# THE POSSIBLE ROLE OF SURVIVIN IN TRANSITIONAL CELL CARCINOMA OF BLADDER

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## ABSTRACT

**Background:** Bladder cancer is third most common malignant tumors in both men and women in Iraq. Transitional cell carcinoma ( TCC) account for > 90% of bladder cancer. Survivin, family member of inhibitor of apoptosis (IAP) that play important role in both cell division and inhibit of apoptosis, Survivin express in vast majority of human cancer but not in normal adult tissues.

**Objective:** to evaluate the possible role of Survivin in patient of transitional cell carcinoma of bladder.

**Materials and methods**: formalin fixed, paraffin embedded tissue from (45) patients with transitional cell carcinoma of bladder from Al-Kadhimiya Teaching Hospital in Baghdad were included in this study. In addition, (16) apparently normal bladder biopsies were as a control groups. Tissue blocks were sectioned for detection of Survivin by Immunohistochemistry(IHC).

**Results**: Survivin was not expressed in normal bladder urothelium, and it was over expressed in 71.1% (32/45) of TCC patients (p=0.001) . Overexpression of survivin was associated with tumor grade (p=0.029), although there was no significant association (p=731), but there was different in reading between Survivin expression and muscle invasion.

**Conclusion:** Survivin is highly expression in TCC in compared with normal urothelium, and play key roles in survival of neoplastic cells by negative regulating apoptosis, and its correlated with poor prognosis in cancer.

**Key words: Survivin, TCC, IHC**

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# INTRODUCTION:

Urinary bladder cancer ranks the fourth most common cancer in men and ninth in women in the United States, accounting for an estimated 70,980 new cases in 2009 [(1)]. Transitional cell carcinoma (TCC) of the bladder remains a significant health problem worldwide, and it account for > 90% of bladder cancer [(2)]. Bladder cancer, in Iraq is the third most common malignancy tumor in both men and women, it's the second most common in men and ninth in women [(3)]. Apoptosis is a physiological cell death process that plays a critical role in development, homeostasis and immune defense of multicellular organisms [(4)], and there are two main apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway [(5)]. Apoptosis is blocked one of the important mechanisms of tumor [(6)], The rate of apoptosis in malignant tumors is an important factor for tumor growth[(7)]. There are several new markers for improving of bladder cancer diagnosis and prognosis, Survivin discovered in 1997 [(8)], its is member of inhibitor of apoptosis protein (IAP), and it is a multifunctional protein that inhibits apoptosis, regulates cell division and enhances angiogenesis [(9)]. it is expressed in a vast majority of human cancer, but not in normal adult tissue, generally high Survivin protein expression correlated with aggressive behavior of tumor cells and in many type cancer[(10)]. Because of this large difference in expression between cancer and corresponding normal tissue, Survivin is an attractive target for both anti-cancer therapy and as a tumor marker [(11)]. Survivin controls both caspase-dependent and caspase independent apoptosis [(12)], and it does not directly bind caspases [(13)]. The precise mechanism by which Survivin suppresses apoptosis, however, is still incompletely understood. Several mechanisms are theorized [(14)]. It is inhibits apoptosis via co-operative interactions with other partners in vivo. An example of these interactions is an IAP–IAP complex between Survivin and XIAP [(15)]. Another possibility is that Survivin requires the cofactor hepatitis B X interacting protein to bind procaspase-9, thus preventing apoptosis via the intrinsic pathway[(16)]. Finally, Survivin may indirectly inhibit caspases via intermediate proteins. Survivin binds to Smac, which is a proapoptotic protein that binds IAPs and thus prevents them from inhibiting caspases [(17, 18)]. Survivin could protect other IAPs such as XIAP from inhibition by SMAC, allowing them to maintain their suppression of caspases [(19)].

