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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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 chromosomes 9p, 9q, 11p, 13q and 17q. 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

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Secondary bladder cancers are mostly metastatic adenocarcinoma from gut, prostate, kidney, or ovary. Histological grading is divided into well, moderately, and poorly differentiated(G1, G2, and G3 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 T1a, where as flat in situ carcinomas are classified as T1b. Tumor that have invaded the lamina propria are classified as stage T1c.

ABSTRACT:
BACKGROUND:
$p_{53}$ gene is the most common mutation in human cancers. In bladder cancer, $p_{53}$ mutations have been associated with high tumor grades and advanced stages. Moreover, $p_{53}$ nuclear over expression appears to be an independent predictor of disease progression and decreased survival after cystectomy.

OBJECTIVE:
To identify the role of $p_{53}$ 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 $p_{53}$ protein 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:
$P_{53}$ over expression was observed in 38 out of 71(54%) patients with superficial tumor of the bladder. A statistically significant relation was noticed between $P_{53}$ over expression and tumor grades, however statistical significant relation between $P_{53}$ negative expression and response to intravesical mitomycin C chemotherapy was noticed, moreover, patients who received intravesical mitomycin C chemotherapy, 71% showed response within 3 month, while 29% showed recurrence. Moreover, it seems that $P_{53}$ 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 $P_{53}$ negative expression.

KEY WORDS: tumor bladder, $p_{53}$ protein, recurrence.
Table (1-1) : TNM Staging of Bladder Carcinoma

<table>
<thead>
<tr>
<th>Marshal</th>
<th>TNM(AJCC-1997)</th>
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</table>
| O       | T\text{in}: Flat non-papillary anaplastic epithelium.  
         | T\text{a}: Papillary tumor confined to the surface epithelium. |
| A       | T\text{1}: invasion to lamina propria (sub mucosa).  
         | T\text{2a}: superficial muscle invasion.  
         | T\text{2b}: deep muscle invasion. |
| B\text{1} | T\text{3a}: microscopic perivesical invasion.  
         | T\text{3b}: microscopic tumor involvement. |
| B\text{2} | T\text{4a}: invasion of prostate, vagina, uterus.  
         | T\text{4b}: invasion of pelvic or abdominal wall. |
| C       | N\text{1}: single LN ≤ 2 cm  
         | N\text{2}: single LN 2-5 cm or multiple LN < 5.  
         | N\text{3}: LN > 5 cm. |
| D\text{1}: regional pelvic LN involvement | M\text{0}: no distant metastasis.  
         | M\text{1}: distant metastasis. |
| D\text{2}: distant LN (above aortic bifurcation) or distant metastasis. |

Transitional Cell Carcinoma is considered clinically as superficial or muscle-invasive:
Seventy percent of tumors are papillary, usually G1 or G2, exhibiting at least 7 transitional cell layers covering a fibro-vascular core (normal transitional epithelium has 5 cell layers). Papillary TCC is usually superficial, confined to the bladder mucosa (T\text{a}) or submucosal (T\text{1}).

**Diagnosis of Bladder carcinoma**

**Symptoms**:
1. Painless hematuria is the presenting symptom in (85-90%) of cases.
2. Irritative voiding symptoms seen to be more common in patients with diffuse Carcinoma In Situ (CIS).
3. Occasionally a pedunculated mass or clot may occlude the bladder neck causing retention of urine.
4. Recurrent urinary tract infections particularly in women in the later decades of life.
5. Symptoms of advanced disease include bone pain from bone metastasis or flank pain from retroperitoneal metastasis. Nerve involvement or ureteral obstruction.

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

The P\text{53} was first discovered in 1979, and it was classified as tumor antigen at that time, however, the realization that P\text{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. Wild type P\text{53} 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).

It's not surprising that it is the most commonly mutated gene in human tumors, including genitourinary malignancies, close to 50% of all tumors has P\text{53} mutation.

The functions of P\text{53} are diverse and complex. The main cellular responses following activation of P\text{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. Loss of cell cycle control underlies the evaluation of tumor toward more locally aggressive and eventually met static
phenotype. \( P_{53} \) 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, \( P_{53} \) induces apoptosis\(^{(1)} \). Which responds to variety of extracellular signals in addition to DNA damage. G2M appear 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 \( P_{53} \) 38 one of them has positive \( P_{53} \) stain.

