A study of p53 expression in transitional cell carcinoma of urinary bladder in Erbil governorate

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Abstract

Background and objectives: This study aimed to evaluate p53 protein expression in both normal bladder epithelium and in cases of transitional cell carcinoma (TCC) of urinary bladder by immunohistochemical study and to correlate p53 expression in urothelial cancer with other clinico-pathological parameters.

Methods: This is a retrospective and prospective study for sample collection during the period from January 2006-May 2009. The samples studied included 105 formalin fixed, paraffin embedded urinary bladder tissue specimens; they consisted of the following diagnostic categories: chronic non specific cystitis (n=5) and urothelial cancer (n=100). In this study the nuclear p53 protein expression was detected in tissue samples by Dako Cytomation. LSAB + System-HRP staining protocol using monoclonal mouse anti human protein DO-7.

Results: None of the chronic non specific cystitis cases showed p53 nuclear immunostaining, while 93% of urothelial cancer specimens examined showed immunopositivity for p53 protein. In this study, a statistically significant correlation was observed between p53 overexpression rate with the tumor grade (p= <0.001) and histological architecture (p= 0.023), but not with other clinico-pathological parameters like age and gender.

Conclusion: Results of the present study showed the validity and simplicity of application of immunohistochemistry in determining the status of p53 protein expression. The results suggest that p53 overexpression is strongly associated with the aggressiveness of urothelial cancer.

Keywords: : Urothelial carcinoma of urinary bladder,P53

Introduction

Urinary bladder cancer is a common disease worldwide. At any point in time 2.7 million people have a history of urinary bladder (UB) cancer; the incidence of UB cancer varies around the world with highest rates in developed communities; but the incidence of UB cancer is increasing in the less developed areas of the world; this increase is a result of global changes in exposure to risk factors for UB cancer and growth of the world population; UB cancer ranks the ninth in cancer incidence.[1] It has been estimated that there is more than 60,000 new cases and more than 14,000 deaths from UB cancer in the United States[2] in 2008. Results of the Iraqi cancer registry in the years 1999-2001 showed UB cancer to be the third most common type of cancer.[3] Approximately 95% of malignant bladder tumors are urothelial cell carcinoma which can be classified as papillary, solid or carcinoma in situ. Papillary lesions are the most common and usually arise in hyperplastic urothelium whereas invasive tumors arise from dysplastic urothelium.[4] Classically bladder cancer has been associated with exogenous and environmental risk factors. The two best known risk factors for bladder cancer are smoking and occupational exposure. Deletion of chromosome 9 is the most common type of chromosomal abnormality in TCC and is

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found in more than 50% of all grades and stages of TCC. [5] TCC as any cancer is thought to develop from genetic changes which affect cell growth and proliferation. Two such genes which can regulate the cell cycle are tumor suppressor genes and proto-oncogenes; the p53 gene located on the short arm of chromosome 17 is one of the most important tumor suppressor genes; this gene is expressed in most cells and important for several reasons.[6] It plays a vital role in apoptosis, cell cycle regulation and DNA repair, it is also involved in cell cycle stability and prognosis of the disease. [7] p53 gene encodes for p53 protein , which increases the time necessary for DNA repair by slowing down the cell cycle at the G1-S transition, and suppresses tumor growth by causing apoptosis. Alteration or inactivation of p53 by mutation can allow a cell to escape from normal into uncontrolled growth leading to cancer development.[8,9]There is a vast amount of evidence to suggest that the dysregulation of p53 is critical in the development of TCC.[10]

Methods

In the current study the materials used were consisted of 105 formalin-fixed, paraffin-embedded urinary bladder biopsy specimens, (70) samples collected from histopathological department of Rizgary Teaching Hospital and(35) samples collected from some major histopathological private laboratories in Erbil city all samples were investigated in Rezgary teaching hospital, during the period from January 2006-May 2009. The cases of urinary bladder biopsy specimens were consisted of 100 cases of previously diagnosed TCC and 5 cases of chronic non specific cystitis. The TCC specimens include 8 cases of total cystectomy and 92 cases of TURBT biopsies. Information about gender, age, type of specimen, and original report were collected from the records of histopathological department in Rizgary teaching hospital and private laboratories.

