Correlation Between Tumor Suppressor Gene P53 And Bladder Cancer In Some Iraqi Patients

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Abstract:

Alterations of tumor suppressor gene p53 were identified in a various types of human cancers, including bladder cancer . So , the present study was designed to investigate the relationship between the tumor suppressor gene p53 in some Iraqi patients with bladder cancer by using two methods , insitu hybridization compare with immunohistochemical assay. A series of 40 bladder tissue cancer collected from different sites (transitional cell and squamous cell) from patients who had undergone cystactomy. These samples were collected during the period between (2012-2014). The histologic types included 21 well differentiated transitional cell , squamous cell, 9 moderately differentiated and ten poorly differentiated carcinomas. By using in situ hybridization , tumor suppressor gene p53 was detected in eighteen out of fourty (45%),otherwise, at immunohistochemistry , it was detected in twenty eight out of fourty (70%). Moreover, the positive results of tumor suppressor gene p53 was related in highly significant to each of stages and sites , significantly related with histologic grade of the tumor by using insitu hybridization method , but there was no significant correlation with each of age and smoking patients. furthermore , in immunohistochemical assay p53 related in highly significant correlation with each of histologic grade, sites, stages of the tumor and the smoking habit. So, it was significantly related with age and gender.

It could be concluded from this study that the two methods of in situ hybridization and immunohistochemistry for detection of tumor suppressor gene , protein p53 is useful in the clinical evaluation of patients with bladder cancer and it suggested that mutations of p53 are involved in the bladder transitional cell and squamous cell carcinomas and it may play an a major role in the malignant transformation of urinary tract cancer.

Keyword: Bladder cancer, Tumor suppressor gene p53, Insitu hybridization, Immunohistochemistry

Introduction:

Inactivation of tumor suppressor gene p53 activity during tumor development is a process of accumulation of its genetic abnormalities (1)

The most common mutations found in human cancers is mutation in the tumor suppressor gene p53 (2). Alteration of this gene or inactivation of wild type of p53 is thought to play a major role in multistep carcinogenesis (1).

More than 95% of these alterations are missense mutations which are confused the control part of this gene(3).

The mutant p53 is a common genetic abnormality in the bladder cancer(4) it has previously been shown that overexpression of p53 occurs in higher grades and higher stages of TCC(4,5). Inactivation of tumor suppressor gene pathway contribute to

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bladder tumor progression and to provide relevant prognostic information to help in the management patients with bladder cancer(6,1).

Worldwide, bladder cancer is the 5th most common cancer in men and 12th most frequent cancer in women , whereas, in developed countries it is 4th most common cancer in men and 9th in women (7).So, there are three kinds of bladder cancer, transitional cell carcinoma (TCC) its also called urothelial carcinoma (8,9),which is begins in urothelial cells that normally makeup the inner lining of the bladder (10). Approximately 90-95% of bladder tumors are transitional cell carcinoma. Squamous cell carcinoma its less frequent than urothelial carcinoma , it's a malignant neoplasm derived from bladder urothelium with pure squamous phenotype(11,12,13).And adenocarcinoma which is less frequent than transitional cell and squamous cell carcinoma.

Smoking is recognized as a major risk factor causing bladder cancer, it is account for 50% of tumors (14). Bladder cancer typically seen in the older patients , more than 90% of bladder

cases occur in patients older than 55 years of age.

The aim of this study was to compared between two techniques(In situ hybridization and Immunohistochemical) to determined the tumor suppressor gene P53 in bladder cancer patients and to evaluate which one is more benefit to use it for clinical evaluation with such patients.

Materials and Methods:

The current study involved of fourty patients with bladder cancer (mean age 62.6 years) ranged between (45-85) years ,with 20 men and 20 women. They were collected randomly from Teaching laboratories\ Medical city in Baghdad. Whom already diagnosed as a (transitional cell carcinoma and squamous cell carcinoma) of bladder cancer by specialist , compared with 10 apparently healthy control where their ages and sex were matched to patients group. P53 suppressor gene and p53 protein were determined in specimens using Insitu hybridization and Immunohistochemistry methods and performed as recommended in leaflet with kits.

- DNA probe Hybridization \ Detection System : Highly – Sensitivity In Situ kit. A complete hybridization and immuno-detection system were purchased from Maxim Biotech , USA . cat. No. (IH – 60001), (IHD – 00S0).

