

Heat Shock Protein70 as Biomarker in Bladder Cancer

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Received 7, August, 2013
Accepted 29, October, 2013

Abstract:

The present study aimed to shed light on the urine HSP70 concentration of patients with urinary bladder carcinoma UBC and control subjects as new urinary biomarker. The second aim was to associate this protein concentration with UBC stage and grade in patients with UBC. A direct ELISA was used to quantify urine HSP concentrations in 58 patients with urinary bladder carcinoma UBC with different grades (G) and stages (T) all malignant of them was transitional cell carcinoma (TCC) type, 15 from patients with urinary Bladder disorders other than cancer UBD and 15 healthy subjects (control). Urine concentrations of HSP70 were elevated in patients with UBC compared to those without UBC (healthy and UBD, $P < 0.5$). There was a high significant between mean level of HSP70 in patients with T2 tumor stage as compared with patients with stage T3. Also, there was a high significant increased mean level of HSP70 in patients with G3 as compared with patients with grad1 G1.

Key words: UBC, HSP, ELISA

Introduction:

Bladder cancer (BC) is one of the most prevalent malignancies in economically advanced countries [1]. It is the seventh most prevalent cancer, accounting for 3.2% of all malignancies [2]. Approximately, 80% of cases of bladder cancer are diagnosed in people over the age of 60. It is the second most prevalent cancer in men 60 years of age or older in the United States [3]. 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 [4].

The gold standard for detection of bladder cancer (BC) is cystoscopy. However, cystoscopy is invasive and costly. Currently, voided urine cytology is the only established noninvasive adjunct to cystoscopy. While cytology is sensitive (70–80%) and highly specific (90–95%) for diagnosis of high-grade of bladder cancer, its sensitivity is as low as 6–38% for low grade tumors. Several

new accurate biomarkers for analysis of voided urine have recently been proved for clinical use, including HSP70, which consider one of new molecule that might has a potential role for the diagnosis of bladder cancer [5]. Such markers can help to detect clinically occult bladder cancer and can increase the interval of cystoscopic evaluation [6].

HSP70 regulates a wide range of protein-associated activities [7]. Expression of HSP70 is enhanced after transformation by oncogenes. Elevated levels of HSP70 protect cells from apoptotic death induced by TNF- α and TNF- β , HSP70 interacts with p53 to stabilize mutant but not the wild-type protein. Conversely, wild-type (but not mutant) p53 down-regulates HSP70 expression [8]. HSP70 could be an interesting biomarker because its overexpression in serum is associated with many cancers. However, these studies are mostly qualitative. HSP70 is considered to be the most universally

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stress inducible HSP with reported inductions of over 200-fold [9] .

Cancer often develops with an associated local inflammatory response [10] . Because the urine of patients with BC is in close contact with tumor cells and adjacent inflamed urothelium, we hypothesized that immune mediators in urine may serve as biomarkers for BC. We therefore examined the urine for heat shock protein70 (HSP70).

The aim of this study was to evaluate urine HSP70 concentration of patients with urinary bladder carcinoma UBC and control subjects . The second aim is to associate this protein with UBC stage and grade .

Materials and Methods:

Urine samples from (58) patients with urinary bladder carcinoma (UBC) that all malignant of them was transitional cell carcinoma (TCC) type from Al-Yarmook Teaching Hospital in Baghdad, and Baghdad Hospital for Specialists Surgeries were included in this study. In addition, (15) patients with urinary bladder disorders other than cancer (UBD) and 15 healthy subjects were as a control groups. The period of study from May-2011 to May-2012 were eligible for this study. The cases were diagnosed clinically by consultant urologists at Al-Yarmook Teaching Hospital, and Baghdad Hospital for Specialists Surgeries. Urine samples were centrifuged at 1500 rpm, for 5 minutes ,the supernatant was frozen at -20°C until the (HSP-70) measurement by ELISA [11]. HSP70 concentrations was quantitatively determined in urine of patients and healthy control subjects by means of ELISA (Enzyme Linked

Immunosorbent Assay) using ready kits manufactured by USBiological company(USA) .

Ethical permission to conduct the research was obtained from these hospitals and from all participants in this study. Selections of the patients were accomplished with the assistance of surgeons in the hospitals .

Statistical analysis

Statistical analysis was performed with the statistical package for social science SPSS19.0. Univariate analysis using one-way analysis of variance (ANOVA) was performed to assess the differences in HSP70 concentrations between groups. When the ANOVA test demonstrated a significant value, post hoc least significant difference analysis was used to determine statistically significant differences between means at significant level ≤ 0.05 .The data were presented in terms of means \pm standard errors (S.E.)

Results and Discussion:

In this study UBC patients with TCC showed a significant($P < 0.05$) increased mean levels of HSP70 ($2.635 \pm SE 0.38819$ ng /ml), as compared with the healthy control and UBD patients ($0.477 \pm SE 0.03197$ ng /ml and $0.985 \pm SE 0.13928$ ng /ml, $p = 0.021, 0.03$, respectively),Figure (1) while there was no significant difference($P > 0.05$) between UBD patients and healthy control ($p = 0.057$). This result agrees with Margel *et al.* who suggested that UBC is associated with increased urinary levels of HSP70 compared with control [12] .

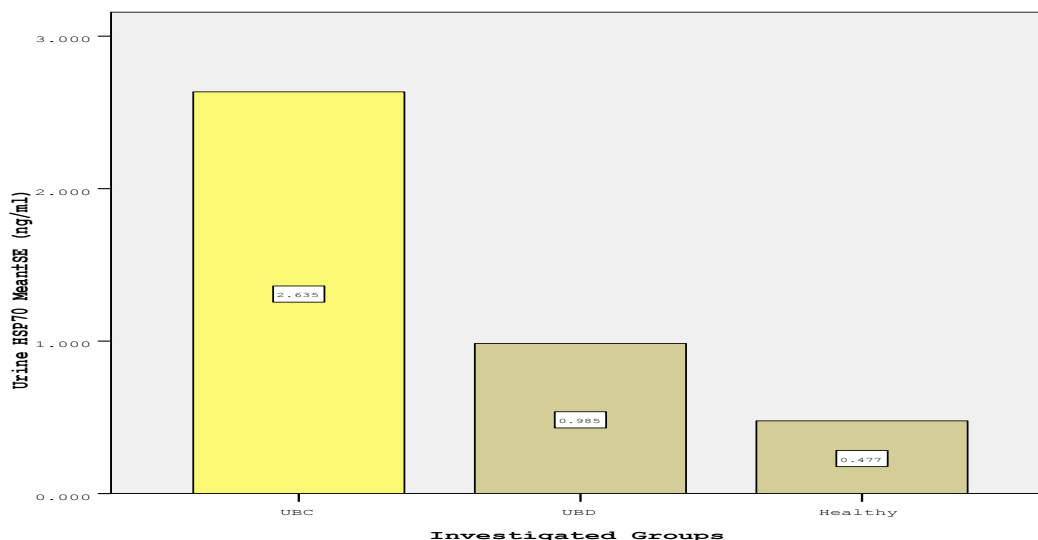


Fig.1: Urine level of HSP70 in patients and healthy controls

The relationship between urine HSP70 mean levels and tumor stages of patients with bladder cancer Figure (2) showed a high significant increased mean level of HSP70 ($3.880 \pm SE 0.75645$ ng /ml) in patients with T2 tumor stage as compared with patients with stage T3 ($1.420 \pm SE 0.20702$ ng /ml , $p=0.009$) while there was no significant static difference between T2

and T1 stages ($3.880 \pm SE 0.75645$ ng /ml and $2.282 \pm SE 0.68508$ ng /ml, respectively $p=0.076$) and no significant static between T3 and T1 patients ($1.420 \pm SE 0.20702$ ng /ml and $2.282 \pm SE 0.68508$ ng /ml, respectively $p= 0.36$), Although the difference in urine HSP70 mean levels were observed between them .

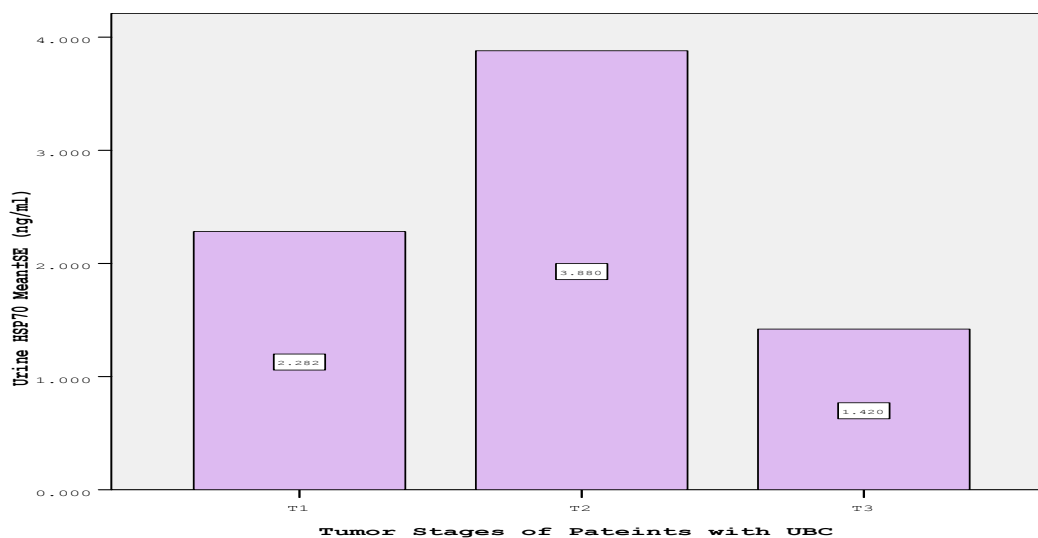


Fig. 2: Urine level of HSP70 in tumor stages of patients with UBC

The relationship between urine HSP70 mean levels and tumor grades of patients with bladder cancer figure (3) showed a high significant increased mean level of HSP70 (3.73 ng /ml \pm

SE 0.83) in patients with G3 as compared with patients with grad1 G1 (1.18 ng /ml $\pm SE 0.24087$, $p=0.009$) while there was no significant static difference between G1 and G2 stages

($1.18 \pm SE 0.24$ ng/ml and $2.815 \pm SE 0.63527$ ng /ml, respectively $p=0.079$) and no significant static between G2 and G3 patients ($2.86 \pm SE 0.64$ ng /ml and $3.73 \pm SE 0.83$ ng /ml, respectively $p= 0.3$), Although the difference in urine HSP70 mean levels were observed between them . The present results compatible with the results of Margel *et al.* [12] ,who suggested that UBC is associated with increased urinary levels of HSPs like HSP-70 , and HSPs were urinary biomarkers for diagnosis and staging of bladder cancer . Syrigos *et al.* [13] have shown that HSP70 was frequently overexpressed in vesical tumoral cells and it could be a useful biochemical marker for bladder cancer , and finally agreed with Yu *et al.* who suggested Prognostic significance of HSPs in

urothelial carcinoma of the urinary bladder [14].

The results demonstrated a significant association between BC and urinary levels of HSP70s . Since this study is limited by the small number of samples analyzed, it needs further validation in a larger cohort, perhaps utilizing a “high throughput” ELISA-based platform. Despite its small numbers, these findings provide a good foundation for the development of a urinary biomarker for BC . In addition, they support a mechanistic insight: BC cells might evade immune surveillance by producing, or inducing other cells to produce HSP molecules resulting in the suppression of effector immunity and suppress apoptosis and thus promote tumorigenesis [8]

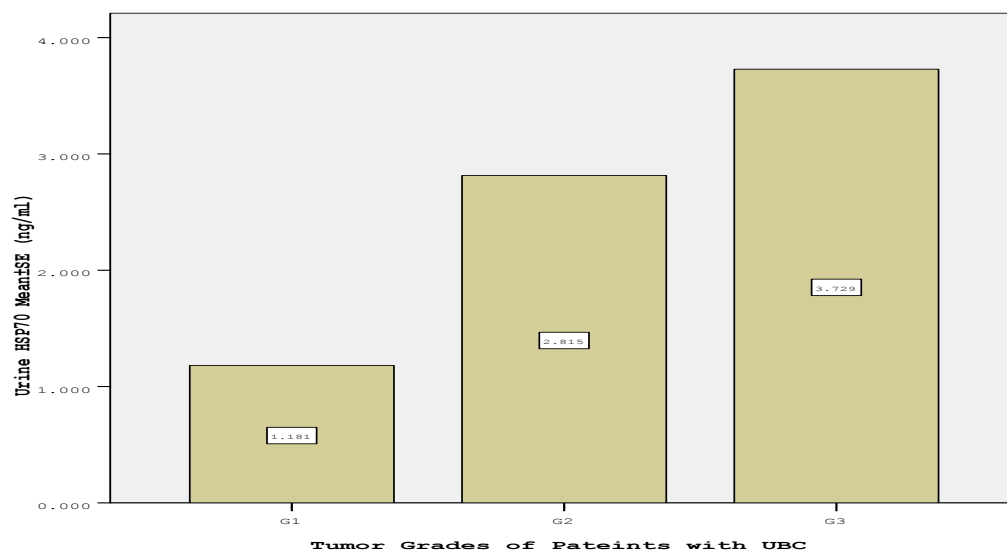


Fig.3: Urine level of HSP70 in tumor grades of patients with UBC

Finally, our results agreed with the fact that HSP70 expression is associations with poor differentiation, lymph node metastasis, increased cell proliferation, block of apoptosis., and higher clinical stage, which are markers of poor clinical outcome . Overproduction of HSP70 causes cells to be resistant against “apoptosis-inducing agents” and leads to increased malignancy and therapy resistance. On

the other hand, down regulation of HSP70 causes increased cell death [15].

References:

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, and Thun MJ .2007. Cancer statistics. CA Cancer J Clin 57: 43–66.