The most representative tissue block was chosen from each case and new sections were made and stained with (H&E) for histopathological evaluation and additional 4 µm sections were made for immunohistochemical staining by Dako Cytomation. LSAB + System-HRP, Code K0679 .

Results

A total of 100 cases of Transitional cell carcinoma of urinary bladder were included in this study.The age distribution of different cases studied is shown in Figure (1), the mean age (±SD) for cases of TCC was (63.2±10.81) years. Out of 100 cases of TCC, the majority of cases (36%) ranged between 60-69 years, and only 1 case (1%) was < 30 years old, while only 2 cases (2%) were>80years. The clinicopathological results for the cases studied were as follows; regarding age, most of the cases were ≥ 60 years old comprising 71%. Regarding gender, most of cases were common among male gender comprising 78% with a male: female ratio equal to 3.5:1. Regarding histological architecture papillary types were slightly more common than solid type forming 58% and 42% respectively. Regarding grading parameter, grade II was the most common comprising 43% followed by grade III comprising 39%. The clinico-pathological characteristics of urothelial cancer cases are shown in Table (1).

Results of p53 immunohistochemical staining:

The expression of p53 immunostaining were divided into 4 groups: group 1 (negative) when there were no p53 staining of tumor cells and there were only 5 cases (5%), group 2 when there were 1-20% of cells with p53 nuclear staining and the result were only 2 cases (2%), group 3 when there were 21-50% nuclear staining of the tumor cells and there were 23 case (23%), group 4 when there were > 50% nuclear staining of the tumor cells and there were 70 case (70%). There were 5 cases of chronic non specific cystitis used as control

for the normal transitional epithelium and the results of p53 nuclear expression were completely negative for all of them Table (2):The correlation between p53 expression and age at diagnosis of TCC is shown in Table (3). Group 4 in which p53 expression was >50%, was the most common group in both age groups (<60 and ≥60) years, comprising 22 (75.9%) and 48 (67.6%) of cases respectively as Table (3).There was no significant correlation between ages of patient with p53 expression in TCC. (x^2=2.428, p=0.488).The correlation of p53 expression with the gender of cases of TCC is shown in Table (3).The p53 expression was higher in group 4 (>50%) in most of the cases for male patients as well as female patients comprising 66.6% and 81.8% respectively .There was no statistically significant correlation between gender of patient and p53 expression in transitional cell carcinoma of urinary bladder (x^2=2.214, p=0.529).The correlation of p53 expression with histological architecture of TCC of urinary bladder is shown in Table (4).Group4 in which p53 expression was >50%, solid histological architecture showed higher p53 expression (78.6%) than papillary type (63.8%).There was a statistically significant correlation between p53 expression and histological architecture of cases of TCC (X^2=9.494, P=0.023).The correlation of p53 expression with grading of TCC of urinary bladder is shown in Table (5). The p53 expression was increasing with increasing grade of tumor in group 4 (33.3% in grade I, 65.1% in grade II and 92.3% in grade III). There was statistically a highly significant correlation between p53 expression and grading of cases of TCC (x^2=24.927, p value < 0.001).

