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VALUE OF P_{53} PROTEIN AS A TUMOR MARKER IN PATIENTS WITH TRANSITIONAL CELL BLADDER CANCER

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Objective: The aim of this work is to determine the value of P53 as a biochemical marker in patients with bladder cancer.

Patients and Methods: This study was conducted on 30 patients with transitional cell carcinoma (TCC) of different grades and 10 healthy men as a control group who had been admitted to the Urology Department, Benha Faculty of Medicine between August 1999 and November 2001. The mean age of the patients was 56.3 years (range 38-80 years). They were evaluated by history taking, clinical examination, laboratory investigations, radiological examination and cystoscopy-guided biopsies. P_{53} was determined in the serum preoperatively and postoperatively after 21 days and 6 months, as well as in the tissue specimens taken by transurethral resection or by radical cystectomy.

Results: The mean serum P_{53} value in the control group was 10.0 $\pm$ 1.83 Pg/ml. In the patients with grade-1 tumors it was 25.6 $\pm$ 4.8 Pg/ml compared to 44.8 $\pm$ 14.73 Pg/ml and 131.1 $\pm$ 15.28 Pg/ml for grade-2 and grade-3 tumors, respectively ($P < 0.05$). In tumors larger than 2 cm the mean serum P_{53} value was 87.54 $\pm$ 10.81 Pg/ml, while in tumors less than 2 cm it was 32.91 $\pm$ 2.32 Pg/ml ($P < 0.05$). The mean serum P_{53} value in a single tumor was 27.8 $\pm$ 7.1 Pg/ml compared to 102.3 $\pm$ 20.4 Pg/ml in multiple tumors ($P < 0.05$). On follow-up after 21 days the mean serum P_{53} value was 14.0 $\pm$ 2.71 Pg/ml in grade-1 tumors, 17.0 $\pm$ 3.79 Pg in grade-2 and 55.3 $\pm$ 12.4 Pg/ml in grade-3 tumors ($P < 0.05$). Eleven patients developed recurrence; their mean serum P_{53} was 125.6 $\pm$ 13.46 Pg/ml preoperatively and significantly decreased to 59.9 $\pm$ 18.2 Pg/ml postoperatively, but then rose again to 91.5 $\pm$ 20.1 Pg/ml. The mean P_{53} in the tissues of the control group was 11.3 $\pm$ 2.31 Pg/ml, while the tissues of the cancer patients showed values of 29.8 $\pm$ 4.42 Pg/ml, 46.6 $\pm$ 11.08 and 140.2 $\pm$ 14.85 Pg/ml for grade-1, grade-2 and grade-3 tumors, respectively ($P < 0.05$).

Conclusion: P_{53} seems to be a promising tumor marker for transitional cell bladder cancer and a valuable tool for identifying subgroups of patients that may have a poor prognosis.

Key Words: bladder cancer, transitional cell carcinoma, P_{53} protein

INTRODUCTION

In nearly 80% of patients with transitional cell carcinoma (TCC) of the urinary bladder superficial disease is diagnosed initially. Within 6-12 months after transurethral resection of the cancer, 70-80% of these patients develop recurrent disease with muscle invasion or metastatic spread in 10-20%. On the other hand, 50-60% of the patients will suffer from recurrences of superficial TCC without developing muscle invasion. Numerous studies have tried to determine prognostic parameters for superficial growth and muscle invading bladder cancer. However, the majority of these factors did not provide prognostic information superior to histopathological parameters such as tumor staging and tumor grading[1].

