The prognostic value of p53 and its relation to O2-free radicals in patients with cancer bladder

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Abstract
The link between p53 as a prognostic marker and O2-free radicals as an aetiological factor for cancer bladder needs to be clarified. The current work was therefore, carried on 50 male subjects. Their ages were ranged from 38-80 years with the mean value 56.3. They were 40 patients with cancer bladder in different grades compared with 10 health male subjects as controls. Our patients were classified histopathologically into 4 groups. Each group comprised 10 patients. Venous blood samples were taken from all patients, pre-operatively and another samples were taken after 21 days, post operatively. Also tissue biopsies with safety margins were taken for determination of malondialdehyde (MDA) and p53 protein.

The results of this work showed that; pre-operative and post operative serum MDA and p53 were significantly increased in all grades compared with the control group (p<0.05) except post operative serum MDA in grade III which showed non-significant difference. Comparative study of post operative serum MDA and p53 versus pre-operative, the data showed statistical significant decrease (p1<0.05). However, tissue biopsies homogenates showed that MDA was significantly decreased in grade III and grade IV (p<0.05) but non-significant in grade I and grade II while p53 was significantly increased in all grades when compared with those obtained from healthy tissues. Correlation study; showed that; pre-operative serum MDA had a significant positive correlation with serum p53 in all grades of cancer (p<0.05). Meanwhile, tissue MDA had a significant positive correlation with tissue P53 in grade IV (p<0.05).

We could conclude that; free radicals, lipid peroxidation and over expression of P53 were inter-related to each others. However, oxy radicals cause genetic changes of P53 resulting in loss of programmed cell death (apoptosis) which play a critical role in tumorgenesis. The rapid decline of serum p53 after radical cystectomy seemed to be a useful prognostic marker for follow up of patients with cancer bladder.
Introduction

Free radicals are potentially dangerous by-products of cellular metabolism that have direct effect on cell growth, development and cell survival, and have a significant role in the pathogenesis of atherosclerosis, cancer, aging and several other conditions including inflammatory disease\(^1\).

The human protein 53 (P53) gene, located on the short arm of chromosome 17, encodes a nuclear phosphoprotein which binds specific DNA sequences in the human genome and appears to have a key role in the control of DNA replication and hence cellular proliferation\(^2\).

There are 2 types of p53, wild type and mutant type. In normal cells, wild type P53 has a short intracellular half-life while mutant P53 products are more stable and less degradable\(^3\).

It has been reported that P53 is a tumour suppressor gene and P53 mutation is the most common genetic mutation in cancer. The mutated gene loses natural tumour suppressor function, allowing damaged cells to divide and go on to become malignant. Aberration of P53 has been associated with various clinical parameters such as short survival or resistant to chemo and radiotherapy but there are controversies concerning the significance of these observations\(^4\).

Muscle invasive transitional cell carcinoma (TCC) of the bladder has a poor prognosis, with half of patients dying from metastatic disease within 2 years of radical cystectomy or radiotherapy for clinically localized disease\(^5\).

Part of the reason for the poor prognosis seen in advanced bladder cancer is the present lack of any prognostic markers to identify tumours that are at higher risk of progression. So, there is intense interest and
pressing clinical need for the development of such markers to allow optimal treatment to be targeted to selected patient groups\textsuperscript{6}.

\textbf{Aim of the work}

The aim of this work is to study the relationship of oxidant stress, lipid peroxidation and P53 as well as the prognostic value of P53 as a biochemical marker in patients with urinary bladder cancer.
Subjects and methods

This study included 50 male subjects. Their ages ranged from 38-80 years with the mean value 56.30. Our patients were selected from Benha University hospital, Urology department from August 1998 to November 2002. They were suffering from cancer bladder in different grades. All subjects included in this study were categorized into the following groups:

Group I: Included 10 healthy subjects served as a control group.
Group II: Included 10 patients with grade I cancer bladder.
Group III: Included 10 patients with grade II cancer bladder.
Group IV: Included 10 patients with grade III cancer bladder.
Group V: Include 10 patients with grade IV cancer bladder.

