The Role of Urinary Fibronectin and Telomerase in Patients with Superficial Bladder Cancer after Transurethral Resection Treated with BCG

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ABSTRACT

Objective: Bacillus Calmette-Guérin (BCG) has been shown to be an effective treatment for superficial transitional cell carcinoma (TCC) of the bladder, but the precise mechanism of action of BCG remains poorly understood. Fibronectin (FN) has been found to play a role in BCG therapy. Although adjuvant BCG has been shown to improve the clinical outcome of superficial bladder cancer (SBC), a significant proportion of patients fail to respond to it. Identification of the subset of patients that are going to develop recurrent disease or stage progression after BCG therapy is very important. Telomerase activity represents a potential tool for tumor detection. The aim of the present study was to give an insight into the diagnostic and prognostic values of the quantitative urinary estimation of both fibronectin (FN) and the catalytic subunit of the complex human telomerase reverse transcriptase [hTERT]) as non invasive tumor markers for early detection of patients with high risk superficial transitional cell carcinoma (TCC) and after trans-urethral resection of bladder tumors (TURBT) that received adjuvant BCG immunotherapy to explore its association with recurrence-free-survival (RFS) or development of invasive disease. Materials and methods: The study was carried out on 10 healthy individuals as a control group (group I) and 20 Egyptian patients with histologically confirmed superficial bladder cancer (group II). Patients were underwent formal TURBT. Morning urine samples were collected from every patient, 1 day before and 2days, two weeks, six weeks, three months, six months and one year after TURBT. Also, morning urine samples were collected from the healthy individuals. Urine samples were used for cytological examination, estimation of fibronectin level using ELISA technique and for the quantitative analysis of hTERT using quantitative real time RT-PCR technique. Results: The results showed that urinary fibronectin level and normalized hTERT- mRNA were significantly higher in SBC patients before TURBT (group II) versus control group (P<0.001). hTERT-mRNA and urinary fibronectin levels were significantly different in group II before and 2 days after TURBT. Urinary fibronectin and normalized hTERT- mRNA were significantly lower 2 weeks and 6 weeks after TURBT than patients group 2 days after TURBT. Both markers were significantly lower in fifteen patients 3 months, 6 months and one year after TURBT when compared with their levels 2 days after TURBT. There was insignificant difference of both markers between the control group and fifteen patient one year

after TURBT. The remaining five patients suffered recurrence of tumor. This approach enabled us to identify cutoff values of urinary fibronectin which characterized by 90% sensitivity and 100% specificity and 93.3% accuracy, and that for urinary normalized hTERT with 85% sensitivity and 80% specificity and 83.3% accuracy. **Conclusion:** *On the basis of these results, it could be concluded that both urinary fibronectin and hTERT- mRNA can be considered from the best markers for diagnosing superficial bladder carcinoma. These parameters can be also used to determine the presence of residual tumor load after TURBT; also they can give an insight about the expected response to BCG immunotherapy after TURBT and detect the BCG nonresponder patients. It seems conceivable that these emerging urine-based tests may progressively replace urine cytology and form a reliable adjunct to cystoscopy in the diagnosis and follow up of patients with superficial bladder cancer.*
Key wards: *Bacillus Calmette-Guérin (BCG), Superficial bladder cancer (SBC), Fibronectin (FN), transitional cell carcinoma (TCC), carcinoma in situ (CIS), human telomerase reverse transcriptase (hTERT), transurethral resection of bladder cancer (TURBT)*

INTRODUCTION

Carcinoma of the urinary bladder is the most common malignant tumor of the urinary tract and the second most common malignancy, after prostatic carcinoma, of the urogenital system. Males are about three times more frequently affected than females[1].

Seventy-four percent of cases are superficial at the time of diagnosis, of which 70% are stage Ta and 30% are stage T1. Low-grade non-invasive tumors may be treated with resection and fulguration. However, despite complete tumor resection, two thirds of patients will develop tumor recurrence in five years and by 15 years, 88% of patients will develop a recurrence. Progression from superficial bladder cancer to deep muscle invasion occurs in 15% of patients. The high rate of tumor recurrence and potential progression provides an opportunity to institute chemoprevention or prophylactic therapy[2, 3].

The initial treatment of superficial bladder cancer (SBC) is transurethral resection of all visible bladder tumors (TURBT) followed by adjuvant intravesical immunotherapy, most commonly with Bacillus Calmette-Guerin (BCG), which is offered to patients at high risk for tumor recurrence and/or development of invasive disease[4].

BCG therapy has been shown to delay or prevent tumor recurrence, stage progression, decrease the need for subsequent cystectomy, and improve overall survival[5]. However, a significant proportion of patients do not respond to intravesical BCG therapy; their tumors persist or recur early after BCG and may become invasive and/or metastatic. Cystectomy is an effective salvage therapy for patients in whom BCG therapy fails, as it has been shown to prevent stage progression and death[6]. In that regard, a tool to predict the

response to intravesical BCG therapy would be invaluable, as it could define a subset of patients that may benefit from early salvage cystectomy [3].

The antineoplastic effect of BCG is most likely the result of a combination of enhanced activity of various arms of the immune system. After intravesical instillation, live mycobacteria attach to the urothelial lining, facilitated by fibronectin, a component of the extracellular matrix. This process leaves bacterial cell surface glycoproteins attached to epithelial cell membranes, and this antigen is thought to mediate the immune response[7,8]. Intravesical instillation of bacillus Calmette-Guérin (BCG) has been established as the most efficient strategy for the prophylaxis of superficial bladder cancer recurrence after complete resection of visible tumors. The antitumor effects of intravesical instillation of BCG vaccine rely mainly on its activation of local immune response, and many factors in both BCG itself and host status are related to therapeutic efficacy [9].

Fibronectin is a glycoprotein with a molecular weight of 440 kDa, found in soluble form in plasma and other body fluids, and in an insoluble (matrix) form in basement membranes and the extracellular matrix. Fibronectin (FN) seems to play a key role in progression and invasion of malignant tumors[10]. BCG cells possess specific high-affinity receptors to fibronectin molecules [9]. The initial crucial step in BCG therapy seems to be the binding of mycobacteria to the urothelial lining which depends on the interaction of a fibronectin attachment protein (FAP)

on the bacterial surface with the fibronectin in the bladder wall [10]. *In vitro* studies showed that a stable BCG/fibronectin interaction was dependent on FAP binding to fibronectin [9]. *In vivo* studies show FAP to be a necessary protein for the stable attachment of BCG to the bladder wall [10]. Pretreatment of BCG with soluble fibronectin competitively blocks the binding of BCG to exposed matrix (insoluble) fibronectin; this could result in the preferential and irreversible binding of BCG to soluble fibronectin. This would decrease the attachment of BCG to the bladder wall and subsequently reduce its efficacy. Moreover, stable binding of BCG *via* FAP within the bladder was shown to be necessary for the expression of BCG-induced antitumor activity[11]. Despite of BCG successful use in the prevention of superficial bladder cancer recurrence, non-responders and certain side effects remain a major obstacle [12].

