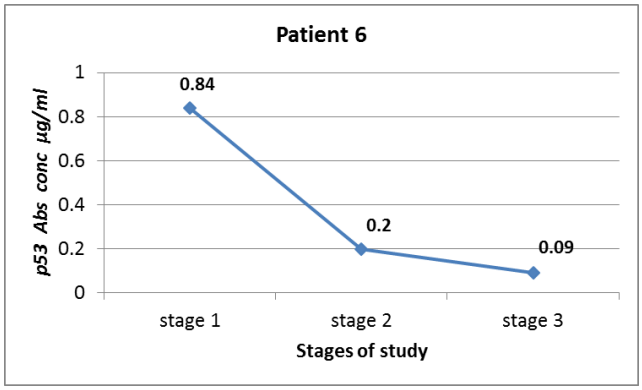

Figure 3: Monitoring of p53 antibodies in patient 6.

than those expressed in Escherichia coli[10]. In the present study, the antigen used in the assay is extracted from human tumor cells. It is not clear why many tumors with p53 mutations or protein overexpression are not immunogenic, but it is clear that the stabilisation and accumulation of mutant p53 proteins are prerequisites for p53 Abs production[11].

Changes in the p53 gene or the p53 protein may be used in clinical-oncological diagnostics for early diagnosis of a tumor, for prognostic purposes, for monitoring the course of a tumor related illness in individual patients and for monitoring therapy[12]. In the course of monitoring developments in patients with progressive tumor disorders, a seroconversion was noted in 40% of the patients over a period of 1-2 years. In some patients, postoperatively a reduction of the p53 antibodies was observed so that p53 may be used for monitoring developments or monitoring therapies. In breast cancer patients the presence of p53 autoantibodies correlates with high level histology and metastasis[13].

It has been claimed that p53 Abs may predict a poor prognosis and cancer relapse, may be of some value in monitoring populations at high risk of cancer, may predate the clinical detection of neoplasiaand may predict response to treatment. Variations of p53 Abs during treatment of patients with cancer have been poorly studied.

Angelopoulou et alreported p53 Abs monitoringin five patients with ovarian cancer andone with breast cancer[14]. We currently reported the results of repeat testing in 4 patients with p53Abs in the three stages of study. Patients (1,3,6) are described in detail.

In the present study, patient 1 had left mastectomy in 2006 and received chemotherapy and radiotherapy. In 2007, the patient had metastases to axillary nodes, underwent surgery, and received chemotherapy. In July 2008 (When we took the sample), the patient had a history of small nodes in the place of the mastectomic breast. Surgery did not performed, the patient has received chemotherapy and radiotherapy until 2009.There was no clinical improvement. The high persistent p53 Abs in patients 1 in the three stages may be due to persistent immunization against p53 protein. Table 1 shows p53 Abs status in patient 1 in stages of study.

In patient3, a temporal decrease in the p53 Abs can be closely correlated with disease progression or regression. Rapid but incomplete decrease patient p53 Abs, followed by a period of a fairly high concentration, was found before cancer progression. When patient received radiotherapy, there were metastases present at the time of tumor resection but undetectable. One study showed that the risk of developing a second cancer after radiotherapy treatment was as high as 57% and that radiotherapy should be avoided in p53mutation carriers[15]. Although it may not be possible to avoid chemotherapy in many situations, radiotherapy sometimes can be avoided by choosing mastectomy rather than lumpectomy for surgical treatment. When radiation cannot be avoided, it is imperative that treating physicians and the patient remain aware of the risk of a second primary tumor in the radiation field[16,17]. Table 2 shows p53 Abs status in patient 3 in stages of study. In patient 6, a decrease of p53 Abs in stage 1 and stage 2 can due to a good respone to treatment. After

lumpectomy in patient 6 in start of stage 1, there was an improvement after chemotherapy in September 2008. She received radiotherapy until April 2009. Tamoxefin was added in December 2009. Until the end of stage 3, patient had normal value of tumor marker CA 15-3 and had no metastases. A postoperative significant drop in p53 Abs may be the result of complete tumor resection and a successful djuvant therapy. Table 3 shows p53 Abs status in patient 6 in stages of study. Patient who has benign mass has ductal hyperplasia, this type may be progressed to a maligance. But we can not depend on the detection of p53 Abs to evaluate the alterations in p53 gene or abnormalities of p53 protein itself , if it is wild or mutated protein. We conclude that p53Abs testing is a convenient method to detect alterations of the p53 gene in patients with braest cancer. The presence of p53 Abs seems to be associated with more progressive cancers and reduced disease-free survival of surgically treated patients. There is accumulating evidence that p53 Abs may be indicative for a poor prognosis and a higher risk of tumor relapse. p53 Abs plays a useful potential biomarker in patient monitoring during therapeutic follow-up.

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