All subjects will be subjected to be the followings:-
1- Full history and clinical examination.
2- E.C.G.
3- Plain X ray and intra venous pyelography (I.V.P)
4- Abdomino-pelvic Ultrasound
5- Computerized tomography (C.T. Scan abdomino-pelvic)
6- Cystoscope guided biopsies: they were graded histo-pathologically & analyzed biochemically for determination of MDA & P53. Also, negative biopsies for cancer were analyzed biochemically for MDA & P53 which served as controls.

A- Tissues preparation for determination of MDA:

Small biopsy of bladder tissue weighing 0.5 gm was homogenized in 1.0 ml Tris buffer (20 mmol) at PH 7.4 using Art Miccra D-8 homogenizer at speed 26,000 rpm. Then 1.0 µl of (0.5M) butylated hydroxy toluene (BHT) in acetonitrile was added to 1.0 ml of homogenate. This homogenate was centrifuged at 3000xg for 10 min at 4°C in a cooling centrifuge. The supernatant was kept frozen at -80°C for later determination of MDA. The concentration of MDA was expressed as
nmol/gm tissue. The chemicals used were supplied by Sigma company.

B- Tissue preparation for determination of P53:

Small biopsy of bladder tissue weighing 0.5 gm was added to 3.0 CC of RIPA buffer which is composed of: (20mM Tris, 0.5mM EDTA (Ethylene Diamine Tetra-acetic Acid), 1.0% Nonidet P40, 0.5% sodium deoxycholate, 0.05% SDS (sodium dodecyl sulphate), 1.0 mM PMSF (phenyl methane sulfonyl fluoride), 1.0 µg/ml Aprotinin, 2.0 µg/ml leupeptin) in a centrifuge tube. Then, the tissue was disintegrated using Art Miccra D-8 homogenizer at speed 26,000 rpm. The homogenates were centrifuged at 10000xg for 10 min. The supernatant was kept frozen at -80°C for later determination of p53. The concentration of P53 was expressed as pg/gm tissue. All chemicals used in the preparation of RIPA buffer were supplied by Sigma company.

7- Urine analysis

8- Sampling:

Venous blood samples (5.0 c.c) were taken from all subjects and divided into two parts. The first part (about 1.0 c.c) was taken on small amount of EDTA powder for determination of complete blood picture. The second part (4.0 c.c) was left to be collected, centrifuged and the sera separated were used for determination of random glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine.

The remaining part of the sera was kept frozen at -80°C until assay of MDA and P53. Another venous blood samples (2.0 c.c) were taken from patients 21 days, postoperatively. Then, centrifuged and the sera separated were kept frozen at -80°C until assay of MDA and P53.
-**Determination of MDA in serum & tissue homogenate by colorimetric method** \(^7\).

Serum proteins were precipitated by addition of trichloroacetic acid then thiobarbituric acid reacts with MDA to form thiobarbituric acid reactive product. Its concentration is proportional with the absorbance of the sample. The procedure was done as mentioned in previous report \(^7\).

-**Determination of P53 in serum & tissue homogenates by enzyme-linked immuno sorbent assay (ELISA) method** \(^3\).(The kit was supplied by Roche company)

The assay was done according to the steps supplied with the kit which based on a quantitative (Sandwich ELISA) principle. The biotin labeled capture antibody is pre-bound to the streptavidine coated microtiter plate. During one single incubation step the P53 containing sample reacts with capture antibody and peroxidase labeled detection antibody to form a stable immuno- complex. Subsequent to the working step, the peroxidase bound in the complex is developed by tetra methyl benzidine (TMB) as a substrate. The photometrically determined colour is proportional to the concentration of P53.

**Statistical analysis:**
The obtained results-were tabulated and statistically analyzed using mean values , standard error , student’s t-test and correlation coefficient (\( r \)). p values < 0.05 were considered significant while p values > 0.05 were insignificant\(^\text{13}\).
RESULTS

