

## Stress Protein Expression in Bladder Tumors Tissues

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### Abstract

This study was designed to compare between the stress protein HSP70 immunoexpression in the urinary bladder cancer (UBC) and bladder benign tumor (BT) , and to show the association between its immunoexpression and the clinical grades in bladder cancer . Paraffin Sections tissues from 50- bladder cancer tissues and 15-bladder benign tumor tissues included in this study, Immunohistochemical technique was done to detect the immunostaining of stress protein HSP-70 . The results indicated that (HSP70) proteins were significantly high expressed in BC compared with BT patients HSP70 (74 % versus 33.3 % ;  $p \leq 0.01$ ) , and the patients with high grades cancer assume elevated positive IHC results compare to patients with low grades cancer with significant difference ( $p \leq 0.01$ ) . Accordingly, HSP70 can be investigated as an omen biomarker in bladder cancer .

**Key Words:** HSP70, IHC, Bladder Tumors.

### Introduction:

Heat shock proteins (HSPs) are stress proteins that are released and activated as a result for natural stress such as fever and, or artificial stressors, like sudden higher temperature and chemotherapy [1] . There are several types of HSPs such as Hsp60, Hsp70 and Hsp90 (the most widely studied HSPs) [2] . They act as a

chaperone play a big role in proteins interaction with each others like protein conformation, protection from assembly damage proteins. HSPs promote inflammation process through transcription and release cytokines to give dangers sign [3] .

Also these proteins have a crucial role in many human cancers, it is suspected to have role in cancer cells proliferation, differentiation and aggressive metastasis in addition to their role as anti-apoptosis proteins on many steps of this process[2],[3] . HSP70s is the most indispensable HSPs for protein folding and is highly associated with tumor advancement , which may also supply therapeutic strategy of cancer [4] .

HSP70 is marginally expressed in normal cells , but is over expressed in malignancy cells, and in general hopeless prognosis, increased tumor grade and drug resistance of various cancers are related with elevated levels of this protein [5],[6] .

So the goal of this Study to detect the significant biological association between bladder carcinoma (BC) and protein tissue expression of HSP70 as a diagnostic biomarker marker in Iraqi patients with BC by using Immunohistochemical technique (IHC) with HSP70 -monoclonal antibody .

## **Material & Methods**

Fifty biopsies from patients with urinary bladder carcinoma (UBC) were included in this study, the patient samples were collected from AL-Yarmook Teaching Hospital and Gazii AL-Harriri Hospital for Specialists Surgeries. 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 performed by specialist pathologist. In addition 15 biopsies from patients with

bladder benign tumor (BT) . For each case, one representative section was stained with Hematoxylin and Eosin and the histopathological diagnosis was revised, while other sections were put on positive charged slides and stained immunohistochemically for HSP70. Immunohistochemical staining was carried out using the Novocastra TM Polymer Detection Systems (Envision technique) by using commercial kit from Novocastra, Newcastle, UK, RE7150-K , the slides were deparaffinized, rehydrated then antigen retrieval carried out by placing the slide in slide steel holder and it was immersed in 1 L of Tris - EDTA buffer pH=9, in plastic container , it was placed in microwave at (800W) for 10 minutes. After cooling the antigen retrieval solution for 15 – 20 minutes at (4°C), the slides were removed and washed by PBS , after that the slides were blocked with peroxides blocking and protein blocking . All of the slides were treated with Monoclonal mouse Anti-human HSP70 monoclonal antibody, dilution 1:400 (Dako, Denmark), then incubated with a post primary block solution for 30 minutes. In the next step the slides were rinsed gently in PBS 2× 5 and tissue sections incubated with a secondary antibody Novolink TM polymer mouse and rabbit immunoglobulins) for 30 minutes, washed in PBS 2× 5 with gentle rocking. After washing, the samples were stained with diluted liquid DAB, and then counter stained with hematoxylin. Slides washed, dehydrated then mounting, and examining under light microscope at 10X,20X,40X magnification.

## Results & Discussion

At the current time, only few Iraqi studies have been focused on HSP expression in UBC. The last two decades, studies that have determined HSPs expression mostly focused on Western blot, mRNA expression and ELISA. The first two tests are costly and time consuming, while the latter is not specific. Thus, IHC for HSP appears to be feasible in the hospitals for detecting UBC.

