Urol Res (2004) 32: 391–394
DOI 10.1007/s00240-004-0415-9

ORIGINAL PAPER

Vibhav Malviya · Harbans Singh · U. S. Dwivedi
Usha · P. B. Singh

# Serum p53 and bladder cancer: can serum p53 be used as a tumor marker?

Received: 11 February 2003 / Accepted: 3 March 2004 / Published online: 19 October 2004


**Abstract** The aim of this study was to find the correlation between serum p53 and carcinoma of the bladder and to investigate whether serum p53 protein can be used as a tumor marker for p53 gene alteration. The study included patients with carcinoma of the bladder and controls. Serum p53 protein estimation was done with an ELISA kit. There were 23 patients with superficial and 17 with invasive carcinoma. The median serum p53 was 31.5 U/ml in superficial and 41 U/ml in invasive cancer. This was significantly higher than the mean value (16.4 U/ml) of controls. Serum p53 rises in patients with carcinoma of the bladder and correlates with the grade of the disease .It can therefore be used as a tumor marker for bladder cancer.

**Keywords** Serum p53 · Bladder cancer · Tumor marker

## Introduction

Transitional cell carcinoma of the urinary bladder exhibits the entire spectrum of biological aggressiveness from superficial low-grade papillary lesions to high-grade invasive disease with high potential for local spread and distant metastasis. The transformation of a normal into a malignant cell is a multistep mechanism, which involves various alterations at the molecular and genetic levels. These acquired alterations in DNA often lead to either the induction of oncogenes or the negation of tumor suppressor genes, resulting in malignantly transformed cells [1]. Molecular markers are being studied to distinguish non-invasive papillary from invasive bladder carcinomas and for identifying individual tumors with a high risk of recurrence or disease progression [2]. The application of various diagnostic tests for cancer risk monitoring is associated with the fact that gene mutations and changes in gene expression correspond to the earliest stages of carcinogenesis. The changes in proto-oncogenes and suppressor genes can be detected either at the genome level, at the level of transferring the genetic information from DNA to protein, or at the level of protein synthesis controlled by genes (oncogenes or anti-oncogenes). In the latter instance, as the concentrations of these proteins are considerably increased, their quantities in tissue or blood serum can be determined by immunohistochemical methods [3]. One of the most common cellular genes which negatively regulates the cell cycle, thus functioning as tumor suppressor gene, is the p-53 gene. The presence of this mutated gene has been correlated with the aggressiveness of several malignant neoplasms including bladder cancer [4]. Although tissue p53 estimation is a well-established procedure and its association with the invasiveness of the tumor and prognosis has been widely studied, serum p53 protein estimation has not been studied adequately. We conducted this study to determine the correlation between serum p53 and carcinoma of bladder, and to investigate whether serum p53 protein concentrations can be used as markers for p53 gene alteration.

V. Malviya · H. Singh · U. S. Dwivedi · P. B. Singh (✉)
Department of Urology, Institute of Medical Sciences,
Banaras Hindu University,
Varanasi, 221 005 India
E-mail: pbsasha2@sify.com

Usha
Department of Pathology, Institute of Medical Sciences,
Banaras Hindu University,
Varanasi, 221 005 India

## Materials and methods

At our university hospital, all of the new and follow-up patients for bladder tumor from March 1999 until February 2001 were studied. The patients were evaluated with urine cytology, ultrasonography and cystoscopy. CT scans were performed if needed. Blood samples were taken for p53 estimation at the time of preliminary evaluation. Age and sex matched normal

healthy volunteers were used as controls. A total of 5 ml of blood was drawn from both the patients and controls; serum was separated and stored in the refrigerator at 2–8°C. Patients with a superficial tumor were treated with transurethral resection (TURBT) with or without intravesical immunotherapy. Patients were checked at regular intervals with cystoscopy and urine cytology. Invasive tumors were treated with either radical cystectomy or radiotherapy.

The serum p53 protein level was measured with a solid phase sandwich ELISA kit (diaclone). A monoclonal antibody specific for p53 had been coated on the wells of microtitre strips provided. Samples, including standards of known p53 concentrations and unknowns, were pipetted into these wells. During the first incubation, the p53 antigen was added to the wells. After washing, biotinylated monoclonal antibody specific for p53 was incubated before the enzyme (streptavidin-peroxydase) was added. After incubation and washing to remove all unbound enzyme, a substrate solution that acts on the bound enzyme was added to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of p53 present in the samples. The assay recognizes both natural and recombinant p53. Statistical analysis was done with the non-parametric Mann-Whitney U-test, Student's *t*-test and the Fischer exact test.