Table (1) showed that the mean values of pre-operative serum MDA in grade I (7.20 ±0.416 nmol/ml vs 2.2 ±0.389 nmol/ml), grade II (7.40 ±0.636 nmol/ml vs 2.2 ±0.389 nmol/ml), grade III (8.90 ±0.809 nmol/ml vs 2.2 ±0.389 nmol/ml) and grade IV (11.50 ±1.249 nmol/ml vs 2.2 ±0.389 nmol/ml) were increased with statistical significant differences (p<0.05) compared with the control group. Also, the mean values of post-operative serum MDA in grade I (3.70 ±0.213 nmol/ml vs 2.2 ±0.389 nmol/ml), grade II (4.20 ±0.757 nmol/ml vs 2.2 ±0.389 nmol/ml) and grade IV (5.10 ±0.657 nmol/ml vs 2.2 ±0.389 nmol/ml) were increased with statistical significant differences (p<0.05) compared with the control group. However, the mean values of post-operative serum MDA in grade III (3.20 ±0.467 nmol/ml vs 2.2 ±0.389 nmol/ml) showed non statistical significant differences compared with the control group. Furthermore, comparative study of post-operative versus pre-operative serum MDA, the data revealed that; the mean values of serum MDA in grade I (3.70 ±0.213 nmol/ml vs 7.20 ±0.416 nmol/ml), grade II (4.20 ±0.757 nmol/ml vs 7.40 ±0.636 nmol/ml), grade III (3.20 ±0.467 nmol/ml vs 8.90 ±0.809 nmol/ml) and grade IV (5.10 ±0.657 nmol/ml vs 11.5 ±1.249 nmol/ml) were decreased with statistical significant differences (p<0.05)

Moreover, the mean values of pre-operative serum p53 in grade I (25.60 ±1.424 pg/ml vs 10.0 ±0.577 pg/ml), grade II (44.80 ±4.659 pg/ml vs 10.0 ±0.577 pg/ml), grade III (131.10 ±4.832 pg/ml vs 10.0 ±0.577 pg/ml), and grade IV (464.10 ±42.284 pg/ml vs 10.0 ±0.577 pg/ml) were increased compared with the control group with statistical significant differences (p<0.05). Also, the mean values of post-operative serum p53 in grade I (14.00 ±0.856 pg/ml vs 10.0 ±0.577 pg/ml), grade II (17.0 ±1.832 pg/ml vs 10.0 ±0.577 pg/ml), grade III (55.30 ±7.336 pg/ml vs 10.0 ±0.577 pg/ml), and grade IV (177.30 ±17.257 pg/ml vs 10.0 ±0.577 pg/ml).
pg/ml) were increased with statistical significant differences (p<0.05) compared with the control group.

Also, comparative study of post-operative versus pre-operative serum p53, the data revealed that; the mean values of serum p53 in grade I (14.00 ±0.856 pg/ml vs 25.60 ±1.424 pg/ml), grade II (17.00 ±1.833 pg/ml vs 44.80 ±4.659 pg/ml), grade III (55.30 ±7.336 pg/ml vs 131.10 ±4.832 pg/ml) and grade IV (177.30 ±17.257 pg/ml vs 464.10 ±42.284 pg/ml) were decreased with statistical significant differences (p<0.05).

Table (2) showed that; the mean values of tissue MDA in grade I (6.30 ±0.895 nmol/g tissue vs 8.70 ±1.375 nmol/g tissue) and grade II (6.70 ±0.761 nmol/g tissue vs 8.70 ±1.375 nmol/g tissue) were decreased with non statistical significant differences. However, the mean values of tissue MDA in grade III (5.20 ±0.389 nmol/g tissue vs 8.70 ±1.375 nmol/g tissue) and grade IV (4.50 ±0.654 nmol/g tissue vs 8.70 ±1.375 nmol/g tissue) were decreased compared with MDA estimated in healthy tissues homogenates with statistical significant differences (p<0.05). Also, the mean values of tissue p53 in grade I (29.80 ±1.397 pg/g tissue vs 11.30 ±0.731 pg/g tissue), grade II (46.60 ±3.503 pg/g tissue vs 11.30 ±0.731 pg/g tissue), grade III (140.20 ±4.695 pg/g tissue vs 11.3 ±0.731 pg/g tissue), and grade IV (478.30 ±42.261 pg/g tissue vs 11.30 ±0.731 pg/g tissue) were increased compared with p53 estimated in healthy tissues homogenates with statistical significant differences (p<0.05).