In this study the positive immunohistochemical expression of HSP70 protein was significantly higher in UBC patients in comparison with that of BT patients (74 % versus 33.3 % ;  $p \leq 0.01$ .), (Table 1). These results compatible with Syrigos *et al.* (2003) who assume that HSP70 was often overexpressed in vesical tumoral cells of bladder than control [7]. The bladder epithelia showed intense staining of HSP70 (Figure 1), and also it was found in all layers of bladder transitional epithelium. Our results corresponds with the data postulated that HSPs may suppress program death and thus promote tumorigenesis [8].

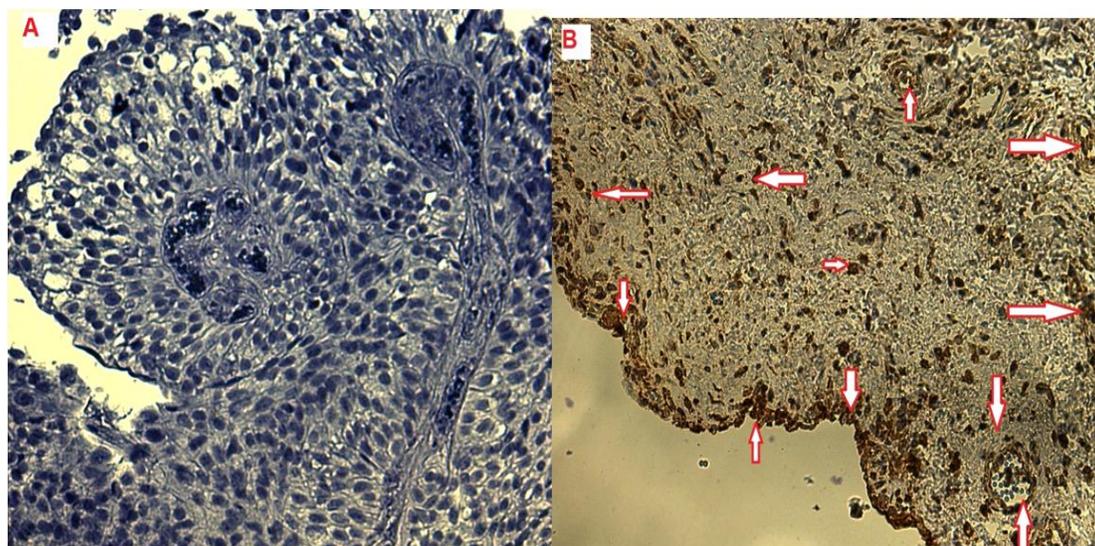
Table (1): HSP70 expression in bladder patients groups

Patients groups	HSP70 immunostaining		Total	Chi-square	P value	
	positive	negative				
UBC	No	37	13	50	8.346	
	Percentage%	74	26	100		
BT	No	5	10	15		
	Percentage%	33.3	66.7	100		
Total	No	42	23	65		0.00393
	Percentage%	64.6	35.4	100		

