

## Evaluation of IGF-2 Gene Expression in Urine and its Potential Use as Biomarker for Bladder Cancer

A.M.El-Abd<sup>1</sup>, H.W.Sherif<sup>2</sup>, O.E.Abdallah<sup>1</sup>, H.M.AbdElkareem<sup>1</sup>, O.Y.Habashy<sup>1</sup>

<sup>1</sup>Medical Biochemistry, Dept., Faculty of Medicine, Benha Univ., Benha, Egypt

<sup>2</sup>Urology, Dept., Faculty of Medicine, Benha Univ., Benha, Egypt

E-Mail:amina@gmail.com

### Abstract

Bladder disease is the tenth most normal malignant growth around the world. The analysis and follow-up of patients require exorbitant obtrusive strategies and because of these costs, bladder disease keeps on being one of the costly malignancies. Early determination is pivotal in bladder disease for what it's worth in different tumors; in this way, non-intrusive biomarkers for early conclusion are vital. The point of the investigation was to analyze urinary insulin-like development factor 2 (IGF-2) levels in persistent pee tests and decide the capability of IGF-2 as a marker for the presence of urothelial carcinoma of the bladder (UCB). Techniques: 50 patients with bladder disease and 20 solid controls. Quantitative constant converse tran-scription polymerase chain response (qRT-PCR) was led to quantify the IGF-2 articulation levels in pee of patients with bladder malignant growth and sound con-trols. Results: patients with UCB have essentially raised degrees of urinary IGF-2.

**Keywords:** IGF-2, bladder carcinoma.

### 1. Introduction

Bladder disease is the ninth most basic malignant growth analysis around the world, with in excess of 330,000 new cases every year and in excess of 130,000 passings each year. In Western nations, momentary cell tumors include 90%-95% of bladder tumors; 3%-7% are squamous cell, and 1%-2% are adenocarcinomas [1].

In Egypt, bladder malignancy has been the most widely recognized disease during the previous 50 years [2].

At the hour of analysis about 75% of tumors are restricted to the bladder mucosa or submucosa and can be taken out by transurethral resection (TUR). Patients stay at a high danger of repeat (41-46%). Consequently, they need a long lasting and regular development [3].

Set up indicative tests are the voided pee cytology (VUC) and cystoscopy, trailed by histopathological assessment. VUC has an affectability of 10-25% for second rate tumors, so it is frequently utilized along with cystoscopy [4].

One significant approach to decrease death pace of bladder disease is to lessen its repeat rate, in this way more compelling and prescient biomarker for malignant growth repeat and anticipation is fundamental.

Insulin like development factor 2 (IGF-2) is a long peptide chemical like insulin and is discharged by numerous tissues, yet especially by liver cells. It has insulin-like metabolic impacts. It is a fetal development factor and a strong mitogen and apoptosis inhibitor [5].

IGF-2 over-articulation is normal being developed of various malignancies, including ovarian [11], prostate disease [15], bosom [14] and colorectal [12].

IGF-2 articulation was one of the markers that can be recognized in pee, however it was not altogether assessed as an individual marker for bladder malignancy [13].

### 2. Subjects and method

This investigation was acted in the Molecular Biology and Biotechnology Unit, Medical Biochemistry Department, Faculty of Medicine, Benha University. furthermore, getting educated assent from included patients. The investigation included 70 subjects of both genders (49 guys and 21 females) chose from Urology Department, Faculty of Medicine, Benha University Hospital. Their ages went from 30-65years. Bladder disease bunch included 50 patients and control gathering of 20 people with typical urothelium.

Pee was gathered in 100-mL sterile cups and set aside momentarily quickly to be conveyed to the research facility inside 2 hours. Tests are kept frozen at -80 °C until to be utilized for discovery of quality articulation levels of IGF-2 by qRT-PCR.