Table (3) showed a significant positive correlation between pre-operative serum MDA and pre-operative serum p53 in grade I (r= 0.652), grade II (r= 0.911), grade III (r= 0.847) and grade IV (r= 0.641). However, pre-operative serum MDA had non-significant correlation with tissue p53 in grade I (r= -0.107), grade II (r= -0.162), grade III (r= 0.331) and grade IV (r= -0.068).
Table (4) showed a non significant correlation between tissue MDA and tissue p53 in grade I (r = 0.299), grade II (r = 0.533) and grade III (r = -0.429). However, a significant positive correlation exists between tissue MDA and tissue p53 in grade IV (r = 0.826) (p<0.05). In addition, tissue MDA showed non-significant correlation with pre-operative serum p53 in grade I (r = 0.019), grade II (r = -0.344), grade III (r = -0.196) and grade IV (r = 0.244)
Table (1): Mean, ±SE and p values of pre-operative and post-operative serum malondialdehyde (MDA) and P53 in different grades compared with the control group and in post-operative versus pre-operative.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Biochemical parameters</th>
<th>serum MDA (nmol/ml)</th>
<th>serum P53 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>-</td>
<td>2.2 ±0.389</td>
<td>10.00 ±0.577</td>
</tr>
<tr>
<td>Grade I</td>
<td>pre-operative</td>
<td>7.20 ±0.416</td>
<td>25.60 ±1.424</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>post-operative</td>
<td>3.70 ±0.213</td>
<td>14.0 ±0.856</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.05</td>
<td>p1&lt;0.05</td>
</tr>
<tr>
<td>Grade II</td>
<td>pre-operative</td>
<td>7.40 ±0.636</td>
<td>44.80 ±4.659</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>post-operative</td>
<td>4.20 ±0.757</td>
<td>17.00 ±1.832</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.05</td>
<td>p1&lt;0.05</td>
</tr>
<tr>
<td>Grade III</td>
<td>pre-operative</td>
<td>8.90 ±0.809</td>
<td>131.1 ±4.832</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>post-operative</td>
<td>3.20 ±0.467</td>
<td>55.30 ±7.336</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N.S</td>
<td>p1&lt;0.05</td>
</tr>
<tr>
<td>Grade IV</td>
<td>pre-operative</td>
<td>11.50 ±1.249</td>
<td>464.10 ±42.284</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>post-operative</td>
<td>5.10 ±0.657</td>
<td>177.3 ±17.257</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.05</td>
<td>p1&lt;0.05</td>
</tr>
</tbody>
</table>

p : Probability versus control group

p1 : Probability versus pre-operative

N.S : Non-Significant (p>0.05).
Table (2): Mean, ±SE and p values of tissue malondialdehyde (MDA) and P53 in different grades compared with healthy tissues.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tissue MDA (nmol/gm tissue)</th>
<th>Tissue P53 (pg/ gm tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy tissues</td>
<td>8.70 ±1.375</td>
<td>11.30 ±0.731</td>
</tr>
<tr>
<td>Grade I</td>
<td>6.30 ±0.895</td>
<td>29.80 ±1.397</td>
</tr>
<tr>
<td>Grade II</td>
<td>6.70 ± 0.761</td>
<td>46.60 ±3.503</td>
</tr>
<tr>
<td>Grade III</td>
<td>5.20 ±0.389</td>
<td>140.20 ±4.695</td>
</tr>
<tr>
<td>Grade IV</td>
<td>4.50 ±0.654</td>
<td>478.30 ±42.261</td>
</tr>
</tbody>
</table>

N.S : Non-Significant (p>0.05).

p : Probability versus healthy tissues
Table (3): Correlation coefficient (r) between pre-operative serum MDA, P53 & tissue P53 in different grades.

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Pre-operative serum MDA</th>
<th>Grade I</th>
<th>Grade II</th>
<th>Grade III</th>
<th>Grade VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-operative serum P53</td>
<td>r</td>
<td>0.652</td>
<td>0.911</td>
<td>0.847</td>
<td>0.641</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Tissue P53</td>
<td>r</td>
<td>-0.107</td>
<td>-0.162</td>
<td>0.331</td>
<td>-0.068</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
</tr>
</tbody>
</table>

N.S : Non-Significant (p>0.05).

Table (4): Correlation coefficient (r) between tissue MDA, P53 and pre-operative serum P53 in all different grades.

