p53 Protein in Serum and Urine Samples of Patients with Bladder Transitional Cell Carcinoma and Its Overexpression in Tumoral Tissue

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Introduction: The aim of this study was to evaluate the levels of p53 protein in serum and urine samples of patients with bladder transitional cell carcinoma (TCC) and their relation with the overexpression of p53 in the tumoral tissue.

Materials and Methods: A total of 39 patients with bladder TCC were evaluated for p53 protein in their serum and urine samples and the overexpression of this marker in their tumoral tissue.

Results: Of 39 patients with bladder TCC, 29 (74.4%) had tissue specimens positive for p53 protein overexpression, 20 (51.3%) had p53 protein in their serum samples, and 27 (69.2%) had this protein in their urine samples. A positive immunohistochemical finding was more common in higher grades of the bladder tumor ($P = .03$), but not in higher stages ($P = .07$). Eighteen of 20 patients (90%) with a positive serum for p53 showed protein overexpression in the tumoral tissue of the bladder ($P = .03$). Of 27 patients with a positive urine sample, 25 (92.6%) had p53 overexpression in their bladder tissue, and of the remainder 12 patients with a negative p53 protein in their urine samples, 8 (66.7%) had no evidence of p53 protein overexpression in their tumoral tissue ($P < .001$). The grade and stage of tumor had no correlation with serum or urinary p53.

Conclusion: According to our findings, a positive serum or urine sample for p53 protein is highly associated with the overexpression of p53 protein in the tumoral tissue of patients with bladder TCC.

Keywords: p53 protein, TP53, bladder cancers, enzyme-linked immunosorbent assay, immunohistochemistry

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INTRODUCTION

Bladder carcinoma is one of the most common malignancies in urology. The most common type of the bladder cancer is transitional cell carcinoma (TCC) that is the fourth most common cancer in men and the eighth in women.[1]

Molecular genetic studies have revealed that mutations in the suppressor genes are responsible for the formation of bladder cancer. One of these suppressor genes is *TP53* which is the most common mutated gene in human beings.[2]

The p53 protein has been shown as a valuable tumor marker in most of the tumors especially bladder tumors.[1,3] The half-life of a normal p53 protein is short (20 to 60 minutes), but with some mutations, it increases and reaches up to 6 hours.[4] The mutated p53 protein prevents formation of the normal type of this protein due to the negative feedback caused by its long half-life.[5] This will result in the accumulation of p53 protein in the nucleus of the cell which can be determined using immunohistochemical methods.[4]

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However, very few tumors with mutated *TP53* will not show the nuclear accumulation of p53 protein by the abovementioned methods.[6] It should be noted that in some cases, immunohistochemical staining may be more sensitive than the molecular methods in the diagnosis of *TP53* mutations and may also reveal the main functional changes of the protein, as well.[6] In the recent years, several studies have been performed for finding more precise methods to determine p53 protein.[7-9] It has been demonstrated that abnormal p53 protein accumulation in the cells may increase its level in extracellular fluids such as serum and urine by an unknown mechanism which can be measured using enzyme-linked immunosorbent assay (ELISA).[9] Several reports have shown different views about the levels of p53 in the serum and urine and their relation with overexpression of p53 protein.[9-14] In this prospective study, we evaluated the relation between the level of p53 protein in the serum and urine samples of the patients with bladder TCC and the pathological and clinical parameters of the disease.

MATERIALS AND METHODS

Between March 2005 and May 2006, 45 patients with TCC referred to the clinics of Imam Reza hospital in Mashhad were selected. The patients provided informed consent and the study was approved by the ethics committee of Mashhad University of Medical Sciences. None of them had the history of previous intravesical bacillus Calmette-Guerin (BCG) instillation, radiotherapy, chemotherapy, or immunocomprising conditions.

Pathology specimens of the bladder were obtained by transurethral resection, cold-cut biopsy, or cystectomy. Tissue passage was performed on the specimens and paraffin blocks were obtained. Four-micrometer tissue cuts were prepared and evaluated by a single pathologist according to the definitions of the World Health Organization.[15] Of 45 samples taken, 39 with TCC were selected for further study. The specimens were then stained by Biotin Avidin immunoenzymatic technique. Those with at least 10% of the tumoral cells stained were considered positive for overexpression of p53 protein and those with less than 10% were considered negative.[16] According to the pathologic stage and radiologic findings, the tumors were categorized into two groups of superficial and deep regarding the invasion of the tumor to the detrusor muscles.

Concurrently, a 2-mL peripheral blood sample and a 2-mL urine sample were also taken. After separation of the serum, they were kept in a temperature of 20°C. Thirty-nine samples with positive pathologic report of TCC were evaluated regarding the presence of p53 protein using ELISA method (Bender MedSystem, Vienna, Austria). Serum and urine samples of 6 healthy age- and sex-matched people without the history of malignancy were also evaluated by ELISA method. Statistical analyses were done using chi-square test and Fisher exact test for comparison of dichotomous variables and a value for *P* less than .05 was considered significant.

RESULTS

Of 39 patients with bladder TCC, 29 (74.4%) had tissue specimens positive for p53 protein overexpression. A positive immunohistochemical finding was more common in high-grade tumors; 4 (44.4%), 10 (71.4%), and 15 (91.8%) patients with grades 1, 2, and 3 had positive results ($P = .03$). Higher stages of the tumors were slightly associated with overexpression of p53 protein ($P = .07$).

Twenty (51.3%) patients showed p53 protein in their serum samples. The grade of tumor had no correlation with serum p53 (Table 1). Eighteen of these patients (90%) showed protein overexpression in the tumoral tissue of the bladder that was indicative of a significant relationship between the serum positivity and protein overexpression ($P = .03$; Table 2).

