The Role of P_{53} nuclear Protein in Prediction of Progression and Recurrence of Superficial Tumor of the Bladder in Response to Intravesical Chemotherapy .

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ABSTRACT:

BACKGROUND :

p53 gene is the most common mutation in human cancers.In bladder cancer, p53 mutations have been associated with high tumor grades and advanced stages.Moreover,p53 nuclear over expression appears to be an independent predictor of disease progression and decreased survival after cystectomy.

OBJECTIVE:

To identify the role of p53 nuclear protein in prediction the progression and recurrence of superficial tumor of the bladder in response to intravesical chemotherapy

PATIENTS AND METHOD :

The expression of p53 protien was studied by immunohistochemical analysis from 71 patients with superficial tumor bladder and all of them were treated by intravesical chemotherapy followed periodically every three months by cystoscopy

RESULT :

P53 over expression was observed in 38out of 71(54%) patients with superficial tumor of the bladder. A statisticaly significant relation was noticed between P53 over expression and tumor grades , however statistical significant relation between p53 negative expression and response to intravesical mitomycin C chemotherapy was noticed , moreover , patients who received intravesical mitomycin C chemotherapy , 71% showed response with in 3 month , while 29% showed recurrence . Moreover, it seems that P53 status did predict response to intravesical mitomycin C chemotherapy.

CONCLUSION :

The response of intravesical chemotherapy in patients with superficial transitional cell carcinoma was higher in p53 negative expression.

KEY WORDS :tumor bladder, p_{53} protein ,recurrence.

INTRODUCTION:

Bladder Tumors : Urinary bladder cancer is the third most common cancer in Iraq between (1995-1997) accounting about (7.5%) of all malignancies[1] .

The most common cytogenetic abnormality is loss of chromosomes9_p,9_q,11_p,13_q and17_q.Activation/amplification of oncogenes(p21ras,c-myc, c-jun, erbB-2).[2]

The vast majority of primary bladder cancer are malignant and epithelial in origin:[2]

90% are transitional cell carcinoma(TCC) ,1-7%are squamous cell carcinoma(SCC)

75%are SCC in areas where schistosomiasis is endemic and 2%are adenocarcinoma

Secondary bladder cancers are mostly metastatic adenocarcinoma from gut,prostate,kidney,or ovary.

Histological grading:is divided in to well,moderately,and poorly differentiated(G1,G2,andG3 respectively).

Staging system for bladder cancer

Two tier system for bladder cancer are currently in wide spread use :

. Jewett and strong (1946) modified by Marshal in (1954) [3]

. American Joint Committee on cancer staging (1997), modifications of the (1987) AJCC [3]

Tumor –Node –Metastasis (TNM), papillary epithelium –Confined tumor are classified as stage T_a, where as flat in situ carcinomas are classified as T_{is} . Tumor that have invaded the lamina propria are classified as stage T_1.

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Table (1-1) :TNM Staging of Bladder Carcinoma

Marshal	TNM(AJJCC-1997)
O	T_{is} :Flatnon- paoillary anaplastic epithelium.
	T_a :Papillary tumor confined to the surface epithelium.
A	T_1 :invasion to lamina propria (sub mucosa).
B_1	T_{2a}: superficial muscle invasion .
B_2	T_{2b} : deep muscle invasion .
C	T_{3a} : microscopic perivesical invasion . T_{3b} : microscopic tumor involvement .
D_1: regional pelvic LN involvement	T_{4a} : invasion of prostate, vagina , uterus . T_{4b} : invasion of pelvic or abdominal wall .
D_2 : distant LN(above aortic bifurcation) or distant metastasis .	N_1 : single LN $\leq$ 2 cm N_2 : single LN 2-5 cm or multiple LN < 5 . N_3 : LN > 5 cm . M_0 : no distant metastasis . M_1 : distant metastasis .

Transitional Cell Carcinoma is considered clinically as superficial or muscle-invasive:

Seventy percentage of tumours are papillary,usually G1or G2,exhibiting at least 7trasitional cell layerscovering a fibro-vascular core (normal transitional epithelium has 5 cell layers).Papillary TCC is usually superficial, confined to the bladder mucosa (T_a) or submucosa (T_1).[2]

Diagnosis of Bladder carcinoma Symptoms :

1- painless hematuria is the presenting symptom in (85-90%) of cases[4].

2- Irritative voiding symptoms seen to be more common in patients with diffuse Carcinoma In Situ(CIS)[4].

3- Occasionally a pednculated mass or clot may occlude the bladder neck causing retention of urine [5].

4- Recurrent urinary tract infections particularly in women in the later decades of life[5].