## Results

During the study, 40 patients with carcinoma of the bladder and 15 controls were studied. Out of 40 cases of bladder carcinoma, 23 were superficial and 17 were invasive The duration of follow-up ranged between 3 months and 2 years .The majority of patients with bladder cancer were in their sixth or seventh decades. The youngest patient was 25 years old and the oldest patient was 84. The median age for control, superficial and invasive tumors was 54.0, 57.5 and 59.0 years, respectively. The male:female ratio was 7:1 for bladder cancer patients and 6.5:1 for controls. The superficial group consisted of four patients with grade 1, 11 patients with grade 2 and two with grade 3 tumors. In the invasive group, 18 patients had grade 2 and five had grade 3 disease (Table 1).

Fifteen patients with superficial disease underwent TURBT, and 12 received intravesical immunotherapy. For muscle invasive carcinoma, radical cystectomy was performed on seven patients, 14 patients received radiotherapy and two patients were lost to follow-up after staging TURBT. After definitive treatment, recurrence was found in seven patients with superficial carcinoma and in three with invasive disease.

The median serum p53 protein concentration in the control group was $16.4 \pm 5.57$ U/ml, whereas it was $31.5 \pm 23.06$ U/ml in the superficial group and $41.0 \pm 29.93$ U/ml in the invasive group (Fig. 1). p53 in patients with bladder carcinoma was significantly higher than in the controls ($Z = 4.28$, $P < 0.001$). Mean serum p53 correlated well with the grade of tumor ($P < 0.01$), but the difference between superficial and invasive tumors was not significant. In patients with superficial disease who relapsed after definitive treatment, the mean serum p53 level was higher (50.0 U/ml) than in patients who did not have recurrence during the period of study (19.0 U/ml).

A value of two standard deviations above the mean serum protein concentration (28 U/ml) of the control group was taken as the cut off value for positive serum p53 protein. A total of 28 (70.0%) patients with carcinoma, including 52.9% with superficial and 82.6% with invasive disease, were positive for serum p53 protein whereas none of the control group had a serum p53 above 28 U/ml (Table 1). The incidence of positive serum p53 is significantly higher in patients with cancer than in controls ($P < 0.001$) and invasive than superficial tumors ($P < 0.05$). Patients with superficial recurrent tumors also had a significantly higher incidence of positive serum p53 ($P < 0.05$).

**Table 1** Median serum p53 concentration and positive serum p53 in different groups

| Groups (*n*) | Median age (years) | Male:female ratio | Serum p53 (U/ml) | | No of patients having serum p53 >28 U/ml |
|---|---|---|---|---|---|
| | | | Median | SD | |
| Controls (15) | 54.0 | 6.5:1 | 16.4 | 5.57 | |
| Carcinoma (40) | 58.5 | 7:1 | 40.25 | 27.77 | 28 (70.0%) |
| Superficial (17) | 57.5 | 7.5:1 | 31.5 | 23.06 | 9 (52.9%) |
| Grade 1 (4) | | | 17.0 | 6.18 | 0 |
| Grade 2 (11) | | | 39.5 | 20.98 | 7 |
| Grade 3 (2) | | | 78.25 | 26.51 | 2 |
| Muscle Invasive (23) | 59.0 | 6.6:1 | 41 | 29.93 | 19 (82.6%) |
| Grade 2 (18) | | | 39.25 | 27.39 | 15 |
| Grade3 (5) | | | 86.0 | 35.60 | 4 |
| Recurrent (10) | 55.5 | 9:1 | 57.25 | 30.75 | 8 (80%) |
| Superficial (7) | | | 50.0 | 24.01 | 6 |
| Invasive (3) | | | 99.0 | 43.59 | 2 |
| Non-recurrent (30) | 59.0 | 6.5:1 | 36.0 | 25.92 | 20(66.6%) |
| Superficial (10) | | | 19.0 | 17.34 | 3 |
| Invasive (20) | | | 41.0 | 27.20 | 17 |

