



VALUE OF BIOCHEMICAL URINARY MARKERS IN DIAGNOSIS AND PROGNOSIS OF BLADDER CANCER PATIENTS

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ABSTRACT:

Background: Determination of tumor markers in body fluids lining the tumor should provide more reliable information on the malignancy, since dilution, metabolism and excretion have very limited, if any, effect on the actual marker concentration, which makes urine the ideal candidate for bladder malignancies.

The present work aimed to evaluate the role of Tumor-Associated Trypsin Inhibitor (TATI), Cytokeratin Fragment Antigen (CYFRA) 21.1, Nuclear Matrix Protein 22 (NMP-22) and Tumor Polypeptide-Specific (TPS) Antigen in urine as potential diagnostic and prognostic markers for bladder cancer, in relation to urine cytology.

The study included 3 groups. Group 1 comprised of 60 patients with primary bladder cancer, group 2 composed of 40 patients with benign urologic diseases and group 3 included 40 healthy volunteers of matching age and sex as a control group. From each subject, one random midstream urine sample was collected before endoscopy. TATI, CYFRA 21.1 and TPS were assayed by radioimmunoassay while NMP-22 was assayed by ELISA. Patients were followed up for 36 months.

Mean levels of all measured parameters were significantly higher in bladder cancer group than those in benign and control groups. ROC curves showed that they were all of diagnostic significance, and at cutoff values of 42 µg/g cr, 21 µg/g cr, 10.5 U/g cr and 152 IU/g cr; respectively, TATI, CYFRA 21.1, NMP-22 and TPS had sensitivities of 82.0%, 80.4%, 73.9% and 68.2% and specificities of 80.5%, 73.1%, 72.4% and 61.5%; respectively. Patients with elevated initial levels of markers at presentation than their respective cutoff values had significantly lower disease-free survival (DFS), also DFS decreased as the number of positive markers increased.

In bladder cancer patients, initial urinary levels of TATI, CYFRA 21.1, NMP-22 and TPS at presentation were significant diagnostic markers with acceptable sensitivities and specificities, and also were significant prognostic markers. When these four markers were joined, DFS declined as the number of positive markers increased.

while it is the second in females. Also the mortality rates resulting from bladder malignancies in Egypt are the highest in the world.⁽¹⁾ Egypt's world-standardized incidence was 37/100,000 for bladder cancer, interpreted as approximately 30,000 new cases per year.⁽²⁾

Despite treatment, 50% to 70% of superficial bladder tumors recur within 5 years after initial resection, while within 2 years, 50% of patients with muscle-invasive cancer develop distant metastasis, and 60% die during 5 years, therefore, long-term follow-up is mandatory.⁽³⁾

Flexible cystoscopy was a more acceptable diagnostic procedure, yet, the procedure remains uncomfortable, expensive and causes urinary tract infections in 16% of patients, in addition, it overlooks microscopic disease. Urine cytology, which detects cancer cells shed in urine, is non-invasive and easy to perform but its diagnostic sensitivity remains disappointing, especially in low grade cancers.⁽⁴⁾

Determination of tumor markers in body fluids lining the tumor should provide more reliable information on the malignancy, since dilution, metabolism and excretion have very limited, if any, effect on the actual marker concentration, which makes urine the ideal candidate for evaluation of markers of bladder malignancies. Several new markers have lately been suggested to improve the diagnostic and

INTRODUCTION

Bladder cancer has always received much attentiveness in Egypt due to

its high prevalence and high mortality rates. In males, incidence rates of bladder cancer are among the highest in the world, thus in males, it is the first site of cancer incidence

prognostic sensitivity in urine, so they would have the potential of serving as an adjunct or even an alternate for cystoscopy.^(5,6)

Tumor-Associated Trypsin Inhibitor (TATI) is a 6-kDa peptide that inhibits trypsin and aprotinin and was found identical to the pancreatic secretory trypsin inhibitor. Elevated TATI levels were found in serum and urine of patients with many types of cancer at advanced stages.⁽⁷⁾ TATI expression was found to be restricted to superficial cells of normal bladder urothelium but increased expression was noted in both dysplastic mucosa and invasive foci of bladder tumors. TATI gene up-regulation was involved in early urothelial tumor development.⁽⁸⁾