Part of the reason for the poor prognosis of advanced bladder cancer is the present lack of any prognostic markers to identify tumors that are at a higher risk of progression. So there are an intense interest and pressing clinical

Table 1: Site and Grade of the Bladder Tumors

Site	No. of Patients			Total	%
	Grade 1	Grade 2	Grade 3		
Trigone	5	8	6	19	63.3%
Lateral wall	3	1	2	6	20.0%
Anterior wall	0	1	1	2	6.7%
Posterior wall	2	0	1	3	10.0%
Total	10	10	10	30	100%

Table 2: Mean Pre- and Post-Operative Serum P53

	Control Group	Grade 1		Grade 2		Grade 3	
		pre-op.	post-op.	pre-op.	post-op.	pre-op.	post-op.
Serum P53 Pg/ml	10.0±1.83	25.6±4.5	14.0±2.71	44.8±14.73	17.0±3.79	131.1±15.28	55.3±12.4
P-value	significant < 0.05						

needs for the development of such markers to allow an optimal treatment in selected patient groups[2].

There is a group of genes involved in the development of cancer called tumor suppressor genes or antioncogenes. The presence of these genes in normal cells is thought to suppress the development of tumors. Mutation of these genes results in a loss of suppression, favoring malignant transformation[3]. A key position in the process of malignant transformation of somatic cells has been attributed to one of these genes, the P_{53} gene[4]. The human protein P_{53} gene, located on the short arm of chromosome 17, encodes a nuclear phosphoprotein which binds to specific DNA sequences in the human genome and appears to have a key role in the control of DNA replication and hence cellular metabolism[5]. There are two types of P_{53}, the wild type and the mutant type. In normal cells, the wild-type P_{53} has a short intracellular half-life and attains a low steady state level that is not detected by histology. Mutant P_{53} products are more stable and less degradable, thus, the identification of P_{53} nuclear over-expression by immunohistochemistry appears to correlate with P_{53} mutation[6].

P_{53} has been reported to be a tumor suppressor gene, and P_{53} mutation is the most common genetic mutation in cancer. The mutated gene loses natural tumor suppressor function, allowing damaged cells to divide and to become malignant[7].

PATIENTS AND METHODS

This study was conducted on 40 patients with a mean age of 56.3 years (range 38-80 year), who were admitted to the Urology Department, Benha Faculty of Medicine between August 1999 and November 2001. They were evaluated by history taking, clinical examination, laboratory and radiological investigation and cystoscopy-guided biopsies. All patients were followed up for 6 months post operatively.

The study group consisted of 30 patients with TCC of different grades and 10 healthy men as a control group. The trigone was the

Table 3: Relationship between Recurrent Cases and Grade and Number of the Tumors

	Grade 1		Grade 2		Grade 3	
	single	multiple	single	multiple	single	Multiple
Recurrent cases	1	1	1	2	2	4
Total (n=11)	2		3		6	

most common site of the bladder tumor (63.3%), followed by the lateral wall (20%), while the anterior wall was the least common site of bladder cancer (6.7%) (Table 1). Seventeen patients (56.7%) were found to have a tumor size of more than 2 cm and 13 patients (43.3%) had a tumor size less than 2 cm. Multiple tumor sites were detected in 8 patients (26.7%) and 22 patients (73.3%) had a single tumor.

The patients were divided into four groups of ten patients each with grade-1, grade-2 and grade-3 cancer, as well as a group with ten healthy men as a control group.

Patients with grade-1 bladder cancer were treated by transurethral resection of the tumor and intravesical instillation of BCG. Patients with grade-2 and grade-3 tumors were treated by radical cystectomy and urinary diversion.

Blood (10 ml) was taken from all cancer patients and the control group, and the cells were sedimented by centrifugation at 2,000 rpm (800g) for 10 min. The supernatant serum was collected and kept frozen at -80ºC.

P_{53} was determined in the serum of the patients preoperatively and postoperatively after 3 weeks and 6 months as well as in the tissue specimens by enzyme-linked immunosorbent assay (ELISA) using P_{53} detection kits supplied by Rochs.

During the operation, a small biopsy of bladder tissue weighing 0.5 gm was added to 3.0cc of RIPA buffer which is composed of 20 mM tris, 0.5 mM EDTA (ethylene Diamine tetra-acetic acid), 1.0% Nonidet P40, 0.05% sodium deoxycholate, 0.05% (sodium dodecyl sulphate), 1.0 mM PMSF (phenyl methane sulfonyl Fluoride), 1.0 ug/ml A protinin, 2.0 ug/ml leupeptin) in a centrifuge tube. The tissue was disintegrated using the Art Miccra D-8 homogenizer at a speed of 26.000 rpm. The homogenates were centrifuged at 1000xg for 10 minutes. The supernatant was kept frozen at a temperature of -80ºC for later analysis.