Table 1. Positive p53 Protein and Grade of Tumor*

p53 Protein	Grade 1 (n = 9)	Grade 2 (n = 14)	Grade 3 (n = 16)	Total (n = 39)	*P*
Tissue specimen	4 (44.4)	10 (71.4)	15 (91.8)	29 (74.4)	.03
Serum sample	4 (44.4)	9 (64.3)	7 (43.8)	20 (51.3)	.51
Urine sample	5 (55.6)	9 (64.3)	13 (81.3)	27 (69.2)	.38

*Values in parentheses are percents.

Table 2. Overexpression of p53 in Tumoral Tissue and p53 Protein in Serum and Urine*

	Serum p53 Protein		Urinary p53 Protein		
p53 Protein Overexpression	Positive	Negative	Positive	Negative	Total
Positive	18 (62.1)	11 (37.9)	25 (86.2)	4 (13.8)	29
Negative	2 (20.0)	8 (80.0)	2 (20.0)	8 (80.0)	10
Total	20	19	20	19	39

*Values in parentheses are percents.

Twenty-seven patients (69.2%) had p53 protein in their urine samples. Although the frequency of p53 positivity was higher in grade 3 compared with grades 1 and 2, there was no significant relation between the tumor grade and p53 protein in the urine (Table 1). Of 27 patients with a positive urine sample, 25 (92.6%) had p53 overexpression in their bladder tissue, and of 12 patients with a negative p53 protein in their urine samples, 8 (66.7%) had no evidence of p53 protein overexpression in their tumoral tissue (Table 2). Thus, a significant relationship was found between the overexpression of the p53 protein in the tissues and the existence of p53 in urine specimens ($P < .001$). Also, in spite of higher frequency of p53 protein detection in serum and urine of the patients with higher tumor invasiveness (63% and 68.4%, respectively), this difference was not significant, either ($P = .07$ and $P = .63$, respectively).

The mean serum level of p53 protein in the patients who were considered to be serum-positive for p53 was 1.17 ± 1.37 U/mL. Mean serum level of p53 in the patients with positive tissue specimens for p53 was 1.46 ± 1.42 U/mL (range, 0 to 5.3 U/mL). In those with tissue-negative specimens, the mean serum level of p53 was 0.32 ± 0.77 U/mL (range, 0 to 2.4 U/mL).

The mean urine level of p53 protein in the patients who were considered to be urine-positive was 1.78 ± 1.67 U/mL. The mean urine level of p53 in the patients with tissue-positive specimens was 2.27 ± 1.6 U/mL (range, 0 to 5.3 U/mL). This was 0.35 ± 0.8 U/mL (range, 0 to 2.4 U/mL) in the patients with negative tissue specimens.

DISCUSSION

Mutations of *TP53* have been reported in human tumors such as bladder carcinoma.[2] Several studies have shown that mutation of this gene is common in bladder tumors and has a relation with the grade and stage of the disease.[1,2] The rate of overexpression of p53 in bladder tumors is reported to be 29% to 78% in different studies.[9,17] In our study, 74.4% patients with bladder TCC showed accumulation of p53 protein in the tumoral tissue. The diversity of these results may be due to the differences in the pathologic stages and grades of the tumors, the process of tissue preparation for the evaluation or antigen retrieval techniques, and finally the definition of positive p53 specimen in the tissues in these studies (cut-off point may be considered 5%, 10%, or 20%).[16] One of the limitations of this study was the lack of a control group, the cut-off value for positive immunohistochemical staining was obtained from previous reports.

A positive p53 protein in serum samples of the patients with bladder TCC has been reported to be 3% to 68% in different studies.[9-12] Also, a positive urine sample for p53 protein has been reported in 60% of these patients by ELISA test.[13] In our study, 51.3% of the patients with TCC had a positive ELISA test for p53 protein in their serum and 69.2% had it in their urine; our relatively high rates may be due to the higher pathologic grades in our patients. In the previous studies a relation had been reported between the p53 protein and pathologic grade.[9-13] In our study, a statistically significant relation was found between the expression of p53 in the tissues and the pathology grade of the bladder TCC. However, such a relation was not found between p53 protein in the urine and serum samples and the tumor stage. Also, although the prevalence of p53 protein was higher in the serum and urine samples of the patients with higher stages, this rate was not statistically significant. More studies in this regard are warranted.

In a study in India, of 18 patients with positive tissues for p53 protein, 17 (94.4%) had positive sera.[9] In another study in Argentina, this rate was reported to be 83.3%.[3] In these studies, the existence of p53 protein was always an indicator of

p53 overexpression in the tumoral tissue; however, this relation was one-way.[9] Our study agrees with the previous studies in this regard. Of 20 patients with a positive serum for p53 protein, 18 showed p53 protein overexpression in their bladder tissue, but of 29 patients with overexpression of p53, 11 did not show the protein in their serum samples. In our study, the relation between the p53 protein in the urine samples, and the tumoral tissues of the patients with TCC was also evaluated. In 29 patients who had overexpression of p53 in their tumoral tissue of the bladder, 25 showed positive urine samples using ELISA method (86%). This relation had also been reported in a similar study by Indulski and colleagues.[13]

CONCLUSION

It seems that serologic evaluation of p53 protein in serum and urine samples is a very sensitive tool for the prediction of p53 overexpression in the patients with bladder TCC. However, negative serologic result does not rule out the p53 overexpression. According to the findings of this study, a positive result of ELISA for p53 protein in urine and serum samples can be considered as p53 protein overexpression in tumoral tissue. This test is cost-effective and simple; thus, performing complicated and expensive immunohistochemical tests can be avoided in the future.

CONFLICT OF INTEREST

None declared.

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