2. El-Mosalamy H. , Salman T.M., Ashmawey A. and Osama N. .2012. Role of chronic E. coli infection in the process of bladder cancer- an experimental study. *Infec. Agen. Cancer*, 7:19.
3. Bernard H, 2008. Epidemiology and etiology of bladder cancer up-to-date. Waltham, MA: up-to-date.
4. Iraqi Cancer Registry (ICR) 2005.
5. Yossepowitch O, Herr HW, and Donat SM. 2007. Use of urinary biomarkers for bladder cancer surveillance: patient perspectives. *J Urol*;177: 1277–82.
6. Anthony C., Woodman , Steve G., Marcus D., Jeremy N., and David T.,2000: Noninvasive diagnosis of bladder carcinoma by enzyme-linked immunosorbent assay detection of CD44 isoforms in exfoliated urothelia, *Clin Cancer Res* . 6:2381-2392.
7. Lanneau,D.,Wettstein,G.,Bonniaud, P., and Garrido,C. 2010.Heat shock proteins :cell protection through protein triage *Sci.World J.*;10 1543–1552.
8. Garrido C, Gurbuxani S, Ravagnan L, and Kroemer G. 2001. Heat shock proteins: endogenous modulators of apoptotic cell death. *Biochem Biophys Res Commun* 286: 433-442.
9. Suzuki,K.,Ito,Y.,Wakai,K.,Kawado, M., Hashimoto,S.,Seki,N.,Ando, M., Nishino,Y.,Kondo,T.,Watanabe, Y.,Ozasa,K.,Inoue,T., and Tamakoshi ,A . 2006.Serum heat shock protein70 levels and lung cancer risk :acase-control study nested in alarge cohort study. *Cancer Epidemiol .Biomarkers Prev.* 15, 1733–1737.
10. Lin WW, and Karin M. 2007. A cytokine-mediated link between innate immunity, inflammation, and cancer. *J Clin Invest*;117:1175–83
11. Sanchez-Carbayo M., Urrutia M., Conzalez de Buitrago J.M., and Navajo J.A. 2000. Evaluation of two new utinary tumor markers: Bladder tumor fibronectin and Cytokeratin 18 for the diagnosis of bladder cancer. *Clin Cancer Res* , 6, 3585-3585.
12. Margel , D. , Pesvner-Fischer, M. , Baniel J., Yossepowitch O., and Cohen I. R. 2011. Stress Proteins and Cytokines are Urinary Biomarkers for Diagnosis and Staging of Bladder Cancer. *Eur. Uro.* (59):113-115.
13. Syrigos KN, Harrington KJ, Karayiannakis AJ, Sekara E, Chatziyianni E. Syrigou EI, and Waxman J. 2003. Clinical significance of heat shock protein-70 expression in bladder cancer. *Urology* 61: 677-680
14. Yu H-J., Chang ,Y- H, and Pan ,C.C.,.2013. Prognostic significance of heat shock proteins in urothelial carcinoma of the urinary bladder . *Histopathology J.* 62,(5): 788–798.
15. Mayer, M.P. and Bukau, B. 2005. HSP70 Chaperones: Cellular Functions and Molecular Mechanism. *Cell Mol Life Sci.* , 62:(6), 670-684.

بروتين الصدمة الحرارية نوع 70 كمعلم حيوي لسرطان المثانة

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الخلاصة :

هدفت الدراسة الحالية على تسليط الضوء على مستوى تركيز بروتين الصدمة الحرارية نوع 70 في مرضى سرطان المثانة البولية ومرضى اضطرابات المثانة البولية والأشخاص الأصحاء كمعلم حيوي حديث في الإدرار ، وثانيا دراسة ارتباطه مع مراحل ومراتب مرضى سرطان المثانة البولية . إذ تم استخدام اختبار الاليزا المباشر لتقدير مستوى تركيز البروتين في الإدرار لـ 58 مريض يعانون من سرطان المثانة البولية بمراحل ورتب مختلفة معظم الاورام كانت من نوع سرطان الخلايا الانتقالي الحرشي، 15مريض يعانون من اضطرابات المثانة البولية و15 أشخاص أصحاء . وجدت الدراسة إن مستوى تركيز معلم الصدمة الحرارية نوع 70 في الإدرار عالي إحصائيا في مرضى سرطان المثانة البولية مقارنة بمرضى اضطرابات المثانة البولية والأصحاء وكانت القيمة الإحصائية ($P < 0.05$) بين المجاميع . الدراسات التجريبية اعتقدت إن بروتين الصدمة الحرارية يلعب دور مهم في بقاء الخلايا السرطانية بوساطة التنظيم السلبي لعملية الاستماتة (الموت المبرمج) وبالتالي يساهم في تعزيز تقدم الورم . وبينت النتائج أيضا وجود علاقة عالية المعنوية بين مرحلة T3 مقارنة بمرضى السرطان مرحلة T2 ، كذلك كان هناك علاقة عالية المعنوية بين مرضى السرطان رتبة G3 مقارنة بمرضى السرطان رتبة G2 وتركيز البروتين .