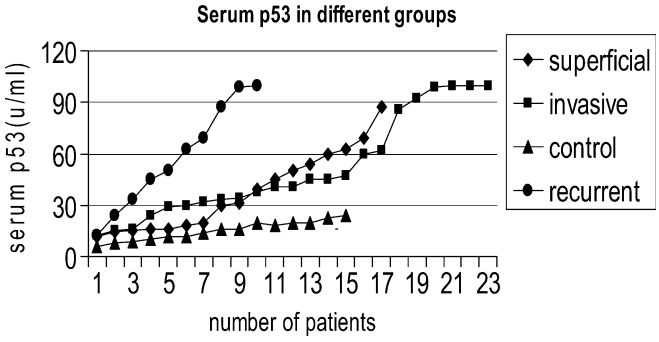


**Fig. 1** Mean serum p53 in the different study groups

## Discussion

The p53 tumor suppressor gene alteration is frequently found in many cancers and leads to the nuclear accumulation of p53 protein. This can be detected by immunohistochemical staining since the altered p53 protein has a longer half-life than wild-type p53 [5]. It has been suggested that this intracellular accumulation also results in high levels of p53 protein in extracellular fluid such as serum. In patients with various malignancies, such as lung cancer [6], head and neck cancer [7], colon cancer [8], and pancreatic cancer [9], significantly higher levels of serum p53 protein than normal have been reported. It was suggested that the analysis of p53 antigen concentrations could detect p53 gene alterations, which could in turn be useful for the selection of treatment regimens. The mean levels in patients with newly diagnosed colon adenomas (0.44 ng/ml) and colon carcinomas (0.55 ng/ml) were significantly elevated compared to with normal colonoscopic examinations and no prior history of colon neoplasia (0.12 ng/ml) [8]. Suwa et al. showed that the mean serum concentration of p53 protein in adenocarcinoma of the pancreas was 0.27 ng/ml, which was significantly higher than in healthy blood donors (0.15 ng/ml) or in patients with chronic pancreatitis (0.15 ng/ml) [9]. Adopting an arbitrary cut off value for the serum p53 protein concentration of 0.37 ng/ml, which corresponded to a value 2SD above the mean of the healthy blood donors, positive serum p53 protein concentrations were found in 23 out of 104 (22.1%) patients with adenocarcinomas, 16 out of 47 (34.0%) patients with carcinomas with distant metastases, but only seven of 57 patients (12.3%) with carcinomas without metastases. In addition, immunohistochemical studies disclosed that the p53 protein was expressed in 28 out of 61 pancreatic adenocarcinomas (45.9%) and serum p53 protein concentrations in the positively immunostained cases were significantly higher than in the negatively immunostained cases (0.35 ng/ml vs 0.15 ng/ml). The mean preoperative serum concentration p53 protein in patients undergoing surgical treatment for head and neck squamous cell carcinoma was significantly higher than in the healthy controls (59.45 pg/ml vs 16.4 pg/ml) [7].

Although the immunohistochemical data on p53 protein accumulation are divergent, possibly because of the use of non-standardized techniques, its prognostic relevance in muscle-invasive bladder carcinoma is well documented [10]. Prevalence of over expression of tumor p53 by immunohistochemical staining has been reported in 29–78% of bladder cancer [11, 12, 13, 14]. Schmitz-Drager et al. in their review compared various studies and found considerable differences caused by technical variation, e.g. the selection of the antibody and the use of different cut-off values, study design and patient selection [15]. Morita et al. [16] reported 63% over expression of tumor p53 but the serum prevalence was 1% (cut off value 50 ng/ml), whereas prevalence has been reported up to 13% in lung cancer, 22.1% in pancreatic malignancy and 32% in colon cancer. In our studies, the median value of serum p53 in patients with bladder cancer was 40.25 U/ml, which is significantly higher than in the controls (16.4 U/ml), and on taking a cut-off value 2SD above the control mean (28 U/ml), 70.0% of patients with carcinoma had positive serum p53. During our study, seven of 17 patients with superficial disease recurred and the median level of serum p53 was significantly higher in this group. Although our study is small, its results clearly suggest that there is an immunological reaction to altered serum p53 protein producing serum p53 antibodies. Our results correlate with the prevalence rate of serum p53 antibodies reported in various cancer types, but is quiet contrary to the figures reported by Morita et al. in bladder cancer patients [16]. This difference can be due to the use of a p53 mutant selective ELISA kit in their study, or it could be because of differences in the gene mutations. Although we could not study its association with survival as our follow-up period was short, our study showed that the prevalence and level of serum p53 is significantly correlated with the grade and stage of disease and with recurrence, similarly to that reported in different tissue p53 immunohistochemical studies. Further large studies are required to demonstrate the relation between tissue p53 and the serum p53 protein and the mechanism of its leakage from tissue to serum.

## Conclusion

Serum p53 concentration was found to be higher in patients with carcinoma of the urinary bladder than in the controls. Patients with recurrent superficial disease had higher median levels of serum p53 than those with non-recurrent superficial disease. Although difference between the median values of serum p53 in the superficial and invasive groups was not significant, significantly higher number of patients with invasive disease had positive serum p53 than in the superficial group. Serum p53 estimation is easy to perform and can be done even before surgical intervention, which is necessary for tissue p53 estimation, hence it can be used as one of the tumor markers for bladder cancer.

## References


1. Brandau S, Bohle A (2001) Bladder cancer. I. Molecular and genetic basis of carcinogenesis. Eur Urol 39: 491
2. Dahse R, Utting M, Werner W, Schimmel B, Claussen U, Junker K (2002) TP53 alterations as a potential diagnostic marker in superficial bladder carcinoma and in patients serum, plasma and urine samples. Int J Oncol 20: 107
3. Krajewska B, Lutz W, Pilacik B (1998) Determination of blood serum oncoprotein NEU and antioncoprotein p53—molecular biomarkers in various types of occupational exposure. Int J Occup Med Environ Health 11: 343
4. Schmitz-Drager BJ, Goebell PJ, Ebert T, Fradet Y (2000) P53 immunohistochemistry as a prognostic marker in bladder cancer. Playground for urology scientists? Eur Urol 38: 691
5. Finlay CA, Hinds PW, Tan TH (1988) Activating mutations for the transformation by p53 produce a gene product that forms an hsc-p53 complex with an altered half life. Mol Cell Biol 8: 531
6. Fontanini G, Fiore L, Bigini D, Vignati S, Calvo S, Mussi A, Lucchi M, Anggeletti CA, Merlo GR, Basolo F (1994) Levels of p53 antigen in the serum of non small cell lung cancer patients correlate with positive p53 immunohistochemistry on tumor sections, tumor necrosis and nodal involvement. Int J Oncol 5: 553
7. Chow V, Yuen APW, Lam KY, Ho WK, Wei WI (2001) Prognostic significance of serum p53 protein and p53 antibody in patients with surgical treatment for head and neck squamous cell carcinoma. Head Neck 23: 286
8. Luo JC, Neugut AI, Garbowski G, Forde KA, Treat M, Smith S, Carney WP, Brandt-Rauf PW (1995) Levels of p53 antigen in the plasma of patients with adenomas and carcinomas of the colon. Cancer Lett 91: 235
9. Suwa H, Ohshio G, Okada N, Wang Z, Fukumoto M, Imamura T, Imamura M (1997) Clinical significance of serum p53 antigen in patients with pancreatic carcinomas. Gut 40: 647
10. Friedrich MG, Riethdorf S, Erbersdobler A, Tiemer C, Schwaibold H, Solter JK, Huland E, Riethdorf L, Conrad S, Hammerer PG, Huland H (2001) Relevance of p53 gene alterations for tumor recurrence in patients with superficial transitional cell carcinoma of the bladder. Eur Urol 39: 159
11. Lipponen PK (1993) Overexpression of p53 nuclear oncoproteins in transitional cell bladder cancer and its prognostic value. Int J Cancer 53: 365
12. Esrig D, Elmajian D, Groshen S, Freman JA, Stein JP, Chen SC, Nichols PW, Skinner DJ, Jones PA, Cote RJ (1994) Accumulation of nuclear p53 and tumor progression in bladder cancer. N Eng J Med 331: 1259
13. Jahnsons S, Risberg B, KarlssonMG, Westman G, Bergstorm R, Pedersen J (1995) p53 and Rb immunostaining in locally advanced bladder cancer: relation to prognostic variables and predictive value for the local response to radical radiotherapy. Eur Urol 28: 135
14. Lu ML, Wikman F, Orntoft TF, Charytonowicz E, Rabbani F, Zhang Z, Dalbagni G, Pohar KS, Yu G, Cordon-Cardo C (2002) Impact of alterations affecting the p53 pathway in bladder cancer on clinical outcome, assessed by conventional and array-based methods. Clin Cancer Res 8: 171
15. Schmitz-Drager BJ, Goebell PJ, Ebert T, Fradet Y (2000) p53 immunohistochemistry as a prognostic marker in bladder cancer. Playground for urology scientists? Eur Urol 38: 691
16. Morita T, Tachikawa N, Kumamaru T, Nukui A, Ikeda H, Suzuki K, Tokue A (2000) Serum anti-p53 antibodies and p53 protein status in the sera and tumors from bladder. Cancer patients. Eur Urol 37: 79