- Mouse anti-Human p53 (tumor suppressor protein, oncogene protein) from Us Biological code (P1001 – 32 C) for (ISH).

- Detection system for p53 protein :-

Universal Dakocytomation labeled streptavidin – Biotin 2 system , Horseradish peroxidase (LSAB – 2 system . HRP) ready to use detection system , code no. K0673 (CA.USA). For (IHC).

- Ready to use N- series primary antibody (Monoclonal mouse Anti- Human p53 protein clone : DO7 . code N 1581). Dako. Denmark.

Immunohistochemical assay:

 Paraffin embedded tissue blocks were sectioned in 5μm thikness by using microtome. All these specimens were deparaffinized and dehydrated. These sections were dewaxed in xylene,then a series of (100,90,70)alcohol and D.W respectively, placed in an endogenous peroxidase block for 25 min., added p53 tumor suppressor gene as a primary Ab in each of samples for 90 min., washing with PBS , secondary Ab was added and incubated for about 1 hr. in humid chamber, streptavidin for 30 min. counterstained by Mayer's hematoxyline , dehydration by using serial of ethanol(70,90,100)% and finally in xylene.

In Situ Hybridization technique

Three steps were involved for detection of p53 by (ISH)

- Prehybridization : tissue sections were cut in 5 μm , all of samples were deparaffinized and dewaxed in xylene, series of ethanol (100,90,70%) and D.W. respectively,then immersed in pre heated (98 Cº) citrate buffer (pH:6). Tissue deprotinization performed by placing it in proteinase K solution, dehydration were done by immersing the slides in D.W. then (70,90,100%) ethanol.

- Hybridization step : done by added p53 probe into each sections , denature the DNA probe by placed it in oven 98 Cº then removed from oven and incubated at room temperature for over night to allow hybridization of probe with target nucleic acid.

- Post hybridization step: protein block buffer using to fall off all the coverslip, conjugate were placed onto sections , substrate used , counterstained by Nuclear Fast Red (NFR), dehydration by 90,100% ethanol , finally with xylene and mounted with DPX.

Statistical analysis:

The ststistical analysis system – SAS (15) was used to effect of differences factors in study parameters. The chi-square χ2 test at the comparative between percentage in this study.

Results:

The series of resected specimens included 40 cases of bladder cancer , twenty five transitional cell carcinoma samples and fifteen case of squamous cell carcinoma were studied insituhybridization and immunohistochemically by using monoclonal antibody to detect p53 protein and DNA hybridization for detection p53 suppressor gene .

The patients ages ranged between (45-85) with mean age of 62.6 years with 20 for both men and women.

(A) Tumor suppressor gene p53 expression by using insitu hybridization in Iraqi patients with bladder cancer presents in 18 out of 40 (45%), however according to their age, gender, tumor stage, site of the tumor, histological differentiation and smoking habit have shown in table (1), there were highly significant association between p53 suppressor gene with bladder cancer patients site and the stage of the tumor (P=0.0038) and (P = 0.0148)respectively at (P≤ 0.01), whereas, there were a significant correlation of p53 with a grade of the tumor (P = 0.0269) at (P ≤ 0.05), otherwise, there were no significant association with age (P = 0.439), gender (P = 0.385) and smoking (P= 0.472) respectively.

(B) As shown in table (2) the p53 expression by using immunohistochemically in Iraqi patients with bladder cancer presents in 28 out of 40 (70%), so, according to their age , sex, histological grade and site , staging and smoking, there were highly significant correlation with histological grade (P=0.0083), tumor stage (P=0.0027), smoking habit (P=0.0133) and site of the tumor (P=0.0128), at (P≤0.01) and significantly association with both of age and gender (P=0.052), (P=0.0319)respectively at (P≤0.05).

The histological differentiation rate in well differentiated is 21 out of 40 for all those cases, the positive count for (ISH) was 11 out of 21 which was (52.3%), the moderate 6 out of 9 which was (66.6%)and poorly differentiated was 1 out of 10 .

By using (IHC) the positive count of the well, moderate ,poor differentiated were (15),(5) and (8)which was (71.4%),(55.5%) and (80%)respectively.

Table (1): Distribution patren of Iraqi patients with bladder carcinoma according to their age, tumor site, gender and tumor grade,smoking habit and stage of their leision in relation with p53 at insitu hybridization (ISH).