5- Symptoms of advanced disease include bone pain from bone metastasis or flank pain from retroperitoneal metastasis Nerve involvemen or ureteral obstruction [4].

Signs [4]

1-No pertinent physical signs owing to the superficial nature of these disease.

2-Patients with large volume or invasive tumors may be found to have bladder wall thickening or a palpable mass.

Bladder carcinoma and risk factors

There are many risk factors can causes the disease:

1 - Cigarette Smoking: is the major cause of bladder cancer in the developed world, in men accounts for (50%) increase risk of cancer than women (31%) . Smokers have a (4 folds) risk compared to non smokers.

2-Occupational exposure : the percentage of risk are(15-35) in men and (1-6) in women [4] and patients who works in contact with chemical dye, rubber, petroleum, leather, and printing industries and these chemical substances include benzidine, beta-naphthlamine, 4-aminobiphenyl [6].

3- Coffee and tea drinking: have been implicated in some but not all studies and considered a risk factor in the presence of smoking [6] .

The P_{53} was first discovered in 1979 , and it was classified as tumor antigen at that time [7] ,however , the realization that **P_{53}** is a tumor suppressor gene came in 1989. Its' located on chromosome 17 p13.1 and encodes a53 Kilodalton nuclear phosphor protein with DNA binding properties [8]. Wild type p53 protein normally has short half life and lasts only very briefly in the cell nucleus, where as the mutated forms often accumulate for longer times and hence are more easily detected by immunohistochemistry(IHC) [8].

Its' not surprising that it is the most commonly mutated gene in human tumors , including genitourinary malignancies , close to 50% of all tumors has **P_{53}** mutation [9].

The functions of **P_{53}** are diverse and complex . the main cellular responses following activation of **P_{53}** include : cell cycle regulation , DNA repair and apoptosis. A loss of cell cycle control allows mutations that result in a growth advantage to be transmitted to the daughter cell [10]. Loss of cell, cycle control underlies the evaluation of tumor toward more locally aggressive and eventually met static

phenotype. $\mathbf{P_{53}}$ response to DNA damage by inducing cell cycle arrest through P21 (a member of cycling dependent kinas inhibitors) and then activating DNA repair enzyme, if the cell cannot arrest growth or repair DNA, $\mathbf{P_{53}}$ induces apoptosis[11]. Which responds to variety of extracellar signals in addition in DNA damage. G2M appearse to respond only to DNA damage and serve as an important point of control for replication errors .

PATIENTS AND METHODS :

This study conducted at surgical specialties Hospital, linked to Baghdad Medical Collage from may 2009 to July 2010.

In our study 120 patients with a mean age of patients was 49 years range in from 18 to 80 years, 94 once are male and 26 are females ,presented to us with symptoms and signs suggesting bladder tumor like hematuria (gross or microscopic),irritative voiding symptoms or presented incidentally on imaging study done for another pathology

All of those patients under goes checking cystoscope with biopsies taken from pathology if present,from 120 patients only 98 patients were found to have T.C.C. on check scope with biopsy , from those 98 patients 2third (71) have superficial tumor ,those with superficial tumor ,their histopathology stained for $\mathbf{P_{53}}$ 38 one of them has positive $\mathbf{P_{53}}$ stain .

All patients with superficial tumor treated with intravesical chemotherapy (mitomycin c) for 6 weeks and then rechecked by cytoscopy . The patients were properly staged according to the TNM staging system.

Statistical analysis

Statistical analysis were performed using chi-square test $(x)^2$.

RESULT:

Twenty three (32%) Patient with superficial tumor who received intravesical mitomycin C chemotherapy showed recurrence within 3 months, while 48(68%) showed no recurrence at that time. (table1)

$\mathbf{P_{53}}$ over expression was more frequent in grade 3 tumors (76%) than in grade 2 (39%) and in grade 1 tumors (33%), however, statistically significant difference was noticed between $\mathbf{P_{53}}$ over expression and tumors grades, P< 0.05 .Table(2).

Among those who were $\mathbf{P_{53}}$ positive, 42% developed recurrence, compared to 18% in those with P_{53} negative expression (table3), and statistically significant relation between intravesical mitomycin C chemotherapy response and $\mathbf{P_{53}}$ negative expression was noticed, P < 0.05 ,p=0.048.However,the result showed no statistically significant between intravesical mitomycin c chemotherapy response and $\mathbf{P_{53}}$ positive expression.X^2=0.0325,p=0.57,p>0.05. table(3)

Table 1: Patients with superficial tumor treated with intravesical chemotherapy.