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Tissue MDA</th>
<th>Grade I</th>
<th>Grade II</th>
<th>Grade III</th>
<th>Grade VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue P53</td>
<td>r</td>
<td>0.299</td>
<td>0.533</td>
<td>-0.429</td>
<td>0.826</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Pre-operative serum P53</td>
<td>r</td>
<td>0.019</td>
<td>-0.344</td>
<td>-0.196</td>
<td>0.244</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
</tr>
</tbody>
</table>

N.S : Non-Significant (p>0.05).
DISCUSSION

Our results showed that; both pre-operative and 21 days post-operative serum malondialdehyde (MDA) was significantly increased in all grades of cancer compared with the control group (p<0.05). However post-operative grade III patients showed a non-significant increase of serum MDA compared with the control group. Post-operative serum MDA was significantly decreased in comparison with the MDA measured pre-operatively in all grades of cancer (p<0.05). On the other hand, MDA in malignant tissue homogenates was significantly decreased in patients with grades III & IV while non-significantly decreased in patients with grade I & II compared with MDA assayed in non-malignant tissue homogenates (p<0.05) (Tables 1 & 2).

The presence of free radicals in biological materials was discovered less than 50 years ago. Soon thereafter, Denham Harman hypothesized that oxygen radicals may be formed as by products of enzymic reactions in vivo. In 1956, he described free radicals as a Pandora's box of evils that may account for gross cellular damage, mutagenesis, cancer, and last but not least, the degenerative process of biological aging. After that, Siu et al. Investigated the oral toxicity of malondialdehyde (MDA), a product of lipid peroxidation found in some foods, in a 90 day study on mice. They found mild dysplasia of the urinary bladder epithelium after oral intake of MDA.

So, free radicals are potentially dangerous by products of cellular metabolism that have direct effect on cell growth and development, cell survival and have a significant role in the pathogenesis of atherosclerosis, cancer, aging and several other conditions, including inflammatory disease.
Free radicals are generated by aerobic organisms during the production of ATP (adenosine Triphosphate) in mitochondria. During the electron-transport steps of ATP production, due to leakage of electrons from mitochondria, reactive oxygen species, superoxide anion (O2\(^{-}\)) and hydroxyl (OH\(^{-}\)) radicals, are generated. These species lead to the production of hydrogen peroxide (H\(_2\)O\(_2\)), from which further hydroxyl radicals are generated in a reaction that seems to depend on the presence of Fe\(^{2+}\) ion\(^{18}\).

In addition, free radicals have both beneficial and harmful actions\(^{17}\). They are needed for signal transduction pathways that regulate cell growth\(^{19,20}\), reduction oxidation (redox) status\(^{18}\), and as a first line of defense against infections by polymorphnuclear leukocytes\(^{17}\). On the other hand, excessive amounts of free radicals can start lethal chain reactions, which can inactivate vital enzymes, proteins and other subcellular elements needed for cell survival and lead to cell death\(^{17,21,22,23}\). Thus free radicals are functionally a double-edged sword\(^{24}\).

Moreover, oxygen free radicals react with polyunsaturated fatty acid residues in phospholipids resulting in the production of a plethora of products, many of them reactive toward protein and DNA. One of the most abundant carbonyl product of lipid peroxidation is MDA which is also, generated as a side product of prostaglandin biosynthesis. It reacts with DNA to form adducts to deoxyguanisine (M(1)G), deoxyadenosine, and deoxycytidine. MDA-DNA adducts are mutagenic in bacteria and in mammalian cells. M(1)G is highly mutagenic when incorporated into viral genome then replicated in E.Coli. It is repaired by the nucleotide excision repair. So, lipid peroxidation appears to be a major source of endogenous DNA damage in humans that may contribute significantly to cancer and other genetic diseases linked to lifestyle and dietary factors\(^{25}\).
However, there was an excessive lipid peroxidation in cancer bladder tissues compared with the normal tissues. These results were compatible with the results of *Cheeseman et al.*, Nagini et al., and *seven et al.*. They found that, LP was significantly decreased in hepatoma cells, oral squamous cell carcinoma and breast cancer, respectively.

Das reported that, there is an inverse relationship between the concentrations of LP and the rate of cell proliferation, i.e. the higher the rate of lipid peroxidation in the cells, the lower the rate of cell division. This is supported by the observation that tumor cells are more resistant to lipid peroxidation than normal cells. Tumor cells have low or almost no superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase enzymes. Hence, it is possible that the relatively high content of vitamin E contributes to the low rate of lipid peroxidation observed in the tumor cells.