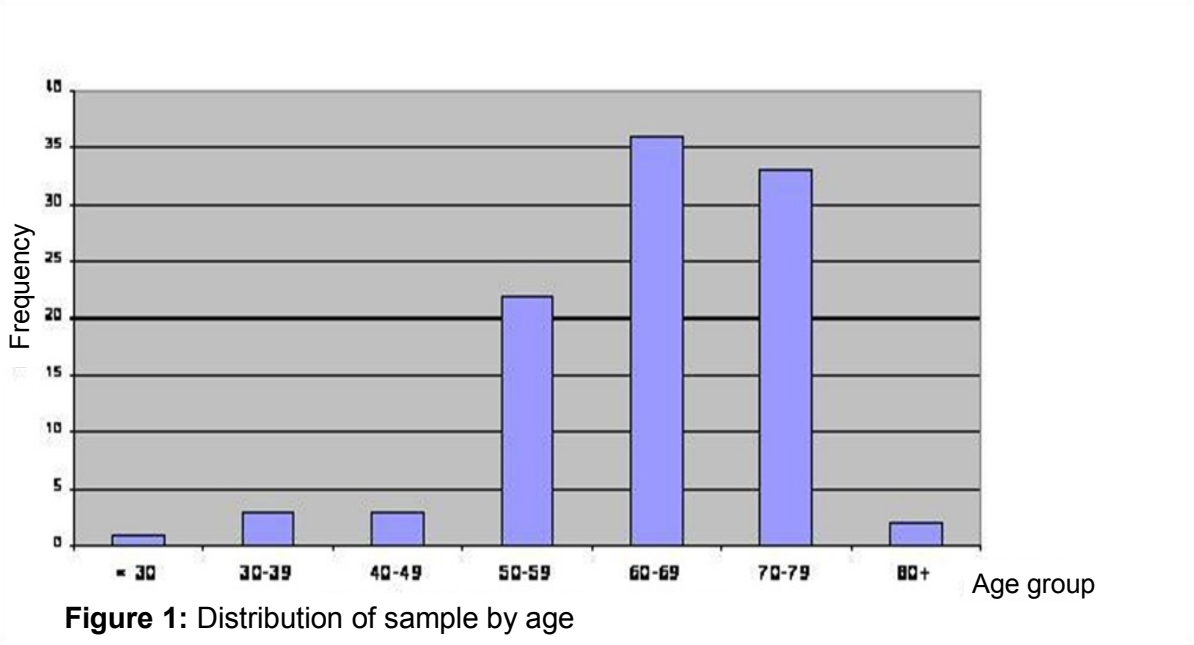

Figure 1: Distribution of sample by age

Table 1: Clinicopatholgical characteristics of urothelial cancer cases

Variables	Categories	Frequency	Percentage
Age	<60	29	29%
	≥60	71	71%
Gender	Male	78	78%
	Female	22	22%
Histological type	Papillary	58	58%
	Solid	42	42%
WHO Grade	I	18	18%
	II	43	43%
	III	39	39%

Table 2: Frequency of p53 scoring groups of TCC cases.

P53 score	Frequency	Percentage %
Negative	5	5
1-20	2	2
21-50	23	23
>50	70	70
Total	100	100

Table3 : Correlation of p53 scoring with age and gender

P53 score	Age		Gender	
	<60	**≥60**	**Male**	**Female**
Negative	2(6.9%)	3(4.2%)	4(5.1%)	1(4.5%)
1-20	1(3.4%)	1(1.4%)	2(2.6%)	0(0%)
21-50	4(13.8%)	19(26.8%)	20(25.6%)	3(13.6%)
>50	22(75.9%)	48(67.6%)	52(66.7%)	18(81.8%)
Total	29(100%)	71(100%)	78(100%)	22(100%)

Table 4: Correlation of p53 scoring with histological architecture of TCC cases

P53 score	Architecture		Total
	Papillary	Solid	
Negative	1(1.7%)	4(9.5%)	5(5%)
1-20	1(1.7%)	1(2.4%)	2(2%)
21-50	19(32.8%)	4(9.5%)	23((23%)
>50	37(63.8%)	33(78.6%)	70(70%)
Total	58(100%)	42(100%)	100(100%)

Table 5: Correlation of p53 scoring with grading in TCC

P53 score	Grade I	Grade II	Grade III	Total
Negative	2(11.1%)	3(7%)	0(0%)	5(5%)
1-20	0(0%)	2(4.7%)	0(0%)	2(2%)
21-50	10(55.6%)	10(23.3%)	3(7.7%)	23(23%)
>50	6(33.3%)	28(65.1%)	36(92.3%)	70(70%)
Total	18(100%)	43(100%)	39(100%)	100(100%)