The Cytokeratin Fragment Antigen (CYFRA) 21 is a fragment of cytokeratin (CK) 19. CKs are the main structural units of the cytoskeleton of epithelial cells and 20 CK have been classified into 2 types, CKs 1-8 as type II, and CKs 9-20 as type I. Microfilaments are heteropolymers constructed of type I and II keratins, and constitute the cytoskeleton. CK19 is a soluble type I CK with the least molecular mass (40 kDa) among all CKs. The CK expression patterns in tissues are well-preserved even during the transformation process from normal to tumor tissue.⁽⁹⁾ CK19 degradation is accelerated in neoplastic epithelial cells due to enhanced protease activity of caspase 3, a regulator of the apoptosis cascade, and fragments are discharged into blood, resulting in increased serum CYFRA 21.1 values.⁽¹⁰⁾

The nuclear matrix protein-22 (NMP-22) is also identified as nuclear mitotic apparatus protein (NUMA-1). NMP-22 supports the structure of the nucleus and ensures the correct separation of genetic material during mitosis into the respective daughter cells through mitotic spindle stabilization. NMP22 is elevated up to 80-fold in tumor cells within the urinary tract. NMP22 is shed into the urine due to cellular apoptosis and other mechanisms of tumor cell death.⁽¹¹⁾

Tissue Polypeptide-Specific (TPS) Antigen closely relates to human cytokeratin 18, which is a crucial component of the protein cytoskeleton of epithelial cells. TPS is synthesized through late S- to M-phase of the cell cycle, then it is released into the blood or other body fluids. Serum TPS concentration is used as an indicator of the rate of cell division and proliferation, as TPS secretion occurs alongside DNA synthesis, protein synthesis, and cell division.⁽¹²⁾

In the present study, we evaluated the role of Tumor-Associated Trypsin Inhibitor (TATI), Cytokeratin Fragment Antigen (CYFRA) 21.1, Nuclear Matrix Protein 22 (NMP22) and Tumor Polypeptide-Specific (TPS) Antigen in urine as potential diagnostic and/or prognostic markers for bladder cancer, in relation to urine cytology.

SUBJECTS AND METHODS

The study involved 140 individuals distributed to 3 groups. Group 1 comprised of 60 patients with primary bladder cancer, proven histologically, of which 41 had transitional cell carcinoma (TCC) and 19 had squamous cell carcinoma (SCC). Group 2 was composed of 40

patients with benign urologic diseases (18 bladder stones, 11 urolithiasis and 11 kidney stones), and group 3 included 40 healthy volunteers of matching age and sex as the control group.

The study was executed following the endorsement of the local ethical committee and each subject delivered an informed written consent. Sampling was done before endoscopy and tumors were classified according to the TNM criteria, (13) and grade was assigned using the 2016 World Health Organization tumor grading system.⁽¹⁴⁾ All patients underwent cystoscopy, and were followed up for 36 months after surgery for recurrence to evaluate disease-free survival (DFS).

From each subject, one random midstream sample of urine was collected. The collected urine samples underwent centrifugation (3000 rpm for 10 min at 4°C) and the cleared solution was stored at -80°C until used, while the sediments were immediately used for cytology. One drop of this sediment was placed on a glass slide and a smear was made, which was fixed and stained with Haematoxylin and Eosin (H&E) stain then slides were examined for the presence of malignant cells shed from bladder tumor.

The TATI was assayed by radioimmunoassay (Orion Diagnostica, Espoo, Finland). According to the manufacturer's instructions, in antibody coated tubes, 50µl of the sample were incubated with 200µl of 125I-labelled tracer and 200µl antiserum over-night, proceeded by washing the tubes and counting them on gamma-counter.⁽¹⁵⁾

The CYFRA 21.1 was assayed using Immunoradiometric kit (SIS Bio International, Schering S.A., France). According to the manufacturer's instructions, 100µl of sample were incubated with 300µl of 125I Anti-CYFRA 21.1 over night in anti-CYFRA 21.1 coated tubes, proceeded by washing the tubes and counting them on gamma-counter.⁽¹⁶⁾

The TPS was measured by TPS@IRMA kit (IDL Biotech AB (publ), Bromma, Sweden) which is an immunoradiometric assay kit. The assay uses beads that were pre-coated with anti-TPS antibodies. According to the manufacturer's instructions, to each tube 100 µl of standards or samples, 200 µl of TPS@IRMA 125I Tracer and 1 bead were added, tubes were incubated for 2h on shaker then aspirated and the beads washed 3 times, then radioactivity was assessed on a gamma counter.⁽¹⁷⁾

The Human Nuclear matrix protein 22 was measured by NMP-22 ELISA Kit (USCN Life Science, Milano, Italy). According to the manufacturer, 100µl of the sample were incubated in wells pre-coated with anti-NMP-22 antibodies for 2 hrs at 37°C. After aspiration, 100µl of Detection Reagent A was incubated for 1 hr at 37°C, after aspiration, 100µl of Detection Reagent B was incubated for 1 hr at 37°C. After aspiration, 90 µl of Substrate solution was incubated for 30 min. at 37°C. 50 µl of stop solution were added and OD was measured at 450 nm.⁽¹⁸⁾

Urine creatinine was measured in 24-hr urine, and all measured parameters were related to the measured creatinine excretion.