The increase of serum MDA in our patients was nearly compatible with that obtained by Huang at al., Seven et al., and Choi et al., who found a significant increase of serum MDA in patients with breast cancer, laryngeal and gastric carcinoma, respectively. Also, our results were in accordance with that obtained by Kaczmarek et al., who found a significantly lipid peroxidation in platelets in patients with bladder cancer and the use of mycobacterium suspension intravesical clusters causes lipid peroxidation inhibition (decrease in MDA concentration).

The data of this work, showed that, Pre-operative and post-operative serum P53 was significantly increased compared with the control group (p<0.05). However, post-operative serum P53 was significantly decreased compared with those obtained preoperatively in all grades of cancer. On the other hand, tissue P53 was significantly increased in all our patients.
with different grades compared with P53 assayed in non-malignant tissue homogenates (Tables 1 & 2)

The P53 tumor suppressor protein is a transcriptional factor that regulates several gene expression pathways that function collectively to maintain the integrity of the genome. Its nuclear localization is critical to this regulation\(^3^4\). Also, the P53 tumor suppressor gene is a check point within the cell cycle because of its ability to induce cell cycle arrest at G1 or programmed cell death (apoptosis) if the cell DNA is damaged\(^3^8\).

Mutation in the P53 tumor suppressor gene are the most common genetic alterations associated with malignant tumors. Overexpression of P53 protein could be considered the result of increased protein stability caused through conformational alteration and has been reported in more than 50% of epithelial malignancies\(^3^9\).

Altered expression of P53 has been described in nearly half of bladder cancer, and P53 mutations are presumed to play a role in the multi-step progression of these tumors\(^4^0\).

Moreover, mutations or altered expression patterns affecting regulatory component described as the (P53 pathway) like those responsible for identification of DNA damage (i.e GADD 45), cell cycle arrest (i.e. P21/WAF1), apoptotic response (i.e. Bax) and other factors such as oncoprotein mdm2 and P14/HARF disrupt cellular growth and apoptosis, leading to neoplastic transformation\(^4^1\).

\[
\begin{align*}
P21 & \quad \text{Cell Cycle arrest} \\
P14 \text{ mdm2} & \quad \text{P53} \\
& \quad \text{Bax} \quad \text{Apoptosis}
\end{align*}
\]

**Schematic representation of the P53 pathways**\(^4^1\).
There are many studies which revealed over expression of P53 in transitional cell carcinoma. Some of these reports revealed that P53 is a good prognostic marker for the relapse and progression free survival in bladder cancer. The observed increase of serum & tissue P53 protein in our patients was in accordance with the results of Morita et al., and Zalabardo et al.

Correlation study revealed that pre-operative serum MDA had a significant positive correlation with serum P53 Protein in all grades of cancer while it has non-significant correlation with tissue P53 in different grades (Table 3). Moreover, tissue MDA had non-significant positive correlation with P53 protein in tissue homogenates in all grades of cancer except grade IV which showed a significant positive correlation (r=0.826 p<0.05). On the other hand, tissue MDA had non-significant correlation with serum P53 in different grades studied (Table 4).

Hussain et al., reported that oxidative stress had linked with chronic inflammation with increased risk of cancer. Oxidative stress and the generation of reactive species can cause mutation in the P53 tumor suppressor gene.

Mutational inactivation of P53 has been found to be involved in various human cancers, which indicates the importance of P53 in human carcinogenesis. It has been reported that >50% of human cancers contain mutations in the P53 gene. The P53 is activated in response to a variety of stimuli such as UV, gamma radiation, hypoxia, nucleotide deprivation, etc. The activation of p53 may cause either cell division, cycle arrest or apoptosis.

The P53 can be considered as one of the oxidative response transcription factors. There are several cysteine residues in the central domain of the P53 protein. These residues are crucial for the P53 protein
binding to specific DNA sequence. Redox modulation at a post-translational level often occurs by reduction or oxidation of the disulfide bond. A reduced state is required for these cysteine residues to ensure that P53 Protein would bind to specific consensus DNA and transactivate target genes\textsuperscript{49,50,51}.