Established approaches for detecting bladder cancer include urine cytology and cystoscopy, used singly or in sequence [10]. However, the invasiveness and relatively high cost of cystoscopic examination and the limited sensitivity of urinary cytology, especially for low-grade superficial lesions, make it of the utmost importance to develop a non invasive, reliable, and simple test to increase the rate of detection of superficial bladder cancer. During the last two decades, the better understanding of the molecular mechanisms involved in carcinogenesis and tumor progression has provided a large number of molecular markers of bladder cancer, with a potential diagnostic and

prognostic value [13]. Among the markers investigated for that purpose, an important role has been played by telomerase activity [14].

Telomerase is a ribonucleoprotein consists of three subunits: an RNA component (hTR), which acts as a template for DNA replication; a telomerase-associated protein (TP1) of unknown function; and the telomerase reverse transcriptase (hTERT), which is responsible for catalytic activity[15]. Activation of telomerase is a critical event in human cell immortalization and carcinogenesis[16]. Telomerase is normally not expressed in normal human somatic cells; however, telomerase activity has been detected in 85% of patients suffering from various cancers[17].

Measurable levels of telomerase have also been observed in body fluids of cancer patients' including the blood sera and in voided urine after prostatic massage in patients with prostate cancer [14,18, 19]. Such clinical tests for telomerase may have great utility as non-invasive, cost-effective methods for the early detection and monitoring of cancer [20].

The aim of the present study was to give an insight into the diagnostic and prognostic values of the quantitative urinary estimation of both fibronectin (FN) and the catalytic subunit of the complex human telomerase reverse transcriptase (hTERT) levels as non invasive tumor markers for early detection of patients with high risk superficial transitional cell carcinoma (TCC) and after transurethral resection of bladder tumors (TURBT) that received adjuvant BCG immunotherapy to explore its association with recurrence-free-survival (RFS) or development of invasive disease.

PATIENTS & METHODS

The present study was carried out on the following groups; Group I (control group) included 10 healthy individuals, 7 males & 3 females; their ages ranged 40-63 years, with a mean of 51.0 ± 8.2. Twenty patients with histologically confirmed superficial bladder cancer (group II), 15 males and 5 females, their ages ranged 46-71 years, with a mean of 56.7 ± 7.5. They underwent formal transurethral resection of bladder cancer (TURBT). Full clinical, surgical and pathological data were prospectively collected from all patients.

Exclusion criteria: Patients with previous intravesical treatment of any kind (TURBT, BCG, and biopsy) within the three months preceding the operation, massive hematuria, documented infection, significant proteinuria and finding of residual/recurrent tumor on the first follow-up cystoscopy were excluded from the study. Written consent was taken from all participants.

From all patients, about 100 ml morning urine samples were collected, 1 day before and 2 days, two weeks, six weeks, three months, six months and one year after TURBT. Samples were not collected on the first day after TURBT because several patients were still connected to bladder irrigation and in some patients the bleeding remained significant. All patients included in this study were selected from Urology Departments,

Tanta University Hospital, Egypt. Also, about 100 ml morning urine samples were collected from 10 healthy volunteers who served as controls (group I). Aliquots of urine samples were stored at -70ºC for the subsequent use.

Urine preparation:

From all subjects, about 100 ml morning urine samples were collected for routine urine analysis, cytological examination and specific investigations. Urine samples were directly transported on ice to the laboratory where they immediately processed. Each urine sample was divided as following; 10 ml for routine urine analysis, 5 ml for culture and sensitivity if indicated, specimen was tested with the hemoglobin dipstick, 15 ml were sent to the Pathology Department, Faculty of Medicine, Tanta University for cytological examination and 10 ml were used for estimation of fibronectin after removal of particulates by centrifugation and storing samples at -70°C until the time of the assay. For the quantitative analysis of the catalytic subunit of the complex human telomerase reverse transcriptase (hTERT) in urine, 12 ml of fresh urine were collected in 15 ml test tube and centrifuged at 2000 rpm, at 4ºC for 5 minutes immediately after collection for reduction of the denaturing effects of RNases and proteases that are present in the urine. After discarding the supernatant, the cell pellet was resuspended in 1400 µl phosphate buffer saline (PBS) (4 ºC) and transferred to 1.5 ml Eppendorf tube. The tube was centrifuged at 2000 rpm, at 4ºC for 5 minutes; the supernatant was discarded [21]. The cell pellet was stored at -70 ºC until RNA isolation.

Patients group didn't show any history of upper urinary tract disorders as confirmed by radiological and laboratory investigations. Superficial bladder cancer diagnosis was based on clinical, radiological, cystoscopy and histopathological examinations. Culture and sensitivity tests for proper antibiotic treatment were done for all bladder cancer cases that showed associated cystitis, consent was taken from all subjects included in the current study.

Serum samples:

Peripheral venous blood samples were taken and transferred to disposable tubes. The tubes were promptly centrifuged at 4ºC, and aliquots of serum were immediately stored at -70ºC for estimation of blood urea and serum creatinine and for indirect hemagglutination test for schistosomiasis.

All subjects were subjected to the following:

1. Thorough clinical examination.
2. Radiological studies including X-ray and ultrasonography for detection of urinary tract stone and mass (for patients only).
3. Routine urine analysis. Culture and sensitivity if indicated.
4. Kidney function: blood urea [22] and serum creatinine [23].
5. Cystoscopy and biopsy (for patients only).
6. Urine cytological examination.
7. Indirect hemagglutination test for schistosomiasis [24].
8. Hematuria screening by hemoglobin dipsticks (hemastix) (Bayer Diagnostic, USA) [25].

9. Urinary fibronectin level [26].
10. Quantitative assay of human telomerase reverse transcriptase – mRNA (hTERT- mRNA) in urine, using real time RT-PCR.
11. BCG treatment of SBC patients started 2 weeks and weekly for six subsequent weeks after TURBT.
12. Follow-up of patients was done for one year after surgery. Follow-up included cystoscopy and biopsy, urine cytology, hematuria screening, quantitative assay of both hTERT- mRNA and fibronectin in urine.
13. Histopathological examination was done for all patients to confirm the diagnosis of malignancy.

Assay of urinary Fibronectin:

Urinary fibronectin levels were determined by the enzyme linked immunosorbent assay kit (ELISA) purchased from MaxiSorp Nunc immunoplates (Nunc, Denmark) according to manufacturer's instructions [26].

Gene expression quantification assay of human telomerase reverse transcriptase – mRNA unit (hTERT-mRNA) in urine, using quantitative real time RT-PCR (Applied Biosystem, USA): hTERT gene expression quantification assays were performed in a two steps quantitative real time reverse transcription-polymerase chain reaction (QRT-PCR). In the reverse transcription step, cDNA is reverse transcribed from RNA samples using random hexamers. In the real time polymerase chain reaction step, products are amplified from cDNA

samples using the AmpliTaq Gold® DNA polymerase and TaqMan® Pre-Developed Assay Reagents primers "PDARs" and TaqMan fluorescent probe. During the reaction, the AmpliTaq Gold DNA polymerase, with its 5'exonuclease activity, cleaves the fluorescent probe between the reporter and the quencher dyes, resulting in increased fluorescence of the reporter. The accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye [27].