All patients with superficial tumor treated with intravesical chemotherapy (mitomycin c ) for 6 weeks and then rechecked by cystoscopy . 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) \( P_{53} \) 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 \( P_{53} \) over expression and tumors grades, \( P< 0.05 \).Table(2).

Among those who were \( P_{53} \) 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 \( P_{53} \) negative expression was noticed, \( P < 0.05 \). However, the result showed no statistically significant between intravesical mitomycin c chemotherapy response and \( P_{53} \) positive expression.\( \chi^2=0.0325,p=0.57,p>0.05. \) table(3)

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

<table>
<thead>
<tr>
<th>Patients with superficial tumor</th>
<th>71</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>55/16</td>
</tr>
<tr>
<td>Recurrence after chemotherapy</td>
<td>23 (32%)</td>
</tr>
<tr>
<td>Response to chemotherapy</td>
<td>48 (68%)</td>
</tr>
<tr>
<td>Risk factors smoking</td>
<td>61/10</td>
</tr>
<tr>
<td>Occupational exposure</td>
<td>26/7</td>
</tr>
</tbody>
</table>

**Table 2:** \( P_{53} \) Immumoreactivity in relation to tumor grade

<table>
<thead>
<tr>
<th>Histological grade</th>
<th>No. Patients</th>
<th>( P_{53} ^{+ve} )</th>
<th>Percentage(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well differentiated</td>
<td>12</td>
<td>4</td>
<td>33%</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>28</td>
<td>11</td>
<td>39%</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>31</td>
<td>23</td>
<td>76%</td>
</tr>
<tr>
<td>Total No.</td>
<td>71</td>
<td>38</td>
<td>54%</td>
</tr>
</tbody>
</table>

\( \chi^2 = 2.867, P< 0.05 \)
RECURRENT OF SUPERFICIAL TUMOR OF THE BLADDER

Table 3: Patients outcome following Intravesical chemotherapy

<table>
<thead>
<tr>
<th>Clinical stage</th>
<th>P53 + ve (No.=33)</th>
<th>P53 - ve (No.=38)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Response</td>
<td>Recurrence</td>
<td>Response</td>
</tr>
<tr>
<td>Tis</td>
<td>3</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Ta</td>
<td>4</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>T1</td>
<td>12</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>14</td>
<td>31</td>
</tr>
<tr>
<td>Percentage</td>
<td>58%</td>
<td>42%</td>
<td>82%</td>
</tr>
</tbody>
</table>

$X^2=2.825$, 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 P53 over expression. Similar results had been observed in the other studies,(Toyoaki etal.-2010)\(^{12}\), studied 119 patients with superficial tuomr and showed that P53 was over expressed in 61%. In Iraq, (Al-Qaysi-2002)\(^{13}\), showed that P53 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 p53staining,ranging from use of different antibodies from rabbit,mouse.
A strong correlation exists between P53 mutations and positive IHC for the P53 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 P53 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 P53 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 P53 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 Tis , Ta and T1 disease treated with intravesical mitomycin C chemotherapy, among P53 positive case, 14 out of 33 patients failed chemotherapy ( 42 %), compared to 7 out of 38 patients in P53 negative group (18%), and a statistically significant relation obtained between P53 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 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 P53 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\(^{7}\). P53 Status and response to Chemotherapy , P53 function may also influence tumor response to chemotherapy via regulation of the cell cycle and apoptosis, however, data regarding P53 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 P53 was thought to confer chemoresistance in bladder cancer via impaired apoptosis as seen in breast, colon, and hematological malignancies.
In contrast, studies by other investigators support the concept that P53 mutations may confer a chemoinsensitive phenotype\(^{15}\).

CONCLUSION:
The following conclusions are drawn:
1- Close to 54% of Superficial bladder tumor showed P53 over expression.
2- P53 over expression was more common among patients with high grade histological tumor.
3- $\text{P53}$ negative expression indicator for response to intravesical mitomycin C chemotherapy.

REFERENCES: