

Research Article



Significance of Serum p53 Antibodies as a Biomarker for Follow-up of Colorectal Cancer and Breast Cancer Patients

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ABSTRACT

The aim of this study was to assay p53 antibodies in the serum of colorectal cancer and breast cancer patients and to assess the prognostic significance of these antibodies in treatment monitoring and recurrence in patients. Serum of One hundred and seventeen patients of colorectal cancer and breast cancer were involved in this study. p53-Abs were assayed using ELISA method. Among 117 patients, 16 (13.6%) had positive p53-Abs. When we followed up patients of positive p53-Abs after surgery, chemotherapy or radiotherapy, there was a swing in p53-Abs concentration during stages of study. Our results may support the role of p53-Abs as a prognostic biomarker for patients with colorectal cancer and breast cancer.

Keywords: p53 gene, p53 antibodies, colorectal cancer, breast cancer.

INTRODUCTION

First described in 1979, and initially believed to be an oncogene, p53 was the first tumour suppressor gene to be identified.¹ The p53 gene is located at chromosome 17p13.1, and it encodes a 53-kDa nuclear phosphoprotein.²

As a transcription factor, activation of p53 triggers the induction of genes, many of which are positive regulators of apoptosis, a highly coordinated form of cell death that constitutes a critical effector arm of p53 tumour suppression. 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 tumours.³

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 tumour disappearance and a decrease in p53-Abs.⁴

In the United States, 106,680 new cases of colon cancer were expected in 2006 (men 49,220; women 57,460), and 41,930 new cases of rectal cancer were expected in 2006 (men 23,580; women 18,350). Colorectal cancer is the second leading cause of cancer related death in the United States with 68,000 deaths annually representing 10% of all cancer deaths.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.⁵

MATERIALS AND METHODS

Patients and controls

One hundred and seventeen patients who were diagnosed with colorectal cancer and breast cancer (57, 60 respectively) and treated at the Al-assad university hospital and Al-bairouny oncology education tumour hospital, Damascus, Syria, from April, 2008 to February, 2011 were enrolled in this study. All 115 patients hadn't received any preoperative adjuvant therapy. Serum samples from 87 healthy individuals with a mean age of 44 years (22-66) were used as a control group.

The following data of patients shown in table 1 were analyzed: age, tumour size, stage of tumour, axillary lymph node metastases, metastases disease and Clinical and pathohistological parameters of the patients and their tumours.

Serum samples

Blood samples were collected from 117 patients with colorectal cancer and breast cancer and (87) individuals. 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:

- Stage 1 (Before surgery) extended from April, 2008 to February, 2009 to investigate p53- Abs.
- Stage 2 (After surgery, and 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.

Table 1: Data on 117 patients with colorectal cancer and breast cancer.

Patients with colorectal cancer	N=57	
	Number	%
Tumour position		
Colon	26	46
Rectal	31	54
Clinical stage (TNM)		
I/II	31	53
III/IV	26	47
Metastatic disease		
Yes	6	11
No	51	89
Patients with breast cancer	N=60	
	Number	%
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

Analysis of p53 antibodies in the serum

We used IN VITRO p53 antibody ELISA for quantitative determination of human p53 antibodies in serum. The special feature of this ELISA is that cell extracts from human tumour 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 use 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 microtitre 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 117 patients with colorectal cancer and breast cancer, 16 patients (8, 8 respectively) (13.7%) had positive p53 Abs. By contrast, all individuals had negative p53- Abs. The cut-off value of positive p53 antibodies is > 0.4 µg/ml.

In stage 1, there were 16 patients positive for p53- Abs (8 colorectal cancer patients and 8 breast cancer patients).

In stage 2, we could follow up:

- Five colorectal cancer patients, two of five (3, 6) remain positive for p53- Abs, three of five (2, 7, 8) were negative for p53- Abs. While two patients (4, 5) did not follow up and patient (1) died.
 - Five breast cancer patients, three of five (9,13,16) remain positive for p53- Abs, two of five (11, 14,) were negative for p53- Abs. While two patients (10, 15) did not follow up and patient (12) died.
- In stage 3, we could follow up:
- Five colorectal cancer patients, two of five (3, 6) remain positive for p53- Abs, three of five (2, 7, 8) were negative for p53- Abs.
 - Five breast cancer patients, three of five (9,11, 13) were positive for p53- Abs, one of five (14) were negative for p53- Abs and patient (16) died.

In evaluation of patients, we depended upon classification of patients according to the status of p53- Abs in the three stages to:



A: Patients had positive p53 Abs in the three stages.

B: Patients had positive p53 Abs in stage 1, while they had negative p53- Abs in stage 2 and stage 3.

C: Patients had positive p53- Abs in stage 1 and stage 3, while they had negative p53- Abs in stage 2.

Table 2 describes the clinical characteristics, follow up and evaluation of patients with p53- Abs.

Table 2: Clinical characteristics, follow up, and evaluation of patients with p53- Abs.

No	Age (Years)	Tumour	Gender	Classification		p53 Abs conc µg/ml (The cut-off value 0.4 µg/ml)			Evaluation
				TNM	Stage	Stage 1	Stage 2	Stage 3	
1	79	Colon	F	T ₃ N ₂ M ₁	IV	2.042	-	-	Died
2	71	Colon	F	T ₃ N ₀ M ₀	II	4.821	0.182	0.173	B
3	52	Rectum	M	T ₃ N ₂ M ₀	III	8.912	5.824	8.247	A
4	56	Rectum	M	T ₃ N ₀ M ₀	II	1.214	-	-	Didn't follow
5	52	Colorectal	F	T ₃ N ₀ M ₀	II	1.101	-	-	Didn't follow
6	75	Rectum	F	T ₃ N ₀ M ₀	II	4.603	4.477	5.89	A
7	71	Rectum	F	T ₂ N ₀ M ₀	I	2.442	0.252	0.210	B
8	81	Colon	M	T ₃ N ₁ M ₀	III	12.83	0.196	0.142	B
9	45	Breast	F	T ₂ N ₃ M _x	III	6.89	3.21	4.9	A
10	50	Breast	F	T ₂ N ₀ M ₀	III	1.603	-	-	Didn't follow
11	52	Breast	F	T ₄ N _x M ₊	IV	1.30	0.1	0.76	C
12	46	Breast	F	T ₂ N ₂ M ₀	III	2.744	-	-	Died
13	44	Breast	F	T ₂ N ₀ M ₀	II	6.090	1.6	0.92	A
14	66	Breast	F	T ₂ N ₁ M ₀	II	0.840	0.2	0.09	B
15	43	Breast	F	T ₄ N ₂ M ₀	III	0.728	-	-	Didn't follow
16	48	Breast	F	T ₃ N ₊ M ₀	III	1.342	0.721	-	Died

DISCUSSION

The present study shows the follow up of colorectal cancer and breast cancer patients and examines p53-Abs status in them. We found that 13.6 % of the patients with colorectal cancer and breast cancer had p53-Abs. The lower rate of p53-Abs in this series could be due to patient selection bias or because of 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 than those expressed in *Escherichia coli*.⁴ In the present study, the used antigen is extracted from human tumour cells. It is not clear why many tumors with p53 mutations or protein overexpression are not immunogenic. It is clear that the stabilisation and accumulation of mutant p53 proteins are prerequisites for p53-Abs production.⁶

Changes in the p53 gene or the p53 protein may be used in clinical-oncological diagnostics for early diagnosis of a tumour, for prognostic purposes, for monitoring the course of a tumour related illness in individual patients and for monitoring therapy.⁷ In the course of monitoring developments in patients with progressive tumour 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.⁸

The monitoring of p53 autoantibodies after surgery and adjuvant chemotherapy of colorectal cancer patients has potential for early diagnosis of tumour relapse.⁹ Likewise, Takeda et al., noticed a significant correlation between curability of colorectal cancer by surgical tumour resection and postoperative disappearance of p53 autoantibodies in these patients.¹⁰

In the present study, patients A the persistent high p53-Abs may be due to persistent immunization against p53 protein.

In patients B, a decrease of p53-Abs can occur in patients who have been treated. Whereas, constant stimulation of the immune system is necessary to maintain a high level of p53-Abs, removing the tumour would prevent such stimulation.

Patients C (patient 11) temporal changes in the p53-Abs can be closely correlated with disease progression or regression. Rapid but incomplete decreases patients p53-Abs, followed by a period of stability at a fairly high concentration, was found before cancer progression. There were metastases present at the time of tumour resection but undetectable.

We conclude that p53-Abs testing is a convenient method to detect alterations of the p53 gene in patients with

colorectal cancer and breast cancer. Monitoring of p53-Abs may help in the early diagnosis and treatment of relapse in patients.

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