Procedure:

Real time QRT-PCR for hTERT-mRNA was done in seven main steps including:

1. Urine preparation [21].
2. RNA isolation [28].
3. Assuring RNA quantity and purity [29].
4. Preparing the human reference total RNA standard curves for the hTERT-mRNA and GAPDH internal control (Stratagene, USA) [30].
5. First step-PCR: cDNA synthesis (RT-Step) using MultiScribe ™reverse transcriptase enzyme [31].
6. Second step-PCR: cDNA amplification.

The TaqMan® pre-developed assay reagents, "PDARs", composed of two primers and TaqMan MGB – fluorescent probe designed for the detection and quantification of the specific genetic sequences of the hTERT-mRNA where target and internal control (GAPDH) assays are performed in separate wells.

The sequences of the primers and probe of the hTERT target are:
hTERT forward primer: 5'-CGGAAGAGTGTCTGGAGCAA-3'
hTERT reverse primer: 5'-GGATGAAGCGGAGTCTGGA-3'
hTERT Probe: Fam-TGCAAAGCATTGGAATCAGAC AGCACT-Quencher MGB

Amplification of GAPDH as an endogenous control is performed to standardize the amount of sample RNA added to a reaction, the quality of RNA and the RT-PCR assay efficiency. For each experimental sample, the amount of hTERT-mRNA and the GAPDH reference is determined from the appropriate standard curve. Then, the hTERT-mRNA amount is divided by the GAPDH reference amount to obtain a normalized hTERT-mRNA [32]. *The sequences of the primers and probe of the **GAPDH** control are:*

GAPDH forward primer: 5'-GAAGGTGAAGGTCGGAGTC-3'
GAPDH reverse primer: 5'-GAAGATGGTGATGGGATTTC-3'
GAPDH Probe: VIC-CAAGCTTCCCGTTCTCAGCC-TAMRA

1- Setting up the plate document with the GeneAmp 5700 SDS software and starting the PCR run.

Statistics: All the statistical analyses were processed using Statistical Program of Social Sciences (SPSS) for windows, version 10.0. Values of the measured parameters were expressed as range & mean value ±SD and the difference between each two groups was determined using unpaired student's t-test for comparing patients' results with controls while paired t-test was used for comparing the results of follow up in patients group, and the significance was considered at p values <0.001 up to <0.05. Multiple receiver operating characteristic curves (ROC curves) were drawn to assess the validity of the tumor markers.

Analysis of the results:

Table (1) showed insignificant difference between the age and sex distribution in all studied groups (p>0.05). All selected patients were suffering from superficial bladder cancer of transitional cell carcinoma type (TCC). As regards pathological stage, pTa represented 55% (n=11), pT1 represented 40% (n=8) and Cis represented 5% (n=1). As regards pathological grade, G1 represented 65% (n=13) and G2 represented 35% (n=7).

Cytological examination of all patients revealed the presence of five positive cases, five suspicious cases and ten negative cases (negative means no atypical cells were detected, positive means the detection of cells with malignant changes, while suspicious means the presence of few suspected cells with atypical changes, suspicious cytology results scored as positive for sensitivity and specificity calculation). In control group, all ten studied reference subjects had negative cytological examination. All patients were negative for cytology, three months after management. Six months after TURBT, five patients showed positive cytological examination (three positive and two suspicious cases as regard the cytology) and tumor recurrence was confirmed histopathologically.

Hemoglobin dipstick of all cases revealed the presence of three patients with negative hemoglobin dipstick results. In control group, five studied reference subjects had positive hemoglobin dipstick results (+). All patients were negative for hemoglobin dipstick one month after management. Three months later, five patients showed positive results with hemastix examination. All selected patients and control were negative for indirect schistosomal hemagglutination test. Blood urea level of control group ranged 27-53 mg/dl, with a mean of 42.1±4.5, while for patients group, it is ranged 36-60 mg/dl, with a mean of 47.7±7.8 with insignificant difference between both groups (P>0.05). Serum creatinine level of control group ranged 0.7-1.3 mg/dl, with a mean of 0.98±0.11, while for patients group, it is ranged 0.8-1.5 mg/dl, with a mean of 1.1±0.19 with insignificant difference between both groups (P>0.05).

In the present study it was shown that urinary fibronectin level and normalized hTERT-mRNA were significantly higher in superficial bladder cancer patients before TURBT (group II) (197.7±66.3 , 4.18±0.62 respectively) versus their corresponding results in control group (group I) (1.58± 0.39 , 0.057± 0.04, respectively) (P<0.001) (table 2).

Urinary fibronectin was significantly increased in patients group II, two days after TURBT (237.7± 71.1) versus patients group II before TURBT (197.7±66.3) (P<0.001). Gradually significant decrease of urinary fibronectin was reported two and six weeks after TURBT (180.7 ±70.6 and 171.9 ±73.2, respectively) versus patients group II two days after TURBT (237.7± 71.1) (P<0.001). There was insignificant difference between patients group (3 and six months after TURBT) (178.5 ±76.1 and 183.2 ± 61.4, respectively) versus patients group before TURBT (197.7±66.3) (P>0.05).

Normalized hTERT- mRNA was significantly decreased in patients group II, two days, 2 weeks, 6 weeks, 3 months and six months after TURBT (3.56±0.56, 2.73±1.97, 1.57 ±0.39 , 1.18±0.45 and 2.33 ± 0.44, respectively) versus patients group II before TURBT (4.18±0.62) (P<0.001).

There was insignificant differences in urinary fibronectin and hTERT-mRNA levels between the patients group, one year after TURBT (formed of 15 non-recurrent cases after TURBT) (1.09 ± 0.82, 0.0, respectively) and the control group (group I) (1.58± 0.39, 0.057± 0.04, respectively) (P> 0.05).

hTERT expression was detected more than 0.33 in two reference subjects of group I, while the other eight control subject were at the zero level so normalized hTERT-mRNA of group I ranged 0.0 – 0.37, with a mean value of 0.057 ±0.04. In patients group II, normalized hTERT was detected positive in 18 cases out of 20 cases≥0.33), while the other two patients were at the zero level. hTERT-mRNA of group II before TURBT ranged 0.0 – 9.04, with a mean value of 4.18± 0.62.