The factor	Number of P53 (ISH) positive Total No.= 18	(%) Patients P53(ISH) negative Total No.= 22	P – value And χ^2 - Value
Age			
60 $\geq$	(38.8%) 7	(50%)11	P = 0.439
60 <	(61.11%)11	(50%)11	χ^2=0.905 NS
Tumor site			
TCC	(44.4%)8	(77.2%)17	P = 0.0038
SCC	(5% .55)10	(22.7%)5	**χ2 = 11.362
Tumor Grade			
Well	(1% .61)11	(45.4%)10	P = 0.0269
Moderate	(3% .33)6	(13.6%)3	*χ2 = 5117
poor	(5% .5)1	(40.9%)9	
Gender			
Male	(5% .55)10	(45.4%)10	P = 0.385
Female	(44.4%)8	(54.5%)12	χ2 = 0.847 NS
Stage			
I II	(7% .77)14	(72.7%)16	P = 0.0148
III IV	(2% .22)4	(27.2%)6	**χ2 =8.533
Smoker	(50%)9	(50%)11	P = 0.472
Non smoker	(50%)9	(50%)11	χ2 =0.914 NS

*(p≤ 0.05), **(p≤ 0.01), NS: Non Significant

Table (2): Distribution of bladder carcinoma patients in relation with p53 protein in immunohistochemical (IHC) method according to their age, gender, site of tumor and grade, smoking habit and stage of the tumor.

The factor	Number of P53 (IHC) positive Total No.= 28	(%) Patients P53 (IHC) negative Total No.= 12	P – value And χ2 - Value
Age			
60 $\geq$	(42.8%)12	(50%)6	P = 0.052
60 <	(57.1%)16	(50%)6	*χ2 =4.924
Tumor site			
TCC	(57.1%)16	(75%)9	P = 0.0128
SCC	(42.8%)12	(25%)3	**χ2 = 9.844
Tumor Grade			
Well	(53.5%)15	(50%)6	P = 0.0083
Moderate	(17.8%)5	(33.3%)4	**χ^2 = 9.625
poor	(28.5%)8	(16.6%)2	
Gender			
Male	(53.5%)15	(41.6%)5	P = 0.0319
Female	(46.4%)13	(58.3%)7	*χ^2 = 5.208

The factor	Number of P53 (IHC) positive Total No.= 28	(%) Patients P53 (IHC) negative Total No.= 12	P – value And χ^2 - Value
Stage			
I II	(75%)21	(75%)9	P = 0.0027
III IV	(25%)7	(25%)3	**χ^2 =11.621
Smoker	(42.8%)12	(66.6%)8	P = 0.0133
Non smoker	(57.1%)16	(33.3%)4	**χ^2 =9.261

*(p≤ 0.05), **(p≤ 0.01)

Discussion:

Urinary bladder cancer is the one of the most common cancer in developed countries(16) and a very common disease worldwide (17), it is incidence like many other types of cancer in certain areas of Iraq after exposure to high levels of depleted uranium after two gulf wars (18).

In this study the results shows the mean age of the patients 62.6 years ranged between 45-85 years . The most cases included patients with age ≥60 years, our study confirmed with (19) who found that the mean age of his patients study was 63.2 years and the majority of his cases were ≥60 years, also confirmed with previous study (20) which reported that the age of their patients ranged between 36-83 years with mean age 66 years . whereas, (21) found that the mean age of patients 63 years ranged between 34-87 years.

In bladder cancer , mutation of p53 gene is a very common genetic abnormalities (22), mutated p53 had along half life , ispite of wild type of p53 which is a short half life (23), many studies reported that p53 mutations is associated with patients of bladder cancer(24,25).

Our finding results shows that the most kind of malignancy was transitional cell carcinoma 25 patients out of 40 which was (62.5%),followed by squamous cell carcinoma which was (37.5%) 15 out of 40 cases .the finding results agrees with (8,26) who reported the most common type of malignancy was transitional cell carcinoma,and also confirmed with (10) who found The most common type of bladder cancer is transitional cell carcinoma with approximately 90-95% of bladder cancer incidence

Regarding to this study , by using insituhybridization, p53 gene expression was detected in 18 out of 40 patients . There were highly significant correlation between positive p53 with each of site distribution ,grade, and stage of the tumor at p= 0.0038 , 0.0269 , 0.0148 respectively , whereas , statistically there were no significant association with age , gender, and smoking. This finding is in agreement with (27)who found highly significant correlation between p53 with tumor grade and stage, also is an agreement with (29) which reported statistically no correlation between p53 and gender, also confirmed with (19) who found highly significant association of p53 overexpression with tumor grade , and stage but statistically no correlation with age and gender.(29) reported no correlation with smoking history and highly significant correlation with stage, site and grade of the

tumor its an agreement with our results.