# MATERIALS & METHODS:

Forty five patients with TCC of bladder, 37 (male) and 8 ( female) with an age ranged from 29 to 80 years, were included in this retrospective study, the patient samples were collected from the archives of histopathology laboratories of Al-Kadhimiya Teaching Hospital in Baghdad, during the period from January 2010 to March 2011. The diagnosis of these tissue blocks were primarily based on the obtained histopathological records of bladder biopsy samples in hospital laboratory. Confirmatory histopathological re evaluation of each obtained tissue blocks was done by specialist pathologist. In addition Sixteen apparently normal bladder biopsies. They were 10 males and 6 females with age range of (35-71) years. For each case, one representative section was stained with Hematoxylin and Eosin and the histopathological diagnosis was revised, while other section was put on

positive charged slides and stained immunohistochemically for Survivin. Immunohistochemical staining was carried out using anti- Survivin monoclonal antibodies (DakoCytomatin, Carpinteria, USA. Number M3624) the slides were deparaffinized, rehydrated then blocked. All of the slides were treated with anti Survivin monoclonal antibody, dilution1:100 (Dako, USA), then incubated over night in refrigerator at 4c°. In the next day the slides were rinsed gently and a secondary biotinyated goat anti-mouse IgG antibody was add. Followed by the addition of the streptavidin-HRP. After washing, the samples were stained with diluted liquid DAB, and then counterstained with hematoxylin. Slides washed , dehydrated then mounting with permanent mounting media (DPX), and examining under light microscope at 400 magnification. We did not differentiate between nuclear and cytoplasmic staining of survivin. The percentages of cells expressing survivin were classified as normal (no reactivity or very few focally positive cells) versus altered (>10% cells expressing survivin) [20].

# STATISTICAL ANALYSIS:

Statistical analysis was performed with the statistical package for social sciences (SPSS) 19.01and Microsoft Excel 2010 for configuration of data, tables and figures. Numerical data were described as mean, standard error, standard deviation; also, comparison between groups done using independent sample t-test. Categorical data were described as frequency and percentage; comparison done by using Chi-square test. P-value of ≤ 0.05 was used as the level of significance.

# RESULTS:

The majority of cases were male (82.2%) while female (17. 8%). Minimum age was 29 years and Maximum age was 80 years (mean age :58.356± 1.728) . the specimens were graded in to two grades ( low grade and high grade ) according to World Health Organization (WHO) and International Society of Urological Pathology (ISUP) [21], Papillary urothelial carcinoma, low grade ( n: 28 ; 62. 2%), and Papillary urothelial carcinoma, high grade ( n: 17; 37.8%). More over, muscle invasion was seen in 24 most of them high grade , while non invasion was seen in the rest 21 cases. Immunohistochemical result for Survivin : the results are summarized in table 1. The immunohistochemical expression of survivin was significantly higher in TCC group in comparison with that of control groups (71.11 % versus 0% ; $p<0.001$.), There was a significant association between the grade of TCC and the immunohistochemical expression of Survivin; p value : 0.029, the majority of high grade tumor cases showed positive immunohistochemical Survivin expression (88.24%), while only 16 cases (57.14%) of low grade tumor showed positive immunohistochemical Survivin expression. In addition the Cases with muscle invasion showed higher frequency of Survivin immunohistochemical expression in comparison with cases with no muscle invasion (71.43% versus 66.67%); nevertheless, this association did not reach a statistical significance. Table (2).