However, reactive oxygen species (ROS) can cause DNA strand breaks, base modification, lipid peroxidation, nuclear transcription factor kappa B (Nf-kB) and P53 activations and subsequent cell injury. The process of P53-dependent cell cycle arrest or apoptosis functions to repair or remove the damaged cells. Because of the fundamental importance of cell arrest and apoptosis, which are regulated by P53, alterations of these pathways can enhance cancer development\textsuperscript{23}.

In vitro study, ROS induce lipid peroxidation and DNA fragmentation and that simultaneous exposure to hypoxanthine / xanthine oxidase and sodium 1-(N,N-diethylamino) diazen-1-ium-1,2- diolate/nitric oxide leads to a \textit{C}:\textit{G} (Cytosine : Guanine) \textarrow{T}:\textit{A} (Thymine : Adenine) transition in the first base of codon 248 of the P53 gene. This mutation induces an amino acid change (Arginine \textarrow{tryptophane}) abolishing the wild type function of P53 which is concomitant with the occurrence of cancer\textsuperscript{52}.

We could conclude that; , free radicals, lipid peroxidation and over expression of P53 were inter-related to each others. However, oxy radicals cause genetic changes of P53 resulting in loss of programmed cell death (apoptosis) which play a critical role in tumorigenesis. The rapid decline of serum p53 after radical cystectomy seemed to be a useful prognostic marker for follow up of patients with cancer bladder.
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الملخص العربي

القيمة الدلالية لبروتين -53 وعلاقته بجذور الأكسجين الحرّة

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تدف هذه الدراسة إلى البحث عن أهمية بروتين -53 كقيمة دلالية في مرضى سرطان المثانة ومدى علاقة بجذور الأكسجين الحرّة كعامل مسبب للسرطان .

أجريت هذه الدراسة على 50 رجلاً من تتراوح أعمارهم بين 38-80 سنة منهم أربعين رجلاً عوالي من سرطان المثانة بحالة مختلفة أما الباقي فهم يبلغ عمرهم عشرة من الأصحاء كمجموعة ضابطة . وقد تم تجميع الدم من المرضى قبل أداء العملية عينة أخرى بعد 3 أسابيع من العملية . كما تم استئصال جزء من المثانة وفحصها بالجراحة للتتأكد من حدوث السرطان وأما الجزء السليم فقد تم الاحتفاظ به للمقارنة مع النسيج الذي يعاني السرطان . وقد تم قياس جذور الأكسجين الحرّة وكذلك بروتين -53 بمصل الدم والأنسجة لجميع الأشخاص تحت البحث .

وقد أظهرت النتائج البحث وجود زيادة ذات قيمة إحصائية في مستوى جذور البدائل أو بروتين -53 في بعض الحالات المثل التي تؤثر على الأنسجة المصاحبة للسرطان وأنها ذات دلالة إحصائية في المرحلة الثالثة والرابعة . وقد وجد أن مستوى بروتين -53 قد زاد تكرارًا بها وأن هذه الزيادة ذات قيمة إحصائية في جميع مراحل السرطان كما تبين وجود علاقة طرديّة بين بروتين كسيدات الدهون وبروتين -53 بمصل الدم قبل إجراء العملية في جميع مراحل السرطان . وأثبتت النتائج وجود علاقة طرديّة ذات قيمة إحصائية بين بروتين كسيدات الدهون وبروتين -53 بالأنشطة في المرحلة الأخيرة لسرطان المثانة كما وجد أيضًا أن مستوى بروتين -53 يقل تكرارًا بمصل الدم بعد إجراء العملية عند مقارنته في مرحلة ما قبل إجرائها.

Aging: a theory based on free radical and radiation chemistry.
أثبت هذا البحث أن هناك علاقة مترابطة بين جذور الأوكسيجين الحرة ومستوى برو
كسيدات الدهون وبروتين -53 بمصل الدم وبالتالي فإن زيادة جذور الأوكسيجين الحرة تؤدي إلى
حدوث طفرات في شريط الحامض النووي الحامل بالجين المسؤول عن تصنيع بروتين -53
مما قد يكون سببا في أن يفقد بروتين -53 وظيفته في إبطاء حدوث السرطان أو الموت
المبرمج للخلايا. كذلك يشير هذا البحث إلى أن بروتين -53 يقل تركيزه بعد استئصال أورام
الثانية مما يجعله مفيدا كقيمة دلالية في متابعة هؤلاء المرضى.