Discussion

Urinary bladder cancer is a common disease worldwide. The incidence of UB cancer varies over the world with the highest rates in developed communities. But the burden of UB carcinoma is increasing in less developed areas of the world. These changes can be attributed to global changes in exposure to risk factors for UB cancer and growth of the world population.[11] UB carcinoma incidence increased as well as other types of cancers in certain parts of Iraq after exposure to high levels of depleted uranium after the two gulf wars.[12]The wild-type p53 protein has a short half-life of 15 to 30 minutes. However, missense p53 gene mutations result in a protein with a prolonged half-life, which is the basis of its nuclear accumulation that is detectable by IHC.[13] Nuclear accumulation of the p53 protein in bladder cancer has been associated with mutations in the gene, although substantial discordance has been demonstrated between the altered p53 protein status.[14,15] Nuclear accumulation of p53 protein is associated with a poor clinical outcome in invasive bladder carcinoma.[16-18] In this study, the age range of patients with TCC was 33-90 years; with a mean age of 63.2 years (Figure 1). In Turkey,[19] found a comparable result with a mean age of 63 years and age range of 34-87 years. In Jordan,[20] also found a comparable result to this study with a mean age 60.6 years mean age and age range 19-91 years . In USA,[21] recorded a mean age of 69 years with age interval of 36-96 years. In another study in USA,[22] found a mean age with 65.3 years and 35-84 years as age range. In Japan,[23] study showed 61.7 years as mean age. Regarding gender, in the current study the male to female ratio was (3.5:1). Other studies showed different values of male to female ratio for example [21], showed

(4:1),[19], showed (5.4:1),[22], showed (1.38:1) and [20], showed (10:1).Five morphologically normal bladder mucosa specimens were tested for p53 nuclear staining, all of them showed absence of nuclear staining in urothelial and stromal cells. Similarly, none of the normal mesenchymal cells in all 100 cases of TCC analyzed showed detectable p53 nuclear reactivity. These findings are consistent with the well-documented short half-life of the wild-type p53 protein, which is normally present at very low steady-state levels and is thus undetectable immunohistochemically.The same negative nuclear staining for normal benign urothelial cells were showed by Sarkis et al.[17]In this study over expression of p53 protein has been demonstrated in approximately 93% of cases of TCC of urinary bladder. In this study, p53 expression was correlated with other clinico-pathological prognostic parameters, like age, gender, histological architecture and tumor grade. p53 nuclear over expression was not correlated with age Table (3). This result was in agreement with results obtained by Sarkis et al[17] from USA and Kwak et al[24] from Korea. While Sinik et al[25] from Turkey showed that p53 over expression was statistically significant with age of patients. The correlation between p53 overexpression with gender was statistically non significant (Table 3). This result was in agreement with Sarkis et al[17] from USA and Kwak et al[24] from Korea. Oppositely Sinik et al[25] from Turkey, found statistically significant correlation of p53 over expression with gender. Also p53 overexpression was higher in female gender ($p< 0.001$) by a study done by Halimi et al[26] in Iran. There was a strong correlation between p53 over expression and grades of TCC of urinary bladder in the cases studied and the result was statistically highly significant ($p < 0.001$). this finding was comparable with studies done by : Halimi et al[26]. While Kwak et al[24] found that there was no significant correlation between p53 nuclear staining and grade of tumors. P53 protein is considered as a prognostic marker that might reflect the . potent of aggressive malignancy, and poor prognosis, the result of current study confirmed it by demonstrating a higher rate of p53 protein-positive staining in high grade TCC of urinary bladder. There was statistically no significant correlation between p53 expression and tumor staging in this study ($p =0.228$). All studies done by others such asHemal et al[27], were in agreement with our results. Whereas other studies such as Kwak et al[24] disagreed with our result. Regarding architectural types of TCC of urinary bladder, in this study there was a statistically significant correlation between p53 over expression and architectural types of TCC especially solid type ($P = 0.023$) which may be do you to the fact that solid type tumor of TCC is more aggressive than papillary type. This result was in agreement with a study done by Lipponen [28], while ($p = 0.35$) reported by Sarkis et al[17] in USA, similarly reported that abnormalities in p53 are not related to the histological architecture of bladder cancer.