By using immunohistochemical assay p53 protein overexpression detected in 28 out of 40 patients , we found highly significant association with grade, stage, site, and smoking at p ≤ 0.05. The study results agree with (27,29,31) they reported that highly significant correlation with each of grade and stage of the tumor.(30) showed that p53 overexpression statistically significant with age, site, gender,and grade of there patients its also confirmed with our results. Whereas, disagree with (20) which found no association between p53 overexpression with all clinicohistopathological aspects like ,age , gender, smoking, grade and the stage of tumor.

The wild type p53 protein have short half life , mutated p53 have long half life , this means the results of that characteristic to accumulation of p53 products and detection of p53 protein by immunohistchmical assay in the nuclei of the cells 15% or 20% only of tumor despite p53 gene products does not accumulation in the nuclei of the cells(22,23)

The results of this study seems that p53 gene alterations will act as an important factor for clinical prognosis of patients with bladder cancer.

The studied data were a small size samples and the significantly correlations observed between all clinico histopathological aspect encourage to perform other research to confirm our data

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العلاقة بين الجين الكابح للورم مع سرطان المثانة في بعض المرضى العراقيين

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الخلاصه:

ان التعبير في الجين الكابح للورم P35 يؤثر في انواع مختلفة من السرطانات البشرية ومن اهمها سرطان المثانة . حيث صممت هذه الدراسة لتقييم العلاقة بين الجين الكابح للورم وبعض المرضى العراقيين المصابين بسرطانات المثانة بأستخدام طريقتي التصبيغ المناعي ومقارنتها بطريقة التهجين بالموقع. اذ تم جمع 40 عينة من مرضى مصابين بسرطان المثانة. اخذت من مواقع مختلفة من المثانة (الخلايا الانتقالية وطبقة الخلايا الحرشفية) من المرضى اللذين خضعوا لعملية استئصال مسبق للمثانة. هذه العينات جمعت خلال الفترة الزمنية بين (2014-2012). شملت هذه الخزعات النسيجية على 21 عينة جيدة التمايز و 9 عينات متوسطة التمايز وعشرة عينات ضعيفة التمايز الورمي.

باستخدام طريقة التهجين بالموقع ، تم تعيين الجين الكابح للورم في 18 عينة من اصل 40 (45%) في حين باستخدام طريقة التصبيغ المناعي تم تعيين الجين الكابح للورم في 28 عينة من اصل 40 (70%) ، النتائج الموجبة للجين الكابح للورم P53 ارتبطت بعلاقة معنوية عالية مع كل من الموقع ودرجة التمايز والحالة المرضية . وارتبط بعلاقة معنوية مع درجة الورم، بينما لم يظهر اي ارتباط معنوي مع كل من العمر والجنس وتعاطي التدخين.

اما باستخ]ام طريقة التصبيغ المناعي اظهرت النتائج ارتباط عالي المعنوية بين الجين الكابح للورم مع كل من درجة الورم ، الحالة المرضية ، الموقع ، تعاطي التدخين وبدرجة معنوية اقل ارتباطا مع العمر والجنس .

نستطيع ان نستنتج من هذه الدراسة بان طريقتي التهجين بالموقع والتصبيغ المناعي لتحديد كل من البروتين والجين الكابح للورم نستطيع ان نستنتج من هذه الدراسة بان طريقتي التهجين بالموقع والتصبيغ المناعي لتحديد كل من البروتين والجين الكابح للورم P53 ذات اهمية للفحوصات السريرية للمرضى المصابين بسرطانات المثانة ، ويعتقد ان الطفرات الحاصلة في الجين الكابح للورم متضمنة في كل من سرطانات المثانة في الخلايا الانتقالية وفي الخلايا الحرشفية المسرطنة ويلعب دورا مهما للغاية .