The mean urinary fibronectin level in the ten healthy volunteers was 1.58±0.391 (0-4 ng/ml). The levels of urinary fibronectin before TURBT in

patients with superficial bladder TCC were used to differentiate them into two subgroups; subgroup A formed of five patients who had significantly elevated fibronectin levels (all were T1) (649.60±268.8ng/ml) if compared with subgroup B which is formed of the other 15 patients (12 patients were Ta, 2 patients were T1 and one patient was Cis) in whom the mean fibronectin value was 47.06±21.07 ng/ml (P<0.001) (Table 3). Two days after TURBT, urinary fibronectin level showed further significant increase in patients subgroup A (727.20±279.9) (P<0.001) (Table 3). No patient in subgroup A had significant gross hematuria after TURBT. There was no association between the magnitude of the increase in fibronectin and pathological status. The subgroup A showed insignificant decrease of urinary fibronectin 2 and 6 weeks after TURBT (668.60±278.5 and 679.80±282.9, respectively) when compared to the same subgroup A before TURBT (649.60±268.8) (P>0.05). Three and six months after TURBT, recurrent significant increase of urinary fibronectin of the same subgroup A was found (710.40±125.9 and 767±137.3, respectively) when compared to the same subgroup A before TURBT (649.60 ± 268.8). While for subgroup B, there was significant increase of urinary fibronectin two days after TURBT (74.53±25.89) when compared to its results before TURBT (47.06±21.07) (P<0.001), followed by significant decrease of urinary fibronectin, two weeks, six weeks, three months, six months and one year after TURBT (18.13±6.26, 2.72±1.57, 1.24±1.01, 1.21±0.24 and 1.09±0.21 respectively)

when compared to the subgroup B before TURBT (47.06±21.07) (P<0.001). Insignificant differences of urinary fibronectin level were found between control group I (n=10) and patients subgroup B (n=15), three months, six months and one year after TURBT.

For hTERT-mRNA, there was insignificant difference between subgroup A (4.19±1.72) and subgroup B (4.15±1.14) before TURBT (P>0.05), but both were significantly elevated above the control (P>0.001). Subgroup A showed insignificant difference of hTERT-mRNA, 2 days , 2 weeks and 6 weeks, three months and six months after TURBT (3.95±1.618, 4.01±1.61, 3.69±1.45, 3.6±1.44, 4.31±1.35, respectively) when compared to the same subgroup A before TURBT (4.19±1.72) (P>0.05), while for subgroup B, there was insignificant differences of hTERT-mRNA, before, two days and two weeks after TURBT (4.15±1.14, 3.43±1.82, 2.31±1.93, respectively) (P>0.05). But significant decrease of hTERT-mRNA of patients subgroup B was reported, six weeks, three months and six months after TURBT(1.88±1.99, 0.95±1.30, 0.0, respectively) when compared to the same subgroup B before TURBT (4.15±3.14) (P<0.05, P<0.001, P<0.001 respectively). Normalized hTERT-mRNA reached to zero in patients subgroup B six months after TURBT and persist as it is in the one year follow up visit.

Figure (1) shows ROC curve of normalized hTERT in urine. The cutoff value for patients group versus control subjects was >0.33 ng/µl, with 85% sensitivity, 80% specificity, PPV

was 89.4%, NPV was 72.7% and 83.3% accuracy. The area under the curve was 0.779. Three of the twenty patients with superficial bladder cancer had normalized hTERT-mRNA ≤0.33 (the cutoff value).

Figure (2) shows ROC curve of fibronectin in urine. The cut-off value for patients group versus control subjects was >20 mg/ml, with 90% sensitivity and 100% specificity, PPV was 100%, NPV was 90% and accuracy was 93.3%. The area under the curve was 0.765. Two patients have urinary fibronectin level below the cut-off value. All control subjects had fibronectin level less than 20 mg/ml (the cutoff value).

Table (4) shows comparison between studied markers (cytology, Hb dipstick, fibronectin and hTERT-mRNA) as regard the sensitivity, specificity, PPV%, NPV% and accuracy for diagnosis of SBC. There was insignificant relation between any of the studied markers with the age or sex in the studied groups (p> 0.05). Complementary usage of normalized hTERT-mRNA expression with cytology raised the specificity from

80% for hTERT-mRNA alone to 100% if combined with cytology. However, complementary usage of normalized hTERT-mRNA expression with Hb dipstick raised the sensitivity from 85% for hTERT-mRNA alone to 90% when combined with Hb dipstick, while the complementary usage of normalized hTERT-mRNA with urinary fibronectin raised both the sensitivity and the specificity to 100%.

Table (5) shows the clinical outcome and the results of studied urine markers during the last two follow up visits before recurrence detection (three and six months after TURBT) in patients subgroup A (n=5) and it shows progressive increase in these markers with no response to BCG therapy. Follow up was done for all patients after TURBT, but the five recurrent cases were excluded from the one year follow up visit. Collectively, out of 20 patients, only five of them showed recurrence with a higher stage or grade within a period of 6 months, the other 15 patients showed no recurrence during the follow up period.

Table (1): Comparison between the mean ages and Sex distribution Progression of disease according to tumor grade and stage of in the studied groups

	Age (years)		Sex			Tumor grade		Tumor stage			cytology		Hb dipstick	
	Range	Mean± SD	Male	Female	M/F ratio	Grade I	Grade II	Ta	T1	Cis	-ve	+ve	-ve	+ve
Group I (n=10)	40-63	51.0±8.2	7(70%)	3(30%)	2.3:1						n =10	n =0	n = 5	n = 5
Group II (n=20)	46-71	56.7±7.5	15(75%)	5(25%)	3:1	13(65%)	7(35%)	11(55%)	8(40%)	1(5%)	n =10	n =10	n = 3	n = 17
	P>0.05		P>0.05											

Group I: Control group.   *Group II: Patients with superficial bladder cancer before TURBT.*

Table (2): Urinary soluble fibronectin and normalized human telomerase reverse transcriptase-mRNA unit (hTERT- mRNA) in the studied groups (Groups bearing different initials are significantly different from each other)(normalized hTERT = hTERT/GAPDH)

	Group I (n=10)	Group II (patients group)							p. value
Urinary Fibronectin (ng/ml)		*before TURBT* (n=20)	*2 days after TURBT* (n=20)	*2 weeks after TURBT* (n=20)	*6 weeks after TURBT* (n=20)	*3 months after TURBT* (n=20)	*6 months after TURBT* (n=20)	*One year after TURBT* (n=15)	
Range	0 - 4	13 - 942	33 - 1001	9 - 951	0 - 880	0 - 969	0 - 989	0 – 2.5	<0.001
Mean±SD	1.58± 0.39[a]	197.7±66.3[b]	237.7± 71.1[c]	180.7 ±70.6 [b]	171.9 ±73.2 [d]	178.5 ±76.1[b]	183.2 ± 61.4[b]	1.09 ± 0.82 [a]	
Normalized hTERT									
Range	0 - 0.37	0 - 9.04	0 - 9	0 - 5.85	0 - 5	0 -4.89	0 - 5.88	0 - 0	<0.001
Mean±SD	0.057± 0.04[a]	4.18±0.62[b]	3.56±0.56[c]	2.73±1.97[d]	1.57 ±0.39[e]	1.18±0.45[e]	2.33 ± 0.44 [d]	0.0 [a]	

Table (3): Level of fibronectin (ng/ml) and normalized hTERT – mRNA in urine of patient subgroups (A and B) before and after TURBT