STATISTICAL ANALYSIS

The results of the studied parameters were expressed as Mean±Standard Deviation (M±SD). Significance of difference between means was assessed by analysis of variance (ANOVA). Probability (p) was then obtained to ascertain whether the difference between the two groups is statistically significant. Association between clinical and pathological aspects and the measured markers was done by Mann-Whitney U test. All tests were two-sided, and the significance level was set to p values <0.05. Sensitivity, specificity and cutoff values were obtained by Receiver Operating Characteristics (ROC) curve. Survival curves were constructed by the Kaplan-Meier method. Multivariate survival analyses were performed by Cox regression. Statistical end points were cancer-specific and progression-free survival measured from the date of operation to the date of death, relapse, or latest follow-up. All statistical analyses were done by the Statistical Package for Social Sciences software (SPSS version 22.0; SPSS Inc.; Chicago, IL, USA). (19)

Table 1: Characteristics of the studies cancer tissues.

Clinical parameter	No.	%
Type of bladder cancer		
TCC	41	68.3
SCC	19	31.7
Tumor grade		
Low-grade	23	38.3
High-grade	37	61.7
Stage		
Ta	5	8.3
T1	13	21.7
T2	25	41.7
T3	17	28.3
Lymph node invasion		
N0	47	78.3
N1	13	21.7
Urine Cytology		
Positive	32	53.3
Negative	28	46.7
Disease Free Survival (DSF)		
Relapse-free	34	56.7
Relapsed	26	43.3

For all subjects involved in the study, TATI, CYFRA 21.1, TPS and NMP 22 were measured in urine samples. The mean levels and standard deviation (SD) were presented in table 2. Urinary TATI levels in µg/g creatinine (cr) were 22.3 ± 18.4 for controls, 31.7 ± 19.1 for benign subjects and 78.1 ± 61.3 in bladder cancer patients. CYFRA 21.1 levels in urine in µg/g cr were 5.3 ± 1.5 in controls, 19.6 ± 14.9 in benign subjects and 54.4 ± 26.9 in bladder cancer patients. TPS levels in urine in IU/g cr were 44.3 ± 16.8 in controls, 72.1 ± 33.5 in benign subjects and 162.7 ± 76.4 in bladder cancer patients. Urinary NMP 22 levels in U/g cr were 7.5 ± 4.6 for controls, 11.2 ± 6.4 for benign subjects and 19.8 ± 7.3 in bladder cancer patients.

Regarding all biochemical markers investigated, benign levels were significantly higher than control levels and

RESULTS

The study included 140 individuals in 3 groups. Group 1 consisted of 60 bladder cancer patients (median age 56.9 years; range 40-71 years), Group 2 consisted of 40 patients with benign urologic diseases (median age 57.3 years; range 42-73 years), and group 3 included 40 healthy volunteers (median age 56.7 years; range 44-72 years) as a control group.

Of the bladder cancer patients, 41 (68.3%) had transitional cell carcinoma (TCC) and 19 (31.7%) had squamous cell carcinoma (SCC). The majority of patients were of clinical stage T2 (41.7%) and the remaining were of stages Ta (8.3%), T1 (21.7%) and T3 (28.3%). The tumor size ranged from 1.8 to 14.6 cm, with a mean and SD of 8.9 ± 7.5 cm. Only 13 patients (21.7%) had lymph node invasion at diagnosis and only 53.3% of patients were positive for urine cytology. After 36 months of follow up, 26 patients (43.3%) relapsed while 34 patients (56.7%) remained cancer-free, table 1.

mean levels in bladder cancer patients were significantly higher than mean levels in both control and benign subjects. The difference between mean levels of TCC and SCC patients was not significant in all the parameters measured.

As for the association of the biochemical markers with tumor pathology, as presented in table 3, higher TPS levels were significantly associated with higher tumor grade (p=0.025), higher clinical stage (p=0.001), larger tumor size (p=0.031), LN invasion (p=0.002), positive cytology (p=0.005) and lower DFS (p=0.001). High levels of TATI were significantly associated with higher clinical stage (p=0.014), LN invasion (p=0.037), positive cytology (p=0.001) and lower DFS (p=0.001). High levels of CYFRA 21.1 and NMP-22 showed significant association only with positive cytology (p=0.027 and 0.042) and lower DFS (p=0.021 and 0.008).