For determination of P_{53}[8] just before the assay, all sera and tissue samples were left at room temperature to be thawed and diluted 1:5 with the diluent supplied with the kit.

Assay procedure:

100µl of standards, diluted sera and the supernatant samples of tissue homogenate were pipetted into the corresponding wells. Then 100µl of anti-p53-POD were added to all wells containing standards/samples. The microtitre plate was covered tightly with the included adhesive cover foil and incubated for 2.0h at 15-25^{0}C on a shaker. The incubation buffer was removed thoroughly by suction. The wells were rinsed five times with 300µl washing buffer which was removed carefully. Then, 200µl of substrate solution were pipetted into the wells. The microtitre plate was covered tightly with the adhesive cover foil and incubated at room temperature on a shaker at 300 rpm until color development was sufficient for photometric detection (10-20 min). After that, 50µl of stop solution was pipetted into each well. The plate was incubated for 1.0 min on the shaker at 300 rpm. The assay results were quantitated spectrophotometrically at 450 nm using the microtitre plate reader (reference wavelength: 690 nm) against air blank. Measuring was carried out within 5 minutes after adding the stop solution. The concentration of P_{53} was calculated from the standard curve. The concentration of P_{53} was expressed as pg/gm for tissue homogenate and pg/ml for the serum.

The data were collected, tabulated and statistically analyzed using Student t-test with $P<0.05$ considered significant.

RESULTS

We examined 30 patients with urothelial bladder carcinoma and 10 healthy volunteers. The clinical data and laboratory analysis data of our patients according to the tumor grade are shown in Tables 1,2 and 3.

Grade of tumor and preoperative serum P_{53} value

The mean serum P_{53} value in the control group was 10.0 $\pm$ 1.83 Pg/ml compared to 25.6 $\pm$ 4.5 Pg/ml, 44.8 $\pm$ 14.73 Pg/ml and 131.1 $\pm$ 15.28 Pg/ml for grade-1, grade-2 and grade-3 tumors, respectively ($P<0.05$) (Table 2).

Size and number of tumors and preoperative serum P_{53}

In tumors larger than 2 cm, the mean serum P_{53} value was 87.54 $\pm$ 10.81 Pg/ml and in tumors less than 2 cm it was 32.91 $\pm$ 2.32 Pg/ml ($P<0.05$).

The mean serum P_{53} value in a single tumor was 27.8 $\pm$ 7.1 Pg/ml, while it was 102.3 $\pm$ 20.4 Pg/ml in multiple tumors ($P<0.05$).

Post-operative follow up:

The mean serum P_{53} value at 21 days postoperatively was 14.0 $\pm$ 2.71 Pg/ml in grade-1 tumors, 17.0 $\pm$ 3.79 Pg/ml in grade-2 and 55.3 $\pm$ 12.4 Pg/ml in grade-3 tumors, $P<0.05$ (Table 2).

Recurrent tumors and P_{53}

In this work, recurrent tumors were detected in 2 patients with grade-1 tumors, 3 patients with grade-2 tumors and 6 patients with grade-3 tumors. Recurrence was more frequent in patients with multiple tumors (7 cases; 63.6%) than in patients with single tumors (4 cases 36.4%) (Table 3).

The mean serum P_{53} value in patients who developed recurrence was 125.6 $\pm$ 13.46 Pg/ml preoperatively and significantly decreased to 59.9 $\pm$ 18.2 Pg/ml postoperatively.

Tissue P_{53}:

The mean P_{53} in the tissue specimens of the control group was 11.3 $\pm$ 2.31 Pg/ml, while it was 29.8 $\pm$ 4.42 Pg/ml, 46.6 $\pm$ 11.08 and 140.2 $\pm$ 14.85 Pg/ml for grade-1, grade-2 and grade-3 tumors, respectively ($P<0.05$).

