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The Relationship Between Bcl-2, and P53 Protiens in Transitional Cell Carcinoma of the Bladder

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Abstract :

Transitional cell carcinoma (TCC) of the bladder in patients has historically a favorable prognosis. Bcl-2 and p53 genes are implicated in cell cycle regulation with roles on programmed cell death. Presence of nuclear accumulation of p53 and cytoplasmic accumulation of bcl-2 were proposed to confer a growth advantage to tumor cells. In this study, we investigated the roles of p53 and bcl-2 as prognostic factors in TCC of bladder.

Method: Investigated 25 patients with transitional cell carcinoma (TCC) and 27 patients with cystitis (control group) for the expression of Bcl-2 and P53 by immunohistochemical staining with specific monoclonal antibodies.

Results: Bcl-2 was significantly expressed in TCC compared to those with cystitis (control). Moreover, P53 was significantly expressed in TCC compared to those with cystitis (control). A significant positive correlation between these two parameters in Tcc.

Conclusions: These observations detect that increased the expression of Bcl-2 and P53 in TCC play a potential role in the development of bladder cancer.

Key words: *Bcl-2, P53, TCC, IHC.*

Introduction:

Transitional cell carcinoma (TCC) of the bladder is the second most common malignancy of the genitourinary tract and the second most common cause of the death from genitourinary tract (1,2).

Acquired and inherited alteration of genes that function as regulation of cell growth and differentiation are considered as crucial steps in the initiation and progression of human malignancies (3)

The neoplastic changes in the urothelium of bladder is a multistep phenomenon, the exact genetic events leading to urothelial transformation involve the activation of oncogenes, inactivation or loss of tumor suppressor genes, and alterations in the apoptotic gene products.

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An initiator and its metabolites induce an alteration in normal cell DNA, programmed cell death is known to play an important role in the cellular response to genotoxic stress; thus, loss of apoptotic response in tumor cells is thought to be one of the mechanisms involved in malignant progression and resistance to chemotherapy (5).

The Bcl-2 gene product is supposed to contribute to oncogenesis by suppressing signals that induce apoptotic cell death (6). Several studies have shown overexpression of Bcl-2 protein in a variety of solid tumors, including prostatic carcinoma, colorectal cancer, squamous cell carcinoma (SCC) of the lung, breast cancer, and nasopharyngeal malignancies (5, 7).

Bcl-2, discovered in human B-cell lymphoma, is a proto-oncogene intrinsically involved in the apoptosis cascade (8). Bcl-2 gene protein overexpression in myeloid malignancies is a consequence of the translocation from its normal location at 18q21 to the immunoglobulin-heavy chain locus at 14q32, resulting in increased production of bcl-2 mRNAs and their encoded proteins Bcl-2 belongs to a family of related genes that regulates the apoptotic pathway,

with Bcl-2 promoting a negative influence(9).

P53 is a play a vital role in the regulation of the cell cycle (11). Upon DNA damage, the level of P53 protein increases causing cell- cycle arrest; this allows for repair of DNA and prevents propagation of the DNA defect (12).

The defective P53 in human cancer leads to the loss of P53-dependent apoptosis, proliferative advantage, genomic instability and DNA repair and angiogenic control loss (13, 14).

Mutation in the P53 gene result in the production of an abnormal and usually dysfunctional protein product with prolonged half- life compared to the wild- type protein and accumulates in the cell nucleus were proposed to confer a growth advantage to tumor cells (11,15).

Furthermore, cells unable to undergo apoptosis may be more susceptible to accumulation of genetic alterations than non diseased cells (16).

This study aimed at investigating the interrelated role of an antiapoptotic protein bcl-2 and tumor suppressor protein P53 in patients with transitional cell carcinoma (TCC) of the bladder.

Materials and Methods:

Fifty two (52) patients, whose median age were 50 years (range 25-75) were examined. According to the histological examination, 25 patients with transitional carcinoma of the bladder and 27 patients complaining cystitis (control group). These patients examined, interviewed and sampled in the Al-Yarmouk and Baghdad Teaching Hospital.

Biopsies were taken from each patient and control biopsies were fixed in 10% formal buffer saline for histological examination and immunological staining for Bcl-2 and P53 protein.

Histology:

The biopsy specimens were embedded in paraffin and stained with haematoxylin – eosin (H&E).

Immunohistochemical analysis (IHC) for detection P53 and Bcl-2 expression in paraffin embedded sections

Principle of the test:

Mucosal biopsies were immunostaining with polyclonal antibodies to Bcl-2 and P53 by the avidin-biotin complex (DakocCrop, Denmark). The primary antibody reacts with antigen in the tissue, and then a biotin labeled secondary antibody (link antibody) binds to the primary antibody. When the conjugate is added, the biotinylated secondary anti-body will form a complex with the peroxidase-conjugated streptavidin and by adding the substrate, which contains 3,3 -diaminobenzidine (DAB) in a chromogen solution, a brown-colored precipitate will form at the antigen site.