Table 2: Urinary levels of TATI, CYFRA 21.1, TPS and NMP 22 (presented as mean± SD).

Parameter	N	Urinary levels	P value		
			p1	p2	p3
TATI (µg/g creatinine)					
Control	40	22.3 ± 18.4			
Benign	40	31.7 ± 19.1	0.012*		
Bladder Cancer	60	78.1 ± 61.3	0.001*	0.037*	
TCC	41	77.5 ± 52.8	0.015*	0.041*	0.825
SCC	19	82.0 ± 66.1	0.009*	0.042*	
CYFRA 21.1 (µg/g creatinine)					
Control	40	5.3 ± 1.5			
Benign	40	19.6 ± 14.9	0.025*		
Bladder Cancer	60	54.4 ± 26.9	0.001*	0.024*	
TCC	41	52.8 ± 26.8	0.001*	0.033*	0.996
SCC	19	55.8 ± 27.0	0.001*	0.030*	
TPS (IU/g creatinine)					
Control	40	44.3 ± 16.8			
Benign	40	72.1 ± 33.5	0.043*		
Bladder Cancer	60	162.7 ± 76.4	0.005*	0.011*	
TCC	41	162.3 ± 77.0	0.011*	0.024*	0.967
SCC	19	162.8 ± 75.7	0.008*	0.029*	
NMP 22 (U/g creatinine)					
Control	40	7.5 ± 4.6			
Benign	40	11.2 ± 6.4	0.038*		
Bladder Cancer	60	19.8 ± 7.3	0.007*	0.037*	
TCC	41	19.5 ± 6.7	0.021*	0.042*	0.826
SCC	19	17.88 ± 7.1	0.028*	0.046*	

* : p is considered significant at $p \leq 0.05$.

p1 : significance when comparing benign and cancer to control levels.

p2 : significance when comparing cancer to benign levels.

p3 : significance when comparing TCC to SCC.

Table 3: Association of clinical and tumor pathological parameters with urinary levels of measured biochemical markers in bladder cancer patients.

	TATI µg/g creatinine	CYFRA 21.1 µg/g creatinine	NMP 22 U/g creatinine	TPS IU/g creatinine
Tumor Grade				
Low-grade (n=23)	64.4 ± 50.7	45.7 ± 24.2	18.4 ± 7.2	84.3 ± 42.7
High-grade (n=37)	87.3 ± 61.8	64.9 ± 29.8	20.0 ± 7.5	232.5 ± 115.3
p	0.082	0.134	0.653	0.025*
Stage				
Ta/1 (n=18)	53.7 ± 37.9	43.5 ± 26.7	17.3 ± 7.7	81.4 ± 37.2
T2/3 (n=42)	111.5 ± 69.7	65.3 ± 30.5	21.8 ± 6.0	233.6 ± 101.8
p	0.014*	0.158	0.130	0.001*
Tumor size				
< 5 cm (n=21)	75.4 ± 56.2	51.7 ± 26.9	16.7 ± 6.3	96.2 ± 44.6
≥ 5 cm (n=39)	88.3 ± 67.9	56.1 ± 25.2	22.1 ± 7.9	221.3 ± 89.7
p	0.651	0.734	0.217	0.031*
LN invasion				
-ve (n=47)	55.3 ± 45.9	49.5 ± 27.5	14.7 ± 7.3	79.4 ± 47.0
+ve (n=13)	100.7 ± 71.1	56.9 ± 29.2	23.7 ± 9.1	240.3 ± 104.1
p	0.037*	0.218	0.086	0.002*
Cytology				
-ve (n=28)	43.8 ± 31.3	38.5 ± 24.2	12.9 ± 6.7	77.6 ± 49.3
+ve (n=32)	108.7 ± 77.1	75.4 ± 29.6	23.1 ± 8.9	238.3 ± 108.7
p	0.001*	0.027*	0.042*	0.005*
DFS				
Relapse-free (n=34)	45.7 ± 32.7	35.7 ± 25.5	12.2 ± 5.3	75.4 ± 42.7
Relapsed (n=26)	105.5 ± 78.5	75.7 ± 31.2	24.6 ± 9.5	243.1 ± 88.1
p	0.001*	0.021*	0.008*	0.001*

* : Mann-Whitney U test, the test is considered significant when $p \leq 0.05$.