Quantitation of target quality mRNA articulation by Real-Time PCR:

- 1- Total RNA Extraction: from pee pellet tests utilizing the PureLink® RNA Mini Kit (Ambion, USA), adhering to the producer's guidelines. Eluted RNA (50µl) was evaluated by Ultraviolet Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA): absorbance at 260nm ought to be >0.15. An absorbance of 1 unit at 260nm relates to 40µg of RNA/ml. The proportion between the absorbance esteems at 260 and 280nm gives a gauge of RNA immaculateness, unadulterated RNA has a proportion of 1.9-2.3 (12). RNA was quickly put away at - 20oC till additional handling.
- 2- Relative quantitation of target quality mRNA by constant PCR: on 2 stages;

The first step was for transformation of RNA into cDNA utilizing RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) (Wiame et al., 2000) as indicated by maker's guidelines. in Veriti™ Thermal Cyler (Applied Biosystems). The PCR blend

for cDNA included absolute RNA (5µl), 5x TransAmp Buffer (4µl), Reverse Transcriptase (1µl) and up to 20µl nuclease free-water with the warm program; 25oC for 10min, 42oC for 15min and 85oC for 5min.

The second step was for quantitation of target quality articulation in Stepone Real-Time PCR System (Applied Biosystem, Singapore). Singleplex responses were finished. This progression was performed utilizing SensiFASTTM Sybr Hi-Rox Kit (Bioline Reagents Ltd, United Kingdom).

#### Data Analysis

The data, produced as sigmoid-shaped amplification plots (the cycle number is plotted against fluorescence on the linear scale), were analyzed by the Relative Quantitation (RQ) manager program 1.2 ABI

SDS software (ABI 7900HT). Because the control samples are used as calibrators, their expression levels are set to 1. But because the expression levels were plotted as log10 values (log10 of 1 is 0), the expression level of the control samples appear as 0 in the graph. Because the relative quantities of the target gene are normalized against the relative quantities of the endogenous control GAPDH gene, GAPDH has no bars in the graph [1].

#### 3. Results

The results of this study have shown that the concentration of IGF-2 in the urine of patients presenting with UCB is significantly higher than in patients without UCB as shown in Table (1).

**Table (1)** Urinary IGF-2 gene expression in bladder cancer group and control group.

Variable	Control group (n=20)	Cancer group (n=50)	Test	P value
Urinary IGF-2 (Mean ±SD)	4.6±1.4	5.6±0.8	3.91	<0.001**
	P<0.05 significant		P>0.05 non-significant	

#### 4. Discussion

Disease is a main source of death and a significant medical issue around the world. Bladder disease (BC) is the tenth most regularly identified malignant growth in both genders and ninth most deadly threat in men [9].

The non-intrusive analysis of new-beginning and repetitive BCa stays a test. VUC, as the current clinical standard strategy for the non-intrusive identification of BCa, is hindered by a deficient affectability between 12–84%, regularly as low as 35% [9].

Our examination included two gatherings with a sum of 50 BCa patients and 20 controls. The general urinary record levels of IGF2 were essentially raised in BCa patients contrasted with control gatherings. The outcomes were in concurrence with an investigation directed by [6]. They tracked down that the general urinary record levels of IGF2 was altogether raised in BCa patients contrasted with control bunches taking all things together tumor stages and grades.

Prior examination by [7] announced that the explicitness and affectability esteems for the IGF-2 urinary measure were 95%, 80 % separately. Urinary IGF-2 groupings of were estimated utilizing IGF-2 ELISA unit in 65 pee tests of 25 patients determined to have BC and 40 BC negative.

Expanded articulation of IGF2 as a result of the deficiency of its engraving is habitually found in an assortment of human tumors. Moreover, strange sign transduction and additionally advertiser enactment was accounted for as a significant system for the IGF2 overexpression in an assortment of tumors including bladder carcinoma, hepatocellular carcinoma, bosom malignant growth, ovarian disease, and prostate disease [8].

As of now, patients go through cystoscopy to analyze and follow-up UCB. A precise pee test for

proteins, for example, urinary IGF-2 would offer patients a less obtrusive and awkward option to cystoscopy for the determination of UCB. Further investigation of this marker in bigger companions of patients with UCB or as a feature of a consolidated board of UCB markers will clarify the maximum capacity of urinary IGF-2 as a component of the progressing look for an exceptionally delicate and explicit urinary biomarker of UCB.

#### 5. Conclusion

Raised relative record levels of IG-F2 in pee could be utilized to distinguish BCa at essential finding and could be utilized as a potential biomarker for determination of bladder disease.

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