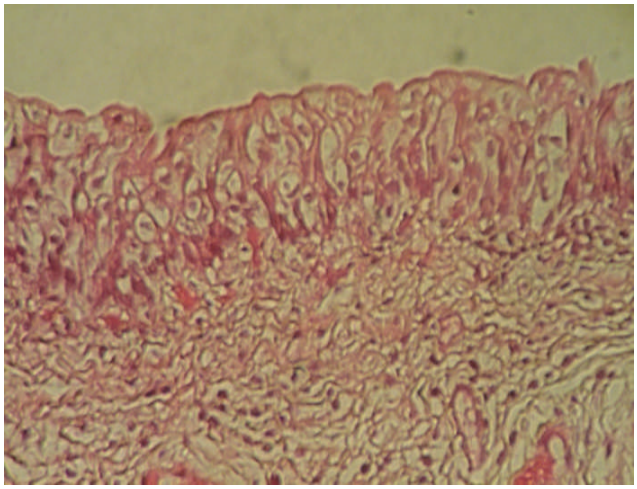

**Figure 1**: Normal urothelium of bladder ( H&E 40X).

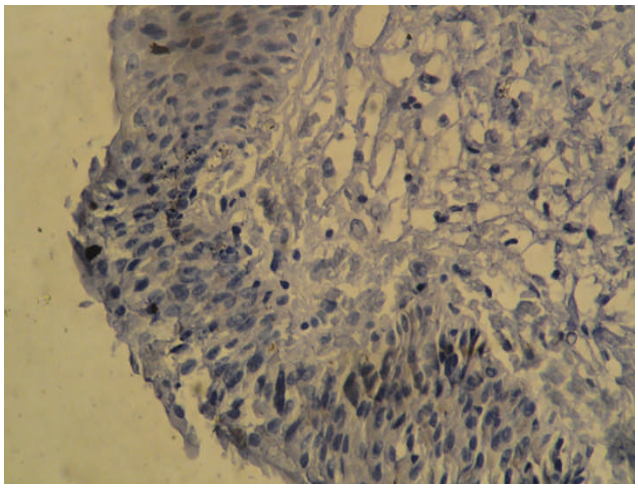

**Figure 2**: Normal urothelium showing the negative immunohistochemical expression of Survivin (x40).

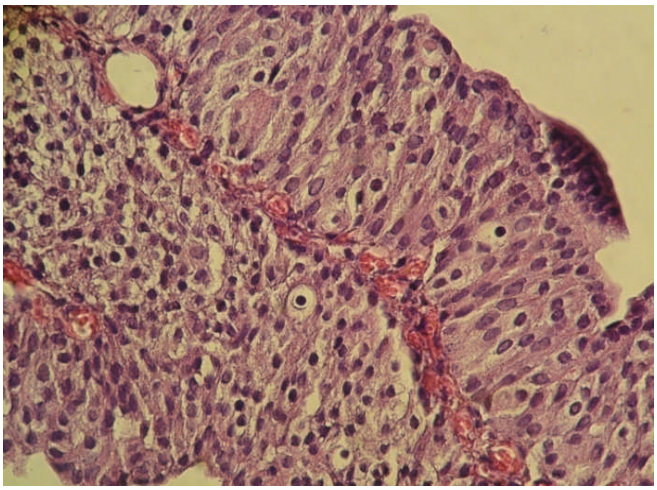

**Figure 3:** Low grade transitional cell carcinoma of bladder( H&E 40X).

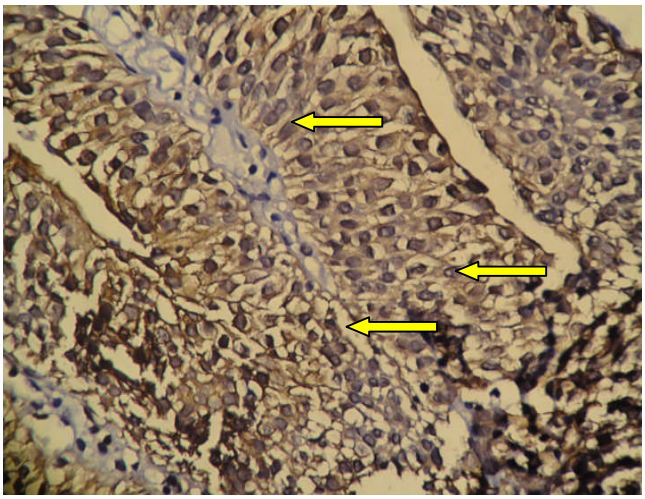

**Figure 4:** Low grade transitional cell carcinoma showing positive immunohistochemical expression of Survivin (brown) (yellow arrows) (nuclear and cytoplasmic)(40x).