Evaluation of the Immunostaining:

Evaluation of the immunostaining was done with the assistance of a histopathologist. The observer was blinded to the clinical diagnosis of the tissues at the time of assessment, and tissues were independently assessed by two observers positive or negative cases, positive immunostaining gave nuclear and/or cytoplasmic dark brown granules.

In the peroxidase secondary detection system, the presence of a brown reaction product at the site of the target antigen is indicative of positive reactivity. Counter stain will be pale to dark blue coloration of the cell nuclei.

The use of universal DakoCytomation streptavidin- biotin system purchased from DakoCytomation (USA) Immunohistochemistry detection kit. The mouse anti-human monoclonal antibodies Bcl-2 protein (code No. / c-2:sc -7382) (santa cruz, cut USA). The mouse anti-human monoclonal antibodies P53 protein (code No. / M7203) (Denmark).

Scoring:

Counting the number of positive cells which gave brown cytoplasmic staining system under light microscope. The extent of the IHC signal was determined in 10 fields (X100magnification). In each field the total number of cells was counted and the extent of cytoplasmic staining cells was determined as a percent. The total staining score was divided by the number of whole cells per field in 10 fields, so the percentage of positively stained cells in the 10 fields was calculated for each case by taking the mean of the percentage of the positively stained cell in the 10 fields.

Statistical analysis:

Student test (t-test) was used for the quantitative data. The relation between the indicators was measured qualitatively by using the correlation coefficient (r). P value of < 0.05 was considered statistically significant.

Results:

The Bcl-2 in bladder cancer patients and control were measured by IHC. The mean percentage of Bcl-2 protein increase significantly in bladder cancer patients than control group (the mean percentages, 34.2 ± 2.9 versus 18.1 ± 1.7, $p < 0.01$) (Table-1, Figure 1).

Highly significant differences between P53 mRNA expressions in bladder cancer patients and in control group (the mean percentages, 25.7 ± 1.9 versus 3.5 ± 0.2 , $p < 0.01$) (Table -2, Figure 2).

Table (1): Comparison of Bcl-2 protein expression in bladder cancer patients and in control group.

Subjects	No.	Mean ± SE.	P - value
Bladder cancer patients	**25**	**34.2 ± 2.9**	**(P < 0.01)***
control group	**27**	**18.1 ± 1.7**	
Total	**52**		

*= *Highly significant difference (P < 0.01)*

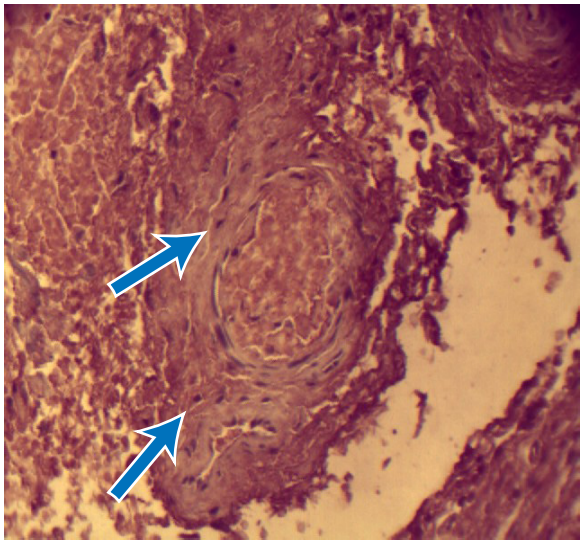

Figure 1: Detection of bcl-2 protein in studied groups by Immunohistochemistry (IHC).

Staining by DAB chromagen (dark brown) counterstained with H& E. Tissue from bladder cancer patients shows positive Bcl-2 signals (X400).

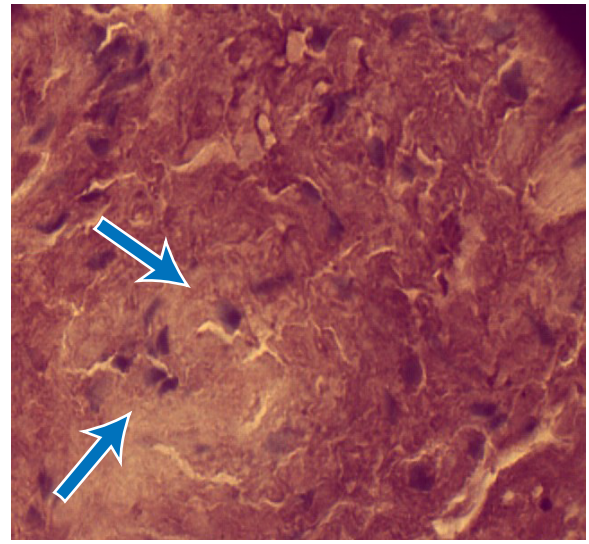

Figure 2: Detection P53 protein in studied groups by Immunohistochemistry (IHC).

Staining by DAB chromagen (dark brown) counterstained with H& E. Tissue from bladder cancer patients shows positive P53 signals (X400).

Table (2): Comparison of P53 protein expression in bladder cancer patients and in control group.