		Urinary Fibronectin (ng/ml)			Normalized hTERT		
		Mean±SD	p. value	t. test	Mean±SD	p. value	t. test
Group II (n=20) *patients group before TURBT*	Group A (n=5)	649.60 ± 268.8^a	<0.001*	9.108	4.19 ± 1.72^a	0.979	0.034
	Group B (n=15)	47.06 ± 21.07^c			4.15 ± 1.14^a		
Group II (n=20) *Two days after TURBT*	Group A (n=5)	727.20 ± 279.9^b	<0.001*	9.436	3.95 ± 1.618^a	0.704	0.386
	Group B (n=15)	74.53 ± 25.89^d			3.43 ± 1.82^a		
Group II (n=20) *Two weeks after TURBT*	Group A (n=5)	668.60 ± 278.5^a	<0.001*	9.953	4.01 ± 1.61^a	0.095	1.765
	Group B (n=15)	18.13 ± 6.26^e			2.31 ± 1.93^a		
Group II (n=20) *Six weeks after TURBT.*	Group A (n=5)	679.80 ± 282.9^a	<0.001*	9.828	3.69 ± 1.45^a	0.05*	1.853
	Group B (n=15)	2.72 ± 1.57^f			1.88 ± 1.99^b		
Group II (n=20) *Three months after TURBT*	Group A (n=5)	710.40 ± 125.9^b	<0.001*	9.979	3.6 ± 1.44^a	<0.001*	3.847
	Group B (n=15)	1.24 ± 1.01^g			0.95 ± 1.30^c		
Group II (n=20) *Six months after TURBT*	Group A (n=5)	767 ± 137.3^b	<0.001*	10.243	4.31 ± 1.35^a	<0.001*	0
	Group B (n=15)	1.216 ± 0.24^g			$0 - 0^d$		
Group II (n=20) *One year after TURBT*	Group A (n=5)	--------	--------	--------	--------	--------	--------
	Group B (n=15)	1.09 ± 0.21^g			$0 - 0^d$		

(Groups bearing different initials are significantly different from each other)
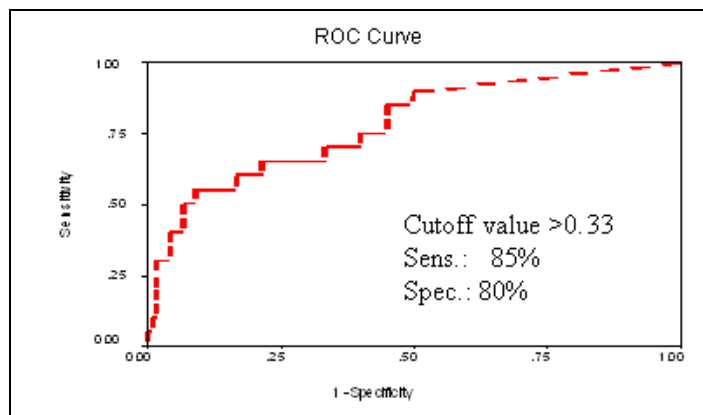
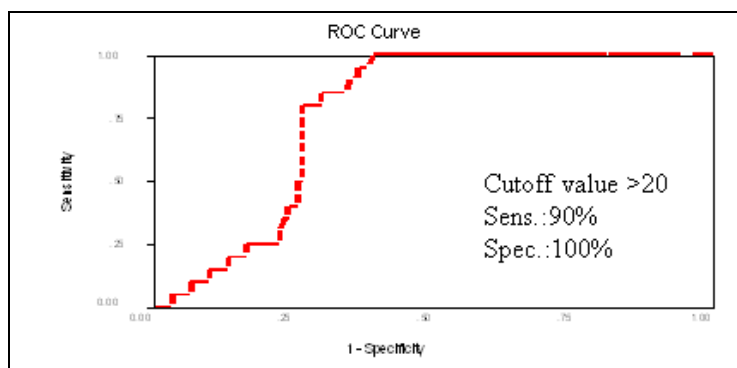**Figure (1):** ROC curve of normalized hTERT in urine (area under the curve 0.779).

Figure (2): ROC curve of fibronectin in urine (area under the curve 0.765).

Table (4): Relations of the studied urine markers in the diagnosis of superficial bladder cancer

Parameters	Sensitivity	Specificity	PPV%	NPV%	Accuracy
Cytology	50%	100%	100%	50%	66.6%
Hb dipstick	85%	50%	85%	70%	73.3%
Fibronectin (Cut-off value >20)	90%	100%	100%	90%	93.3%
hTERT-mRNA(Cut-off value >0.33)	85%	80%	89.4%	72.7%	83.3%
hTERT-mRNA and Cytology	85%	100%	100%	76.9%	90%
hTERT-mRNA and Hb dipstick	90%	80%	90%	80 %	86.6%
Fibronectin and hTERT-mRNA	100%	100%	100%	100%	100%

Table (5): The histopathologcal outcome and the result of studied urine markers for the recurrent five cases (subgroup A) during the follow-up (A+B)

A)

Case no.	Initial Histopathology	Cytology		Hb dipstick		Interval to recurrence	Recurrent Histopathology
		3month	6 month	3month	6month		
1.	TCC/PT1/I	-ve	suspicious	++	++	6 month	TCC/PT1/II
2.	TCC/PT1/II	-ve	+ve	Trace	+	6 month	TCC/PT1I/III
3.	TCC/PT1/II	-ve	+ve	+	++	6 month	TCC/PT1I/III
4.	TCC/PT1/I	-ve	+ve	Trace	Trace	6 month	TCC/PT1/II
5.	TCC/PT1/II	-ve	suspicious	+	+	6 month	TCC/PT1/II

B)

Case no.	Urinary Fibronectin		Normalized hTERT-mRNA	
	3month	6month	3month	6month
1.	1001	1120	1.52	1.96
2.	579	599	3.52	4.47
3.	801	876	3.14	4.66
4.	290	329	4.97	5.2
5.	881	911	5.00	5.27
Mean±SD	710.4±125.9	797±137.3	3.63±1.44	4.31±1.35
	p. value <0.05*		**p. value** <0.05*	

* Significant

DISCUSSION

Carcinoma of the bladder is the most prevalent cancer in Egypt and in most African countries[33]. Bladder cancer has always received much attention in Egypt due to its high prevalence with high mortality rates[34]. Diagnosis is usually done at a late T stage when therapy is rarely curative[35]. It has been suggested that detecting bladder cancer earlier will decrease the rate of progression[36].

Intravesical bacillus Calmette-Guérin (BCG) has been shown to be an effective treatment for superficial transitional cell carcinoma (TCC) of the bladder, but the precise mechanism of action of BCG remains poorly understood[37,38]. Fibronectin (FN), an important component of the extracellular matrix, has been found to play a role in BCG therapy. Although adjuvant BCG immunotherapy has been shown to improve the clinical outcome of SBC, a significant proportion of patients fail to respond to it[5, 39]. Identification of the subset of patients that are going to develop recurrent disease or stage progression after BCG therapy (BCG non-responders) is very important, since aggressive therapy with early salvage cystectomy has been shown to improve overall survival[6,40].

