Immunohistochemical Expression of p53 Biomarker in Urinary Bladder Urothelial Carcinoma among Sudanese Patients

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Abstract

Background: Urothelial (transitional cell) bladder carcinoma UBC is the most common malignancy of the urinary tract and the seventh leading cancer in men worldwide. Mutations in P53 gene are the most common genetic abnormality in human cancer. Mutation of P53 gene results P53 protein accumulation in cells' nuclei. This accumulation is detectable with Immunohistochemical methods and correlates with P53 gene mutation. **Aims:** The study aimed to detect the expression of p53 in Urothelium cell carcinoma of urinary bladder using Immunohistochemical method, and correlate the expression of p53 with the gender, age of patient and histological grade. **Methods:** Forty paraffin embedded blocks previously diagnosed as transitional cell carcinoma were collected. This retrospective analytical study conducted at, Elalim Medical Laboratory Center and histopathology and cytology lab of ElRibat University Hospital, Soba University Hospital during the period from September 2015 to December 2016. **Result:** Immunohistochemical expression of p53 was revealed positive result of expression of p53 in 7 (17.5%) of high grade sample, and negative in 15 (37.5%) of them. While 4 (10%) of low grade sample show positive expression of p53 14 (35%) of sample show negative expression. 27 (67.5%) of patients were males, 5 (12.5%) of them were positive for p53 expression and 22 (55%) of them represent negative of p53 expression and 13 (32.5%) patients were female 6 (15%) of them were positive for p53. Patient from 15 to 35 years were 2 (5%) patients, 2 (5%) were negative expression of p53, from 36 to 55 years were 11 (27.5%) patients, 5 (12.5%) were positive expression of p53 and 6 (15%) negative from 56 to 75 years were 23 (57.5%) patient, 5 (12.5%) were positive expression of p53, 18 (45%) negative from 76 to 95 years were 4 (10%) patients, 1 (2.5%) were positive expression of p53, 3 (7.5%) were negative expression of p53. **Conclusion:** the correlation between the p53 expression and histological grades of the tumor show insignificant statistical association (p value=0.449). The correlation between the expression of the p53 with gender showed insignificant statically association (p value=0.067). The correlation between the expression of the p53 with age showed insignificant statically association (p value=0.402).

Keywords

Urothelial bladder carcinoma UBC, P53 gene Mutation, paraffin embedded blocks

1. Introduction

Bladder cancer is one of several types of cancer arising from the tissues of the urinary bladder [1], and it is the most common urinary site of malignancy and the second most common reason of cancer deaths from the genitourinary system.

urinary tract after prostate cancer in the United States, with 81,400 new cases and 17,980 deaths in the year 2020 [2]. Globally there are about 430,000 new cases diagnosed each year [3]. Bladder cancer is tumor of old adults (>60) [4].

Bladder cancer represents an important health problem with an age-standardized, incidence rate (per 100,000 person-years) of 9 in men versus 2.2 in woman and an age-standardized mortality rate (per 100,000 person-years) of 3.2 and 0.9, respectively [5, 6, 7].

Tumor protein p53 (Tp53) is the common oncosuppression gene mutated in all human cancers [8].

Bladder carcinogenesis is closely associated with tumor suppressor dysfunction and the inactivation of TP53 [9]. Therefore, p53 has been studied as a marker of urothelial cell carcinoma recurrence and progression. The p53 protein is encoded by TP53 tumor-suppressor gene which located at 17p13.1 [9].

The aim of our study was designed to determine of Expression of p53 in urothelial carcinoma of bladder among Sudanese patients in Khartoum State by using immunohistochemical stain technique.

2. Materials and methods

This is analytical retrospective hospital based study, conducted in Khartoum State, including Elalim medical laboratory center, histopathology and cytology lab of Soba University Hospital and El Ribat University hospital, fourty samples of formalin fixed paraffin wax embedded blocks of patients with urinary bladder urothelial carcinoma were collected from storage room, Data obtained from patients files (sex, age and histological grade), from each block two sections were been cut (3u) by using rotary microtome. All sections were stained with Haematoxyline and eosin staining (H&E) and immunohistochemistry p53 biomarker. One section (3u) from formalin—fixed paraffin embedded tumor was cut and mounted onto glass slides than incubated in oven for 30 minutes. Following deparaffinization in xylene for 10 minutes, slides were rehydrated through graded series of alcohol and placed in distilled water. Samples were stained with haematoxylin (mayers) for 10 min, bluing in running tap water 10 minutes, then stained with eosin for 3 minutes, dehydrated cleared and mounted. The diagnosis and tumor histological grade were confirm by pathologist. Immunohistochemistry procedure was done as the follows; sections (3 micro miter) from formalin fixed paraffin embedded blocks were cut and mounted on to Stalinated slides (dako).

Following deparaffinization in xylene, slides were rehydrated through graded series of alcohol and were placed in running water. Sample were steamed for antigen retrieval for p53. Briefly, slides were placed in the PT Tank containing enough tris buffer (ph. 9) to cover the section, and then the machine was been turned on at 20 minutes to start heating from 65c until reach 95c and boiling at high temperature (95c) for 20 minutes. Sections allowed to cool to 65c and endogenous peroxidase activity was block with peroxidase blocking reagent (3% hydrogen peroxide and methanol) for 10minutes. The slides were incubated with (100-200 micro liter) of primary antibody for 20 minutes at room temperature in moisture chamber, and then rinse. In phosphate buffer saline, the primary antibody for p53 was concentrated, then 1 micro ml of primary antibody was diluted to 250 micro liter of antibody Diluents (dako, carpintera), after washed with PBS for 3minutes, binding of Antibodies was detected by incubating for 20 minutes with polydextran labeled polymer (Dako). Finally, the sections were washed in three changes of PBS, followed by Adding 3,3 diaminobenzidine tetra hydrochloride (DAB) (Dako) as a Chromogen to procedure the characteristic brown stain for the Visualization of the antibody/enzyme complex for up to 5 min. Slides were counter stained with haematoxylin for one minute, then dehydrated cleared and mounted. The samples were diagnosed for p53 expression by the pathologist, based on immunohistochemistry protocol for staining p53 antibody.