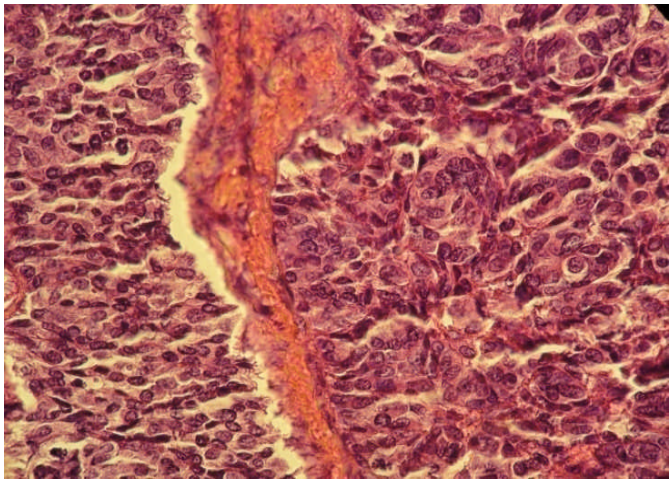

**Figure 5:** High grade transitional cell carcinoma of bladder (H&E 40X**).**

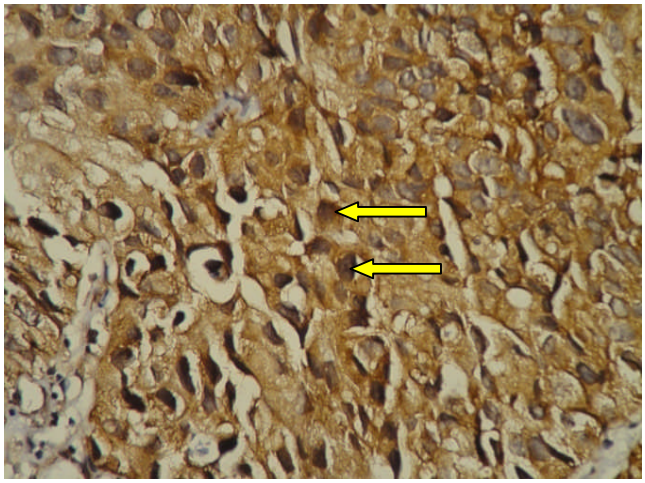

**Figure 6**: High grade transitional cell carcinoma showing the positive Survivin immunohistochemical expression (brown) (yellow arrows) (nuclear and cytoplasmic)(40x).

# DISCUSSION

Bladder cancer, in Iraq is the third most common malignancy tumor in both men and women , it's the second most common in men and ninth in women [3]. Different biomarkers have shown promise as non invasive marker of bladder cancer, and some may be useful in prognosis and therapy [22]. Since its discovery in 1997, Survivin has granted significant interest due to its possible role as IAP [8], several studies suggest that the measurement of Survivin in tissue may be valuable in cancer diagnosis, prognosis and prediction of response to therapy [2]. Our study focus on the possible role of Survivin, in Iraqi patients. Our study showed that most of patients presented with low grade tumor (62%), in addition the patient with muscle invasion(53.3%), but most of theses cases were high grade, this agreed with several studies done in Iraq as: (Kadhim, 1999; Al Naib, 1999; Al Qaysi, 2002; Kadhim, 2004) [24,25,26,2], the possible explanation, most cases of muscle invasion with high grade tumor, or may be small sample size. There was a highly significant expression of Survivin in our patients with percentage of 71.1% in TCC cases in compared with control group, as there is no previous studies in Iraq regarding Survivin in bladder cancer, we can not correlate our results locally, while this result internationally in agreement with Swana, *et al.,* 1999; Zizhong, *et al., 2007;* Nouraee, *et al*., 2009[27,28,29], which predict the role of Survivin in the occurrence and development of bladder cancer. Because of this large difference in expression between cancer and corresponding normal tissue, Survivin is an attractive target for both anti-cancer therapy and as a tumor markers. The association of Survivin with tumor grade was a significant as the majority of high grade tumor cases showed positive immunohistochemical Survivin expression (88.2%), while only 16 cases (57.1%) of low grade tumor showed positive immunohistochemical Survivin expression, this was agreed with Swana, *et al., 1999;*Yin, *et al., 2006;* Zizhong, *et al., 2007;* Nouraee, *et al*., 2009[27,28,289], and this reflect the role of Survivin in differentiation of the tumor to its grade. While this association does not agree with Ku, *et al*., 2004[30], in which they studied 88 case of TCC and this disagreement could due to sample size. Although there was no significant association between Survivin expression and muscle invasion by the tumor, but the our readings showed some difference as 71.4% of the positive case had no muscle invasion, while 66.7% of same group had muscle invasion this agreement with [30], which may indicate the poor association of Survivin with tumor stage according to muscle invasion. While the study of Shariat, *et al.,* [20] showed a highly significant association of Survivin with muscle invasion as there study contain 726 patients in a multi-institutional study. This may explain the disagreement with our results.