Patients with superficial tumor	71
Male/Female	55 /16
Recurrence after chemotherapy	23 (32%)
Response to chemotherapy	48 (68%)
Risk factors smoking	61 /10
Occupational exposure	26 /7

Table 2: P_{53} Immumoreactivty in relation to tumor grade

Histological grade	No. Patients	No. $\mathbf{P_{53}}$ $^{+}{}_{ve}$	Percentage(%)
Well differentiated	12	4	33%
Moderately differentiated	28	11	39%
Poorly differentiated	31	23	76%
Total No.	71	38	54%

X^2 = 2.867, P< 0.05

Table 3: Patients outcome following Intravesical chemotherapy

Clinical stage	P_{53} + ve (No.=33)		P_{53} - ve (No.=38)		Total
	Response	Recurrence	Response	Recurrence	
T_{is}	3	4	9	1	16
T_a	4	4	6	2	15
T_1	12	6	16	4	40
Total	19	14	31	7	71
Percentage	58%	42%	82%	18%	

X^2 =2.825, P< 0.05, p=0.048

DISCUSSION:

Carcinoma of the urinary bladder is the third most common malignant tumor in Iraq (1995-1997) according for about 7.5% of all malignancies[1]. The patients requiring long term follow up early diagnosis and careful follow up after treatment is essential feature in the management of this disease.

In our study the results, 54% of the patients with superficial tuomr showed **P_{53}** over expression. Similar results had been observed in the other studies,(Toyoaki *etal*-2010)[12], studied 119 patients with superficial tuomr and showed that **P_{53}** was over expressed in 61%. In Iraq, (AI-Qaysi-2002)[13], showed that **P_{53}** over expressed in 23 out of40 (58%) bladder tumor.

This difference may be due

a-Variation in technique for enhancing epitope expression.

b-Non uniform methodology for **p_{53}**staining,ranging from use of different antibodies from rabbit ,mouse.

A strong correlation exists between **P_{53}** mutations and positive IHC for the **P_{53}** nuclear protein, and significant advantage of IHC over DNA sequencing is that IHC is commonly used for the assessment of other antigen as tumor markers in many pathology laboratories.

In our study **P_{53}** over expression was more frequent in high grades tumors than low grade tumors (76%) in grade 3, and only (33%) in grade 1 tumor , was obtained (table3). A statistically significant relation between P_{53} over expression and tumor grade (P=0.049). These results were in a agreement with (Al-Qaysi-2002)[13] showed significant correlation (P< 0.05) between **P_{53}** over expression and tumor grade. However, these results were disagreement with (Toyoaki *et.al* -2010)[12] which showed no statistically significant relation.

The bladder tumor does not recur suddenly; the malignant transformation is a gradual and continuous process. The probability of recurrence is greater after resection of tumor but careful follow up after treatment is an essential feature in the management of this disease.

After three months of follow up, 71 patients with stage T_{is} , T_a and T_1 disease treated with intravesical mitomycin C chemotherapy, among P_{53} positive case, 14 out of 33 patients failed chemotherapy (42 %), compared to 7 out of 38 patients in **P_{53}** negative group (18%), and a statistically significant relation obtained between **P_{53}**negative expression and intravesical mitomycin C chemotherapy response, P < 0.05, (table 3). These, results were inagreement with (Shim *et.al*.-2008)[14], they observed that superficial tumor cases domenstrated late recurrences and progression with relatively lower frequencies, a finding that represent, statistical variations of recurrence and progression depend on many factors, including follow-up intervals, a definition of recurrence and progression, and sample size. However, reaching an accurate diagnosis may be most important. So data regarding **P_{53}** status as a predicator of response of bladder cancer to chemotherapy is contradictory and appears to depend on the specific mechanism of action of the chemotherapeutic agent as well as tumor type[7].

P_{53} Status and response to Chemotherapy , **P_{53}** function may also influence tumor response to chemotherapy via regulation of the cell cycle and apoptosis, however, data regarding **p_{53}**status

As a predictor of response of bladder cancer to chemotherapy is contradictory,and appears to depend on the specific mechanism of action of the chemotherapeutic agent as well as tumor type .

Initially **P_{53}** was thought to confer chemoreisstance in bladder cancer via impaired apoptosis as seen in breast, colon, and hematological malignancies.

In contrast, studies by other investigators support the concept that **P_{53}** mutations may confer a chemosensetive phenotype[15].

CONCLUSION:

The following conclusions are drawn:

1- Close to 54% of Superficial bladder tumor showed **P_{53}** over expression.

2- **P_{53}** over expression was more common among patients with high grade histological tumor.

3- **P_{53}** negative expression indicator for response to intravesical mitomycin C chemotherapy.

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