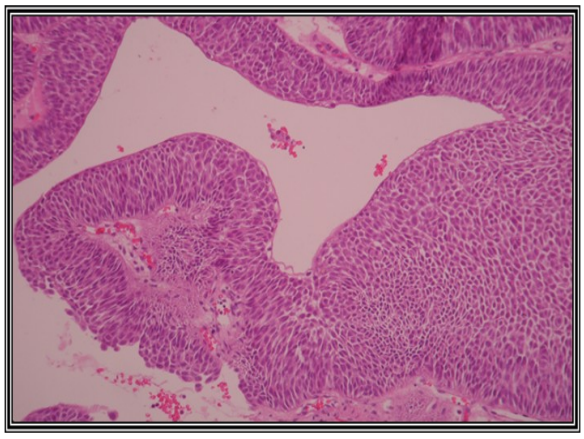

Figure 2:TCC, Grade II, H&E, x200

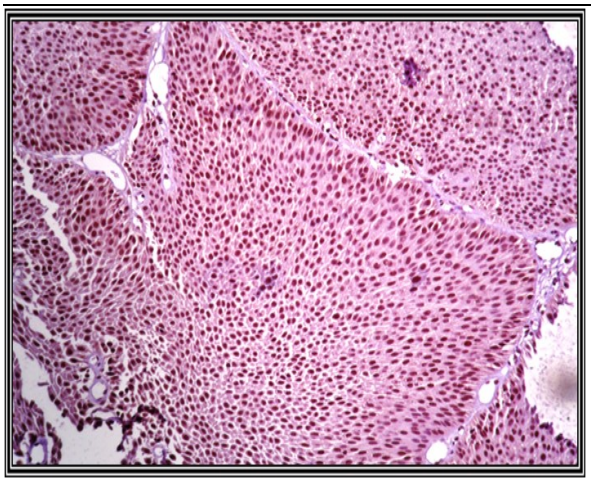

Figure 3:TCC, Grade II showing nuclear p53 immunostaining. (P53 IHC staining x200)