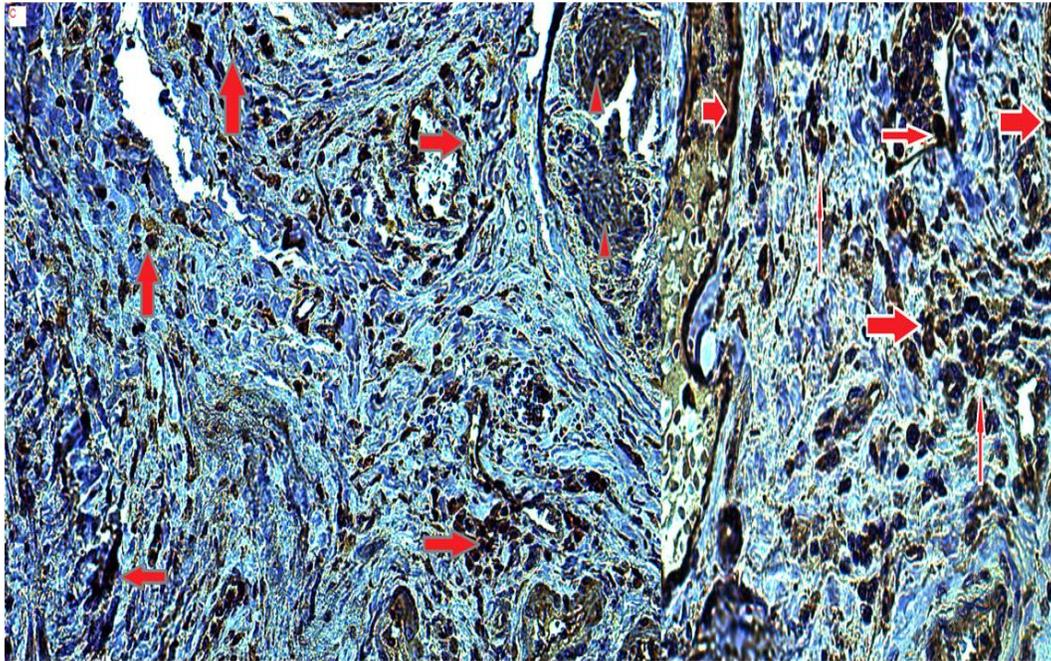
\*\* ( $P \leq 0.01$ ).high significant

In terms of scores, UBC patients with the score ++ represented the highest frequency (48.6%), and a similar observation was made in BT patients but the frequency was higher (60%). However, (Table 2) showed the frequency

distribution of HSP70 expression scores in group subjects. Chi-Square test showed that there was significant difference ( $p \leq 0.01$ ) between urinary bladder carcinoma and bladder benign tumor for HSP70 IHC scores in tissue sample taken from each case. Regarding the tumor grade of UBC, HSP70 was detected in 10 out of 18 of grade-1, 16 out of 19 of grade-2, and 11 out of 13 of grade-3. There was a high significant association between the grade of UBC and the immunohistochemical expression of HSP70 ( $p \leq 0.01$ ), the majority of high grade tumor cases showed positive immunohistochemical HSP70 expression 27(84.4%), while only 10 cases (55.6%) of low grade tumor showed positive immunohistochemical HSP70 expression, (Table 3).



**Figure( 1):** A: Immunohistochemical tissue expression of HSP70 in bladder tissues showing no detectable HSP70 immunostaining (score 0 negative). B: Showing strong positive HSP70 immunostaining in bladder epithelia (Score ++ brown) ( arrow) (20X).



**Figure( 1) : C:** Invasive transitional cell carcinoma , poorly differential(Grade II) showing positive HSP70 immunostaining (Score +++ brown)( arrow) (20X,40X) .

**Table (2):** Frequency of HSP70 IHC scores in patients groups

HSP70 Score	UBC		BT		Total	
	No.	%	No.	%	No.	%
+++	11	29.7	0	0	11	26.2
++	18	48.6	3	60	21	50
+	8	21.6	0	0	8	19
Scatter ≤ 10	0	0	2	40	2	4.8
<b>Total</b>	37	88.1	5	11.9	42	100
<b>P-value</b>	---	<b>0.006</b>	---	<b>0.0012</b>	---	<b>0.002</b>
<b>χ<sup>2</sup> -value</b>		<b>7.519</b> **		<b>10.85</b> **		<b>8.928</b> **

\*\* (P<0.01).High significant

An increased expression of HSP70 has been reported in a number of tumours, such as osteosarcoma bladder cancer [7],[9].The patients with cancer have high levels of HSPs that are associated with poor prognosis , and treatment resistance as

these proteins protect tumour cells from therapeutic stressors such as androgen or oestrogen withdrawal, radiation, metastases, and cytotoxic chemotherapy [10],[11].

In this study our results are in correspondence with the study results of Cappello *et al.* (2006) who demonstrated a significant association of the high grades ( $G \geq 2$ ) of UBC with the percentage of tumor cells positive HSP70 [12]. Syrigos *et al.* (2003) investigated the expression of HSP70 in a series of UBC, finding that this protein was extremely expressed in advanced grades of bladder cancer and assume its function as biomarker [7]. Moreover, other published studies confirm a relation between high HSP-70 expression and tumor aggressiveness and propagation [13]. HSP-70 is proposed to inhibit the energizing of caspases, crossing apoptosis and thus allowing a survival reward of transformed cell [14].

## Conclusion

The high significant differences in the IHC expression score of HSP70 in BC compared with BT and elevated expression in patients with high grades of UBC have led to HSP70 being investigated as a prognostic biomarker in bladder cancer .

**Table (3 )** Association between HSP70 IHC expression with tumor grade

parameter		HSP70 EXPRESSION		Total	P-value $\chi^2$ -value
		positive	negative		
Grade of UBC	Low grade ( $G \leq 1$ )	10(55.6%)	8(44.4%)	18	0.0025 7.418 **
	High grade ( $G \geq 2$ )	27(84.4%)	5(15.6%)	32	
	Total	37(74%)	13(26%)	50	
** ( $P \leq 0.01$ ) high significant					

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