# CONCLUSION:

Survivin is highly expression in TCC in compared with normal urothelium, and play key roles in survival of neoplastic cells by negative regulating apoptosis, and its correlated with poor prognosis in cancer. SBecause of large difference in expression between cancer and corresponding normal tissue of bladder, survivin is an attractive target for both anticancer therapy and as tumor marker,

# TABLES

**Table(1):**showing the immunohistochemical expression of Survivin in both TCC and control groups.

| Study groups | | Survivin Cytoplasmic Immuno-score | | Total | P value |
|---|---|---|---|---|---|
| | | Negative | Positive | | |
| TCC | No. | 13 | 32 | 45 | p value: <0.001 |
| | Percentage% | 28.89% | **71.11%** | 100.00% | |
| Control | No. | 16 | **0** | 16 | |
| | Percentage% | 100.00% | 0.00% | 100.00% | |
| Total | No. | 29 | 32 | 61 | |
| | Percentage% | 47.54% | 52.46% | 100.00% | |

**Table (2):** showing the association of immunohistochemical expression of Survivin with tumor grade and muscle invasion.

| Parameters | | Negative score | Positive score | Total | P value |
|---|---|---|---|---|---|
| Grade of TCC | Low grade | 12 (42.9%) | 16 (**57.1%**) | 28(100%) | p value =0.029 |
| | High grade | 2(11.8%) | 15(**88.2%**) | 17(100%) | |
| Muscle invasion | No muscle invasion | 6 (28.6)% | 15 (**71.4**%) | 21(100%) | p value =0.731 |
| | Muscle invasion | 8(33.3)% | 16(**66.7**%) | 24(100%) | |

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# دور السيرفايفين SURVIVIN في سرطان الخلايا الانتقالية للمثانة البولية

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## الخلاصة :

يعد سرطان المثانة ثالث اكثر الاورام الخبيثة شيوعا في العراق لكلا الجنسين ، يشكل سرطان الخلايا الانتقالية اكثر من (٪ ٩٠) من سرطان المثانه . ان معلم السيرفايفين هو احد بروتينات مانعه الاستماته ( الموت المبرمج للخلايا)، ويلعب دور مهم في انقسام الخلايا ومنع عملية الموت المبرمج للخلايا. حيث له القابليه على عبور غالبية السرطانات ولكنه يفقد هذا العبور في الخلايا النسيجية الطبيعية.

**الهدف من الدراسة** : تحديد الدور المحتمل لمعلم السيرفايفين Survivin في تشخيص سرطان الخلايا الانتقالية (TCC)في المثانة البولية.

**المواد وطرق العمل** :

جمع ٤٥ عينة نسيجية شمعية مثبتة بالفورمالين تم استردادها من المواد الأرشيفية لمستشفى الكاظمية التعليمي في مدينة بغداد ، خلال الفترة من كانون الثاني ٢٠١٠ إلى آذار من العام ٢٠١١. اضافة الى ١٦ عينة من نسيج المثانة الطبيعي التي جمعت كمجموعة سيطرة. تم اختيار هذه الكتل النسيجية لتحديد السيرفايفين بطريقة التصبيغ المناعي النسيجي الكيميائي.

**النتائج :**

وجدت الدراسة ان معلم السيرفايفين ليس له القابلية لعبور الانسجة الانتقالية الطبيعية في المثانة البولية ، بينما كانت له القابيلة على العبورفي ٣٢ عينة من اصل ٤٥ (٧١,١%) وكانت القيمة الاحصائية (P:0.001) وان هذا التعبير له علاقة وثيقة برتب السرطان (P:0.029)، بالرغم من عدم وجود علاقة احصائية معنوية (P:0.731) ، لكنه هناك اختلاف في القراءة بين تعبير السيرفايفين و غزو العضلات لسرطان الخلايا الانتقالية للمثانة البولية .

**الاستنتاجات:**

ان معلم السيرفايفين كان عالي التعبير في سرطان الخلايا الانتقالية مقارنه مع الخلايا الطبيعية للنسيج الانتقالي بالمثانة البولية ، وهذا يلعب دور مهم في بقاء الخلايا السرطانية بوساطة التنظيم السلبي لعملية الاستماته. وكذلك يلعب دور مهم في المآل الردي للسرطان


*فرع الاحياء المجهرية / كلية الطب / جامعة ذي قار

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