References

1. Ploeg M, Aben K, and Kiemeney L The present and future burden of urinary bladder cancer in the world. World J Urol 2009; 27:289-293.
2. Nieder A, Mackinnon J, Fleming L, Kearney J, Hu J, Sherman R *et al* Bladder cluster in Florida: Identifying population at risk. The Journal of Urology 2009; 182:46-51.
3. Results of Iraqi Cancer Registry Iraqi Cancer Board: Baghdad, Iraq 2001.
4. Luis N, Knowles-L E, Real F Molecular biology of bladder cancer. Clin Transl Oncol 2007; 9:5-12.
5. Pasin E, Josephson D, Mitra A, Cote R, and Stein J Superficial bladder cancer: An update on etiology, molecular development, classification, and natural history. Rev Urol 2008 ;10(1):31-43.
6. Fernando SE, Wu X, Peersa LS P53 over expression receptor and steroid hormone status in endometrial carcinoma. Int J Surg Pathol 2000; 8:213-222.
7. Etebary M, Jahanazad I, Mohagheghi MA, Azizi E Immunohistochemical analysis of p53 and its correlation to the other prognostic factors in breast cancers. Acta Medica Iranica 2002); 40(2): 88-94.
8. Schlemper RJ, Riddel RH, Kato Y, Borchard F, Cooper H S, Dawsey SM *et al* The Vienna Classification gastrointestinal epithelial neoplasia; GUT 2000; 47: 251-255.
9. Kamaran A and Pirim I Predictor of Progression in Gastric Carcinoma. The Internet Journal of Genomics and Proteomics; 3(1): (2007); 1540-2630
10. Bai L and Zhu WG p53: Structure, Function and Therputic Applications; 2006; 2(4):141-153.
11. Martine Ploeg, Katija K. H. Aben, Lambertus A. Kiemeney The present and future burden of urinary bladder cancer in the world. World J Urol 2009; 27:289-293.
12. Al-Azzawi SN. Depleted uranium radioactive contamination in Iraq: an overview. Global Research. Vol 1. August 2006:4.20-25
13. George B, Datar R, Wu L, Cai J, Patten N, Beil S *et al* P53 gene and protein status: The role of p53 alteration in predicting outcome in patients with bladder cancer. J Clin Oncol 2007; 25: 5352 -5358.
14. Ersig D, Spruck C, Nichols P, Chaiwun B, Steven K, Groshen N *et al* p53 nuclear protein accumulation correlates with mutations in the p53 gene, tumor grade, and stage in bladder cancer. American Journal of Pathology 1993; 143:1389-1397.
15. Lu M, Wikman T, Orntoft T, Charytonowicz E, Rabbani F, Zhang *et al* Impact of alteration affecting the p53 pathway in bladder cancer of clinical outcome, assessed by conventional and Array-based methods. Clinical Cancer Research; 2002; 8: 171-179.
16. Ersig D, Elmajian D, Groshen S, Freeman J, Stein J, Chen S *et al* Accumulatiion of nuclear p53 and tumor progression in bladder cancer. The NEW ENGLAND JOURNAL of MEDICINE 1994; 331:1259-1264.
17. Sarkis A, Dalbagni G, Cordon-Cardo C, Zhang Z, Shienfeld J, Fair W *et al* Nuclear over expression of p53 protein in transitional cell bladder carcinoma: A marker for disease progression. J Natl Cancer Ins 1993; 85:53-59
18. Malats N, Bustos A, Nascimento C, Fernandez F, Rivas M, Puente D *et al* P53 as prognostic marker for bladder cancer. Lancet Oncol 2005;6: 678-686.
19. Emrbağci A, Yağci F, Sarica K, Karkök M, and Erturhan S. Predictive value of p53 and NAT2 enzyme for disease free survival in patients with superficial bladder cancer. Turkish Journal of Cancer 2002; vol 32/ no. 3.
20. Matalka I, Bani-Hani K, Shotar A, Bani Hani O, and Bani-Hani I Transitional cell carcinoma of the urinary bladder: a clinicopathological study. Singapore Med J 2008; 49 (10): 790.
21. Cheng L, Neumann R, Nehra A, Spotts B, Weaver A, and Bostwick D Cancer heterogeneity and its biologic implications in the grading of urothelial carcinoma. American Cancer Society 2000; 88:1663-1670.
22. Boudreaux K, Clark P, Lowrance W, Rumohr J, Barocas D, Cooksoon M *et al* Comparrision of American Joint Committee on Cancer Pathological Stage T2a Versus T2b Urothelial Carcinoma:Analysis of Patient Outcomes in Organ Confined Bladder Cancer. The Journal of Urology 2009;181(2):540-546.
23. Oh B, Sim J, Park CH, Ruy S, and Park Y Prognostic value of expression of mutant p53 and EGFR in transitional cell carcinoma of the bladder. J Korean Cancer assoc 1997; 29: 663-672.

24. Kwak D, Ha J, Chang H, Choi M, Park Ch, and Kim Ch Clinical implications of the expression of Survivin and p53 in superficial transitional cell carcinoma of the bladder, Korean J Urol 2009; 50: 12-17.
25. Sinik Z, Alkibay T, Ataoglu Ö, Biri H, Sözen S, Deniz N *et al* (Nuclear p53 overexpression in bladder, prostate, and renal carcinomas. Int J Urol 1997; 546-551.
26. Halimi M, Salehi A, Baybordi H, and Nezami N Immunohistochemical positive stained p53 protein in bladder transitional cell carcinoma. Indian Journal of Pathology and Microbiology 2009; 52 (2).
27. Hemal A, Khaitan A, Dinda A, Gupta P, Seth A, Dogra P *et al* Prognostic significance of p53 nuclear overexpression in patients of muscle invasive urinary bladder carcinoma treated with cystectomy. Urol Int 2003; 70: 42-46.
28. Lipponen PK Overexpression of p53 nuclear oncoprotein in transitional cell bladder cancer and its prognostic value. Int J Cancer 1993; 53: 365-370.