Subjects	No.	Mean ± SE.	P - value
Bladder cancer patients	**25**	**25.7 ± 1.9**	**(P < 0.01)***
control group	**27**	**3.5 ± 0.2**	
Total	**52**		

Table 3, revealed a significant correlation between Bcl-2 and P53 in Bladder cancer patients (r = 0.297, p<0.05) and significant correlation in control group (r = 0.138, p<0.05).

Table 3: Relation between the mean percentage of Bcl-2 and P53 in Bladder cancer patients and control group.

Variable	subjects	Correlation coefficient	P - value
Bcl -2 and P53	Bladder cancer patients	0.297	< 0.05
	control group	0.138	< 0.05

*= *Highly significant difference (P < 0.01)*

Discussion:

Bcl-2 and p53 proteins expression was a frequent finding in TCC evidenced by IHC expression. The tumors showed positive bcl-2 expression comparing with cystitis (control group). These findings are in agreement with the study of Atug et al (18) who stated that the positive immunostaining of bcl-2 was observed in bladder cancers which confirmed the significant bcl-2 association with the tumor. Other studies have reported an association between greater bcl-2 protein expression, with both higher tumor grade and/or higher-stage disease (17, 18, 19).

Healthy bladder tissues express low bcl-2 concentrations, we can hypothesize that altered expression of bcl-2, and the consequent block of apoptotic pathways, may represent a first step in bladder carcinogenesis (5).

In addition, the result of Shiina et al. (21) was different too form our results. They showed that the expression of bcl-2 was observed only in 24.7% of bladder cancer cases and stated that this expression inversely correlated with tumor grade and was not correlated with tumor stage. One potential explanation for the high levels of bcl-2 expression in bladder cancer patients is the loss of p53 function. The loss of p53 function may enhance the expression of bcl-2, by relieving it from the transcriptional repression of the wild type p53 protein (22). This was confirmed by the highly significant direct correlation found between the IHC expression of bcl-2 and that of p53 (P<0.05).

Given the main reason of the high p53 positive expression in bladder cancers is the formation of dysfunctional mutated p53, therefore the higher the p53 in bladder cancer the higher the mutated p53 leading to less functional wild p53.

Accordingly, less wild p53 leads to higher bcl-2 and decreased apoptosis. Bcl-2 prevents the interaction between Bax and Bak, which in turn prevents the release of the cytochrome c from mitochondria, resulting in the prevention of apoptosis (23). Moreover, bcl-2 sequesters caspase activators and this prevents caspase activation which subsequently prevents apoptosis and the tumor cell then proliferate (23)

It is of interest that these findings are similar to those previously reported, in which a similar survival correlation with p53 and bcl-2 status was observed in TCC patients (24). Other author showed that bcl-2 overexpression is probably one of the most important mechanisms by which tumor cells escape p53-mediated apoptosis (25).

The effect of the mutated p53 is to inhibit the wild type function, namely cell cycle arrest and apoptosis, driving tumors to proliferate and increase in size. This increment in the tumor stroma will affect the microenvironment circulation in the surrounding normal tissue, reducing the infiltration of immune cells to the area with reduction in the cytokines level.

Hence, a state of local immunosuppression will take place (26). The process of angiogenesis helps increase the tumor size and facilitates metastasis, dysfunction of p53 also gives hand to angiogenesis and hence metastasis (27). Therefore in bladder cancers, the overexpression of P53 represents the accumulation of mutated dysfunctional version of the protein leading to a state of high rate proliferation, immunosuppression, where all increase the invasiveness and aggressiveness of the tumor as what is found in this study.

In conclusion, p53 has the upper hand in the bladder oncogenesis. P53 was overexpressed in bladder cancer which was most probably a mutated type. P53 was associated with tumor progression. P53 mutation affects other cell cycle proteins, like bcl-2, which aggravates the disease condition.

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العلاقة بين بروتين Bcl- 2 و P53 في امراضية سرطان المثانة الانتقالي

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الخلاصة:

الهدف : قياس مستوى تعبير بروتين Bcl-2 وp53 لمرضى سرطان المثانة الانتقالي .

الطريقة : استخدمت طريقة التحليل الكيميائي المناعي لكشف وتحديد مستوى بروتين p53, Bcl-2 في المقاطع النسيجية المطمورة في البرافين والتي تم الحصول عليها من 25 مريضا مصابا بسرطان المثانة الانتقالي Tcc)) والمجموعة الثانية تشمل 27 مريضا مصابا بالتهاب المثانة حيث اعتبروا محموعة سيطرة.

النتائج : التحليل الكيميائي المناعي لمستوى بروتين Bcl-2 يزداد زيادة معنوية في حالة سرطان المثانة الانتقالي عن مجموعة السيطرة . ومستوى تعبير بروتين ال p53 يزداد ايضا معنويا مقارنة مجموعة السيطرة .وهناك ارتباط موجب بين BCL-2 و P53 .

الاستنتاج : بروتينات لP53, Bcl-2 تلعب دورا مهما في مرضى سرطان المثانة الانتقالي وان تعبير Bcl-2 يرتبط ارتباطا موجبا مع تعبير 53P .