DISCUSSION

The P_{53} tumor suppressor protein is a transcriptional factor regulating several gene expression pathways that function collectively to maintain the integrity of the genome. Its nuclear localization is critical to this regulation[9].

P_{53} is a critical regulator of the G_1 S-check point. The critical protein in cell response to DNA damage or extracellular growth regulatory signals is the transcription factor P_{53}. Active P_{53} binds to the promoter region of the P_{53}-responsive genes and stimulates the transcription of genes responsible for cell cycle arrest, repair of DNA damage and apoptosis. P_{53} responds to DNA damage by inducing cell cycle arrest and then transcriptionally activating DNA repair enzymes. If the cell cannot arrest growth or repair the DNA, P_{53} induces apoptosis[10].

Mutations in the P_{53} tumor suppressor gene represent the most common genetic alteration associated with malignant tumors. Overexpression of P_{53} protein could be considered the result of increased protein stability caused by conformational alteration and has been reported in more than 50% of epithelial malignancies[11].

Mutational inactivation of P_{53} has been found to be involved in various human cancers which indicates the importance of P_{53} in human carcinogenesis. It has been reported that over 50% of human cancers contain mutation in the P_{53} gene. P_{53} is activated in response to a variety of stimuli such as ultraviolet rays, gamma radiation, hypoxia, nucleotide deprivation etc. The activation of P_{53} may cause cell division, cycle arrest or apoptosis[12].

An altered expression of P_{53} has been described in nearly 50% of bladder cancer cases, and P_{53} mutation is presumed to play a role in the multi-step progression of these tumors[13].

The data of this work showed that preoperative and postoperative mean serum P_{53}

values were significantly increased compared to the control group ($P<0.05$). However, the mean serum P_{53} value increased with grade, size and number of the tumors. The post operative mean serum P_{53} values in our patients were significantly decreased compared to those obtained preoperatively in all grades of bladder cancer. These results are comparable to those of many investigators who found that P_{53} over-expression in the serum of bladder cancer patients was dependent on stage, grade, pattern of growth and focality of the tumor[1,11,14-16].

In this study, the mean tissue P_{53} was significantly elevated in all patients with different grades of bladder cancer compared to the mean tissue P_{53} value of the control group. The same finding was reported by Zalabardo and associates[17].

In our recurrent cases, the mean serum P_{53} value decreased significantly in the early postoperative period (after 21 days), then it increased again significantly in the late postoperative period (after 6 months) ($P<0.05$). Similar results were recorded by other researchers and led to the conclusion that P_{53} accumulation in transitional cell carcinoma predicts the risk of recurrence and mortality independent of stage, grade and lymph-node status[15-19].

The inactive P_{53} mutants lose their ability and therefore allow uncontrolled cell proliferation with uninhibited replication of defective DNA. Such mutations also lead to an accumulation of P_{53} due to reduced proteolytic degradation reaching the threshold level for immunohistochemical detection[20]. In addition, the P_{53} status in primary tumors may predict not only the likelihood of recurrence but also whether patients will respond to chemotherapy[21].

In conclusion, P_{53} protein status in serum and tumors seems to be a promising tumor marker for transitional cell carcinoma of the bladder and may be a significant tool for identifying subgroups of patients that may have a poor prognosis. In addition, knowledge of the P53 status might also help to decide on the therapeutic options.

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Editorial Comment:

This paper gives a nice report on a limited number of patients with bladder cancer, but with very striking results concerning P53. It can add to a number of other papers that believe that P53 is an important marker. However, the role of P53 as a prognostic marker for bladder cancer remains controversial. A randomized study based on P53 and coordinated by Dr. Stein from the University of South California is ongoing in the United States and Europe. The results of this important study need to be awaited before drawing any definitive conclusions.

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Due to the fact that this paper was accepted on short notice, the translation of the abstract will be published in Vol. 11, No. 2, 2005.

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