The mainstay for diagnosing bladder cancer is the combination of cystoscopy, biopsy, and voided urine cytology. Cytology, although useful for detecting high grade tumors, lacks sensitivity in low grade lesions[41,42]. Limitations of cytology include a significant false negative rate in about half of cases, mainly in patients with low-grade or early-stage tumors[43], as the low-grade well-differentiated tumors can appear cytologically normal[44,45,46]. In addition, the invasiveness of cystoscopy led to search for more accurate and non invasive biomarkers for early detection of bladder cancer and long-term follow up[47, 48,49].

During the last two decades, the better understanding of the molecular mechanisms involved in carcinogenesis and tumor progression has provided a large number of molecular markers of bladder cancer, with a potential diagnostic and prognostic value[15].

Telomerase is a ribonucleoprotein complex that catalyzes the addition of telomeric repeats to the end of chromosome DNA, thereby preventing the loss of telomeric sequences at each cell division and contributing to the maintenance of cell immortality and to the uncontrolled growth of cancer cells, by elongating telomere ends[16]. Because of its central role in carcinogenesis and for the high frequency of its activation in human cancers, telomerase has been expected to be a new and promising marker for cancer diagnosis and therapy[50,51,52]. Recent evidence has suggested that telomerase activity in urine is a potentially useful marker for the early detection of bladder cancer[53,54].

Therefore, the aim of the present study was to give an insight into the diagnostic and prognostic values of the quantitative urinary estimation of both fibronectin (FN) and the catalytic subunit of the complex human telomerase reverse transcriptase (hTERT) levels as non invasive tumor markers for early detection of patients

with high risk superficial transitional cell carcinoma (TCC) and after transurethral resection of bladder tumors (TURBT) that received adjuvant BCG immunotherapy to explore its association with recurrence-free-survival (RFS) or development of invasive disease.

In the present study, there was insignificant difference in age and sex distribution between the studied groups. Male predominance among bladder cancer patients was manifested with a male: female ratio 3:1. This predominance was also reported by **Wallance**[55] and **Ghoneim**[56] who explained that finding by the fact that males are more exposed to environmental and occupational risk factors than females. Most of the cases of the present study were in the sixth decade of life and all cases were transitional cell carcinoma (TCC). It has been reported recently that there is an   increase in the incidence of changing in the pattern of bladder cancer from squamous to transitional cell carcinoma as TCC represented 62.8%, while squamous cell carcinoma (SCC) represented 26.8% of all reported cases [57]. The cause of the increased incidence of TCC even among bilharzial associated bladder cancer in the last few years may be due to the appearance of new environmental factors among Egyptian population; however this needs further studies [56, 57].

Histopathologic classification of bladder carcinomas revealed that 11 out of 20 TCC cases had superficial non-invasive tumors (Ta) and represented (55%), 8 out of 20 TCC cases had pT1 (the cancer has started to grow into the connective tissue

beneath the bladder lining and represented (40%) and one case out of 20 had *carcinoma in situ* (CIS) and represented (5%). As regards pathological grade, G1 represented 65% (n=13) and G2 represented 35% (n=7).

Cytological examination of all patients revealed the presence of five positive cases, five suspicious cases and ten negative cases. In control group, all ten studied reference subjects had negative cytological examination. All patients were managed by TURBT followed by BCG vaccine instillation started from the second week after TURBT and continued for six subsequent weeks. All patients were negative for cytology six weeks after TURBT. Six months later, five cases showed positive cytological examination (three positive and two suspicious cases) where tumor recurrence was confirmed histopathologically.

Cytological examination of the studied cases showed low sensitivity (50%) but high specificity (100%) for diagnosis of superficial bladder cancer with an accuracy 66.6%. That finding was in agreement with those of **Halling et al.** [58] who reported that the low sensitivity of cytology could be contributed to the difficulty of cytology in detecting well differentiated tumors, or the presence of few cancer cells that may be insufficient for cytological evaluation.

Hemoglobin dipstick of all cases revealed the presence of three patients with negative hemoglobin dipstick results. In control group, five studied reference subjects had positive results. All patients were negative for Hb dipstick one month after management.

Three months later, five cases showed positive or trace results with hemoglobin dipstick. Hemoglobin screening of the studied cases by hemoglobin dipstick test (hemastix) revealed relatively good sensitivity (85%) and low specificity (50%) in the diagnosis of superficial bladder cancer. Although hematuria is a common presentation of bladder cancer, it is also a common finding in other genitourinary disorders such as urinary tract infections, benign prostatic hyperplasia (BPH) and stones; this may explain the low specificity of hemastix [59].

In the present study it was found that urinary fibronectin level was significantly higher in superficial bladder cancer patients before TURBT (group II) when compared to the control group (group I). There was significant increase in urinary fibronectin in the patients group, two days after TURBT when compared to its value before TURBT. Urinary fibronectin level showed progressive significant decrease in patients group, 2 weeks, 6 weeks, three months, six months and one year (15 patients only) after TURBT when compared to patients group, two days after TURBT. The values of urinary fibronectin of patients did not reach to the control values and remained high till the sixth months visit and the lowest level achieved only by fifteen patients (1.09 ± 0.82) who showed insignificant difference when compared to the control group (1.58 ± 0.39) in the one year visit ($P > 0.05$). The remaining five patients during the sixth month follow up visit showed recurrent tumor growth, so they were

excluded from the statistics in the one year follow up visit.

These results could be explained as follows; the turnover of the suburothelial matrix might be responsible for the low but measurable urinary fibronectin levels in healthy individuals. The precise explanation for increased urinary fibronectin level in the presence of TCC of the bladder, in the present study, is the effect of a tumor-derived protease which may be responsible for increasing the release of fibronectin from bladder matrix during the multistep process in tumor invasion and metastasis [60,61]. The study demonstrated higher urinary concentration of fibronectin in patients with superficial bladder cancer than healthy subjects. *Sanchez-Carbayo et al.* [62] also reported a significant differences among malignant, benign and normal control groups and reported that the degenerative change or exfoliation of bladder cancer cells can cause release of fibronectin or its fragments into urine [63], this can explain the marked differences among malignant versus control groups in the present study and in previous studies [64,65].

Peak levels of fibronectin in all patients occurred immediately after surgery and continued for 2 days. That increase represents large amounts of fibronectin leaking into the bladder during and immediately after surgery. Theoretically, a high level of fibronectin after surgery may originate from three sources. The first is persistent bleeding from the resected areas, accompanied by soluble fibronectin originating from plasma [65,66]. The second is the

surgical destruction of bladder basement membranes and extracellular matrix, both of which are rich in fibronectin[67]. The third is tumor tissue itself, which has high fibronectin content[60]. In agreement with the current results, *Laufer et al.*[68] who reported an increased urinary fibronectin levels after transurethral resection of noninvasive bladder tumors and then decreased 2 weeks after the operation but he did not follow up the patients later. The rapid increase in urinary fibronectin, two days after TURBT in the present study might be related to the surgical intervention, hematuria or the high fibronectin tumor content but hematuria cannot be regarded as the only source of fibronectin in the urine samples of our patients due to the persistent high fibronectin levels after regression of hematuria. Also, the persistence of high levels of fibronectin even after the healing of the bladder urothelium (two weeks later), supports the suggestion that the high fibronectin level was produced by the tumor cells.

Insoluble (matrix) fibronectin has an important role in the adherence and activity of BCG. Matrix fibronectin is found in the basement membranes and submucosa of the bladder, and is absent from the healthy luminal surface [60]. Tumor resection exposes fibronectin molecules to the bladder contents. The high-affinity receptors of BCG can react with both soluble and matrix fibronectin, and this binding is essentially irreversible at normal urinary pH [66]. Therefore, the presence of significant amounts of soluble fibronectin in the urine released from the tumor tissues, as

noted here, could result in the preferential and irreversible binding of BCG to soluble fibronectin. This would decrease the attachment of BCG to the bladder wall and subsequently reduce its efficacy, so that a possible role of fibronectin in the failure of BCG therapy was concluded.

Urinary fibronectin levels decreased significantly in the follow up visits but their levels collectively did not reach to the normal range. This could be explained by the persistent high level of urinary fibronectin in five out of twenty SBC patients which affecting the final statistics results, so patients with superficial bladder TCC in the present study (group II) were differentiated into two subgroups according to the levels of urinary fibronectin before TURBT; *group A* formed of five patients who had significantly elevated fibronectin levels and did not normalized after TURBT (5 patients were T1), group *B* which is formed of the other 15 patients (12 patients were Ta, 2 patients were T1 and one patient was Cis) in whom the fibronectin level was significantly lower than group A and normalized within 6 weeks after TURBT. Furthermore, within group A there was no association between fibronectin levels before TURBT and the presence of gross hematuria or the estimated volume of the tumor. BCG immunotherapy started from the second week post TURBT and continued weekly for six subsequent weeks. Higher level of group A fibronectin was associated six months later with recurrence of carcinoma and so this high level may be related to the

residual malignant tissues after TURBT which also causes resistant BCG therapy and causing failure of BCG and recurrence. There are insufficient data to suggest whether this urine marker is of value in anticipating virulent progression of cancer but proved data for affection of BCG therapy was obtained[9,10,11]. Subgroup B, one year after TURBT (n=15), showed insignificant difference in urinary fibronectin level (1.09 ± 0.21) when compared to the control group (1.58± 0.39)(p<0.001). While for subgroup A with persistent high level of urinary fibronectin followed by recurrence of the tumor, most probably due to the high level of soluble fibronectin which can compete efficiently with the matrix form of fibronectin for binding to the specific receptors on the mycobacteria (FAP) and could consequently diminish the effect of BCG and increase the possibilities for recurrence.

ROC curve analysis for urinary fibronectin level gave the optimum cut-off value for patients group versus control subjects which was >20 mg/ml, with 90% sensitivity and 100% specificity and 93.3% accuracy. The area under the curve was 0.765. All patients with superficial bladder cancer had fibronectin >20 (the cutoff value).

In the present study, hTERT-mRNA was quantified by using real-time RT-PCR and it was detected in the urine of two control subjects (group I), whereas the remaining samples were below the cut-off value (cut-off value >0.33). This was in disagreement with the study of *De-Kok et al.*[69] who reported zero hTERT expressions in tissue biopsies from healthy bladders. This seems reasonable as telomerase activity appears to be strictly regulated during development and during embryonic differentiation in most somatic cells[70]. The positive data with detectable hTERT was restricted to two subjects out of five females with associated chronic cystitis. These results are in agreement with those of *Sanchini et al.*[53] who attributed this to the presence of a higher fraction of inflammatory and non-bladder epithelial cells in women than in men. This is probably due to the shorter female urethra, which favors the entrance of bacteria into the bladder and which could, at least in part, explain the false positive hTERT-mRNA assay results. Therefore, once the contaminating elements largely present in urine samples has been demonstrated, it will be possible to improve diagnostic accuracy in women by subtracting the telomerase activity belonging to non-tumor epithelial cells from the total hTERT-mRNA. *Lancelin et al.*[71] reported telomerase activity in 2 of 4 including chronic cystitis. Also, *Leber et al.*[72] attributed the hTERT expression in non malignant bladder disorders to activated lymphocytes in these inflammatory disorders.

In the present study, it was found that urinary normalized hTERT- mRNA was significantly higher in patients group before TURBT (group II) versus control group (group I). The role of hTERT in bladder cancer has been presented in many studies[20,21]. Increase in the replicative potential of tumor cells alone do not ensure tumor growth, as cells progress through a limited

number of replications before they show telomere shortening, stop dividing and become senescent (telomere molecular clock). Therefore, tumor cells, including bladder cancer, up regulate the expression of telomerase enzyme to maintain telomere length above a critical threshold to allow unlimited replication potential (immortalization)[73]. In addition, *Rahman et al.*[74] reported that increase of constitutive hTERT expression has an antiapoptotic activity by inhibition of p53-induced apoptosis, independently of telomerase activity. Telomerase activity has been detected in more than 90% of various human cancer tissues [17,18]. Concerning urological tumors, hTERT has been detected in renal cell carcinoma, bladder cancer, and prostate cancer [19,20,21]. These studies showed that hTERT was detectable not only in progressive or metastatic cancer but also in approximately 80% of cancers in a very early stage. Thus, hTERT might be useful in the diagnosis of early cancer.

There was significant decrease in urinary hTERT- mRNA in patients subgroup B received BCG after TURBT until it reached to zero one year after TURBT. On the other hand, apoptotic cells were markedly increased in the treated cases. This could be explained as BCG has been documented to induce down-regulation of telomerase activity in a variety of in vitro models (including bladder cancer cell lines) raising the possibility that hTERT may be a marker of response to BCG [37,38]. Also, hTERT activity has been shown

to provide prognostic and predictive information in a variety of epithelial neoplasms[75,76,77,78]. At the same time, patients of subgroup A, showed insignificant difference in hTERT-mRNA before and after TURBT, even after BCG instillation and by follow up for this subgroup, there was a resistant high level of hTERT followed by recurrence six months after TURBT. Failure of BCG by high level of soluble fibronectin causing suppression of BCG down-regulation activity on telomerase resulting in upregulation of telomerase and inhibition of p53-induced apoptosis resulted in recurrence. These results suggest that the reduction of telomerase activity is related to the mechanism of BCG effects. Possible mechanisms to be considered are either that BCG inhibits telomerase first, or that it induces apoptosis and decreases telomerase activity as a result of that induction [38].

Patients with high hTERT tumors had a worse recurrence free survival compared to patients with low hTERT tumors. Baseline higher pre-BCG expression of hTERT was associated with subsequent development of invasive disease after BCG. Also, the persistent rise of telomerase may be considered as indicator of the future resistance of the tumor to BCG therapy or increase the possibility of recurrence.