Statistical analysis:

The data were analyzed electronically by using computer programme SPSS version 16. Chi-Square test was used and considered statistically significant when the p-value ≤ 0.05 .

Ethical consideration

The study was performed after agreement to use tissue blocks from AL Elaim medical laboratory center, Soba university Hospital and El Ribat national hospital also after approval from the ethics committee of ALneelin University.

3. Results

Total of 40 samples of patients with urothelial Carcinoma were investigated in Elalim medical laboratory center and histopathology and cytology lab of soba University Hospital, ElRibat University Hospital. The age of study population ranged between (15-95) years with mean age 60.55 years. Patients from 15 to 35 years were 2 (5%) patients, 2 (5%) were negative expression of p53, from 36 to 55 years were 11 (27.5%) patients, 5 (12.5%) were

positive expression of p53 and 6 (15%) negative from 56 to 75 years were 23 (57.5%) patient, 5 (12.5%) were positive expression of p53, 18 (45%) negative from 76 to 95 years were 4 (10%) patients, 1 (2.5%) were positive expression of p53, 3 (7.5%) were negative expression of p53. The genders of study population represent that 27 (67.5%) of patients were males, 5 (12.5%) of them were positive for p53 expression and 22 (55%) of them represent negative of p53 expression and 13 (32.5%) patients were female 6 (15%) of them were positive for p53 expression and 7 (17.5%) of them represent negative of p53 expression. 22 (55%) of patients were high grade and 18 (45%) were low grade. p53 revealed positive expression in 7 (17.5%) of the high grade sample and 15 (37.5%) were negative for p53 expression, and the percentage of expression in low grade sample 4 (10%) were positive for p53 expression and 9 (22.5%) were negative for p53 expression.

The relation between the expression of p53 with patients age have urothelial cell carcinoma show insignificant statically association p-value (0.402) ($p > 0.05$) as indicated in Table 1. The relation between the expression of p53 with patient gender Show insignificant statically association p-value (0.067) ($p > 0.05$) as indicated in Table 2. The relation between the expression of p53 and histological grade show insignificant statically association p-value (0.449) ($p > 0.05$) as indicated in Table 3.

Table 1. Showed the Correlation between the P53Expression and the Age

		P53Expression		Total	
		Positive	Negative		
Age	15-35 years	Count	0	2	P value (0.402)
		% of Total	0%	5%	
	36-55 years	Count	5	6	
		% of Total	12.5%	15%	
	56-75 years	Count	5	18	
		% of Total	12.5%	45%	
	76-95 years	Count	1	3	
		% of Total	2.5%	7.5%	
	Total	Count	11	29	
		% of Total	77.5%	22.5%	

Table 2. Showed the Correlation between the P53Expressionand the Sex

		P53Expression		Total	
		positive	Negative		
Sex	Males	Count	5	22	P value (0.067)
		% of Total	12.5%	55%	
	Female	Count	6	7	
		% of Total	15%	17.5%	
	Total	Count	11	29	
		% of Total	27.5%	72.5%	

Table 3. Showed the Correlation between the P53Expression and the Grade

		P53Expression		Total	
		Positive	Negative		
Grade	High grade	Count	7	15	P value (0.449)
		% of Total	17.5%	37.5%	
	Low grade	Count	4	14	
		% of Total	10%	35%	
	Total	Count	31	9	
		% of Total	27.5%	72.5%	

4. Discussion

In this study of 40 sample of patients with bladder transitional cell carcinoma were investigated by immuno-chemical method 22 of them were high grade representing (55%) and the remaining 18 (45%) were low grade. Regarding to gender that males are more affected with urothelial cell carcinoma than females representing (67.5%) and (32.5%) respectively is agreed with male to female ratio found in this study is 2:1. This is consistent with result of study done in the United State [10]. They reported that bladder urothelial cell carcinoma more common among males than females 3:1 and this supported by study done in the United State [11]. The age of study population ranged between 15 years to 90 years with mean of 55 years patients less than 55 years were 13 (32.5%) and older than 55 years were 27 (61.5). This means that urothelial cell carcinoma of urinary bladder occurs in older age (65-75). This result is support by study done in India [12]. They reported that the commonest affected age of presentation of urothelial cell carcinoma of bladder is 50 to 70 years Bladder urothelial cell carcinoma revealed negative expression of p53 in 15 (37.5%) of high grade samples, and positive expression of p53 in 7 (17.5%) too. While 14 (35%) of low grade sample show negative expression of p53 and 4 (10%) show positive of p53 expression, concluded that there are no association between expression of p53 and grade of tumor show (p value=0.449), This result consist by Ibrahim et al. [13]. Others against reported significant correlation with higher grades [14, 15, 16, 17].

This study show that no correlation between the gender with expression of (p value=0.067). This result consist with study done in Chennai [18] and other against [19].

Statistically, there was no significant correlation between p53 expression and sex of the patients. Statistically, there was no significant correlation between p53 expression and the age of the patient's p-value (0.402). This is consist with the conclusions of [18] and other against [19].

5. Conclusion

Bladder urothelial cell carcinoma in male is more common than female and more common in above 56 years old, there is no correlation between expressions of p53 with grade and age and gender.

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