The present study showed 85% sensitivity and 80% specificity, for hTERT expression in the diagnosis of superficial bladder cancer. *De-Kok et al.*[69], also reported high specificity for hTERT but in bladder biopsies from different non-neoplastic bladder disorders using real time PCR. *Bowles*

et al.[79] reported a specificity of 93.5% for hTERT expression in urinary sediments from different non neoplastic bladder diseases using multiplex hTERT/GAPDH RT-PCR.

Previous studies have reported several candidate predictive factors of response to adjuvant BCG but a practical marker with good independent predictive value has not been identified[80]. In that regard, traditional pathological tumor characteristics [81], molecular markers such as p53[82] and immunological markers such as purified protein derivative skin reaction (PPD)[83] do not appear to have a consistent predictive value. Expression of hTERT would be a good predictor of BCG response for three reasons. Firstly, BCG has been documented to induce down-regulation of telomerase activity in a variety of in vitro models, raising the possibility that telomerase may be a marker of response to BCG [37,38]. Secondly, in the case of hTERT, the association between nucleolar telomerase activity and hTERT mRNA expression has been well documented and validated [84,85]. Thirdly, hTERT activity has been shown to provide prognostic and predictive information in a variety of epithelial neoplasms including gastric, renal, hepatocellular, lung and colon cancers[75,76,77,78]

CONCLUSION

The assessment of fibronectin and hTERT-mRNA in urine represents a highly sensitive tool for early detection of SBC. It seems conceivable that these emerging urine-based tests may progressively replace urine cytology and form a reliable adjunct to cystoscopy in the diagnosis of bladder cancer. Both urinary fibronectin and hTERT mRNA in addition to being both of them from the best markers for diagnosing bladder carcinoma, can be used to determine the presence of residual tumor load after TURBT of bladder TCC. These parameters also can be used for follow up of patients with superficial bladder cancer after TURBT and to predict the sensitivity of a tumor to BCG instillation before therapy. They could be used as predictors for selecting the appropriate patients for BCG treatment.

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دور كل من الفيبرونكتين والتليومريز في البول في مرضي سرطان المثانة السطحي بعد استئصاله عبر مجري البول ومعالجتهم بمصل البي. سي. جي

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المقدمة:

يعد سرطان المثانة من اكثر الاورام انتشارا في مصر والعالم ، ومن اكثر الصعوبات التي تصاحب هذا النوع من الاورام وخاصة السطحي منها هو قابليته للانتشار السريع وظهوره بعد استئصاله ومن ثم فإن التشخيص المبكر والمتابعة الدورية لهما اهمية كبري في مثل هذه الحالات وبالرغم من استخدام منظار المثانة في التشخيص المبكر فإنه لا يمكن الاعتماد عليه لما يسببه من جروح و اصابات نتيجةالاستخدام المتكرر وكما ان التغيرات الشكلية التي تحدث نتيجة الالتهابات المزمنة والحصوات تؤدي الي نتائج غير دقيقة. ان التغيرات التي تحدث في كل من الفيبرونكتين وزيادة نسبة انزيم التليومريز من اهم العوامل المصاحبة لسرطان المثانة وبالتالي فإن دراسة هذه التغيرات من الممكن ان يكون لها اهمية في التشخيص المبكر ومتابعة مرضي سرطان المثانة السطحي وتحديد مستقبلي لدرجة استجابة المرضي للعلاج بمصل البي سي جي الذي يتبع استئصال الورم وهل هناك احتمالات لمقاومة الورم للعلاج ومن ثم عودة المرض مرة اخري

الهدف من البحث: القاء الضوء علي دور كل من انزيم التليومريز والفيبرونكتين في البول كدلالات اورام غيرنافذة في التشخيص المبكر والمتابعة الدورية لمرضي سرطان المثانة السطحي ومتابعتهم بعد استئصال الورم عن طريق مجري البول وتحديد درجة استجابة المرضي للعلاج بمصل الابي سي جي واحتمالية حدوث مقاومة من المرض للعلاج بالبي سي جي ومن ثم حدوث انتكاسة اخري وعودة المرض

مواد وطرق البحث:

اشتمل هذا البحث علي مجموعتين:

(1) المجموعة الاولي : تتكون من عشرة اشخاص من الاصحاء كمجموعة ضابطة.

(2)المجموعة الثانية : شملت عشرون مريض (جميعهم ليسوا من مرضي البلهارسيا) يعانون من سرطان المثانة السطحي .

وتم اجراء الفحوصات التالية لكل افراد المجموعات قبل اجراء استئصال الورم من المثانة عن طريق مجري البول وبعد الاستئصال بيومين ، اسبوعين ، ست اسابيع ، ثلاثة شهور، ست شهور، وعام كامل

1-تاريخ مرضي

2- فحص اكلينيكي

3- الفحوص الروتينية المعملية(بولينا وكرياتنين ونسبة الاجسام المضادة للبلهارسيا بالدم)

4- اشعة عادية وموجات فوق صوتية

5- منظار المثانة مع اخذ عينة للتحليل الهستوباثولوجي

6- تحليل سيتولوجي للخلايا بالبول

7- فحص الدم بالبول

8- قياس مستوي الفيبرونكتين بالبول

9-قياس مستوي انزيم التليومريز بطريقة الريال تايم- بي سي ار بالبول

وقد اظهرت الدراسة النتائج التالية:

*ان كل من انزيم التليومريز بطريقة الريال تايم- بي سي ار و الفيبرونكتين في البول لهما من الحساسية والنوعية العالية في اكتشاف سرطان المثانة في المراحل المبكرة وخاصة في المرضي الذين لهم القابلية للتحول السرطاني

* ان الفحص السيتولوجي للخلايا الموجودة في البول لا يتمتع بالحساسية الكافية مما يقلل الاعتماد عليه في تشخيص سرطان المثانة السطحي علي الرغم مما له من قدرة نوعية عالية للتشخيص

اٌ فحص الدم بالبول لا يمكن الاعتماد عليه في تشخيص هذا المرض حيث يعد صفة مشتركة مع كثير من امراض المثانة الاخري

*بين امكانية استخدام كل من ان انزيم التليومريز بطريقة الريال تايم- بي سي أر و الفيبرونكتين في البول في متابعة مرضي سرطان المثانة السطحي بعد استئصال الورم مع تحديد امكانية الحاقه بالحقن لمصل البي سي جي بالمثانة عن طريق تحديد مدي استجابة او مقاومة المريض للعلاج المناعي بمصل البي سي جي حتي يتسني للطبيب المعالج تحديد طريقة العلاج اما بالاستئصال للورم والحاقه بمصل البي سي جي بالمثانة لست مرات متتابعة تبدأ من الاسبوع الثاني بعد الاستئصال او باستئصال المثانة اوجزء منها اذا كان هناك مقاومة للورم للعلاج واستعداد لحدوث ظهور للورم مرة اخري.

*نأمل بأن تفتح هذه الدراسة بابا جديدا لطرق تشخيص ومتابعة مرضي سرطان المثانة السطحي حتي يمكن التوصل الي طرق وقائية وعلاجية اكثر فائدة.