To set cutoffs for the studied parameters as diagnostic markers, ROC curves were established as shown in figure 1 and table 4. All biochemical markers were statistically significant (0.011, .018, 0.027 and 0.032 for TATI, CYFRA 21.1, NMP 22 and TPS; respectively). The highest AUC, 0.83, was found for TATI, which at a cutoff value of 42 $\mu\text{g/g cr}$ showed a sensitivity of 82.0% and specificity of 80.5%. CYFRA 21.1 was also a good

marker for bladder cancer with AUC of 0.81, and at a 21.0 $\mu\text{g/g cr}$ cutoff value, it showed 80.4% sensitivity and 73.1% specificity. Followed by NMP 22 with an AUC of 0.78 at a cutoff level of 10.5 U/g cr with a sensitivity and specificity of 73.9% and 72.4%; respectively. TPS had the least AUC of 0.75 and at a cutoff value of 152 IU/g cr, its sensitivity and specificity were 68.2% and 61.5%; respectively

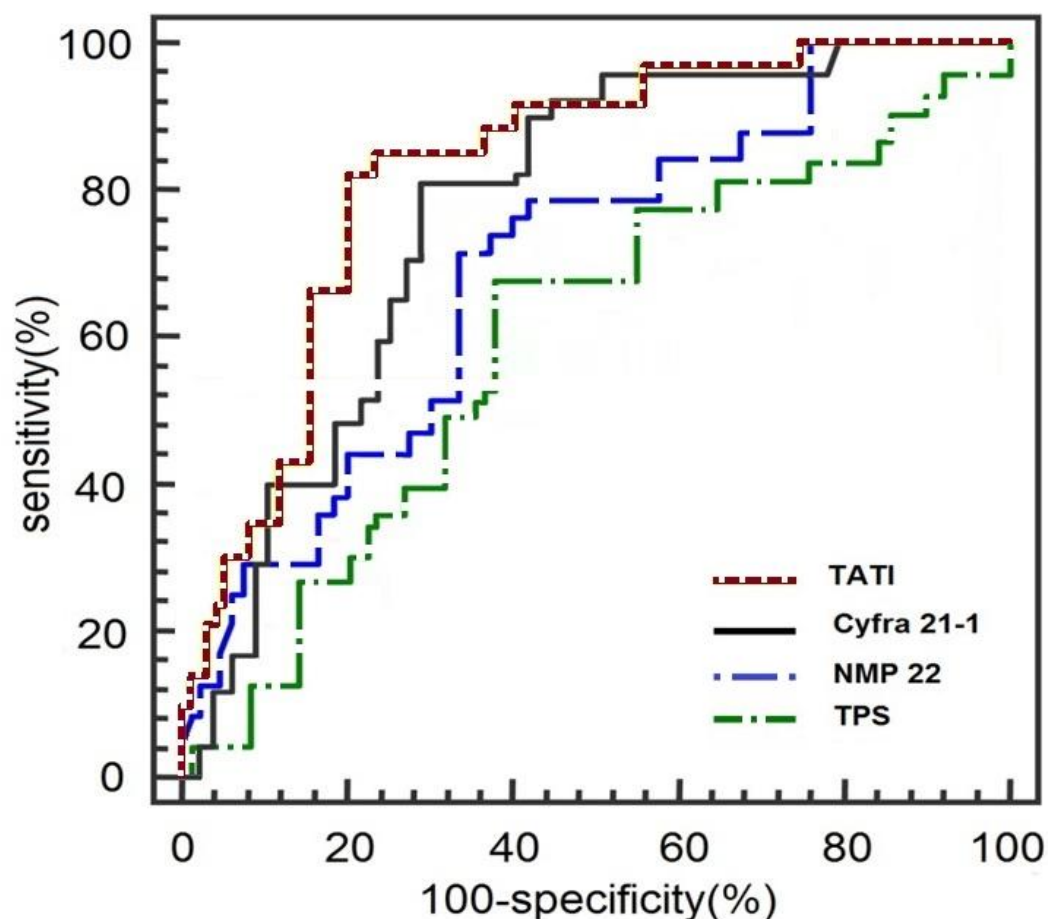


Figure 1: ROC curve for urinary levels of studied urinary markers.

Table 4: AUC, optimum cutoff, sensitivity and specificity of urinary TATI, CYFRA 21.1, NMP 22 and TPS for the detection of bladder cancer.

Parameter	TATI	CYFRA 21.1	NMP 22	TPS
p value	0.011	0.018	0.027	0.032
AUC	0.83	0.81	0.78	0.75
Cut off	42 $\mu\text{g/g cr}$	21 $\mu\text{g/g cr}$	10.5 U/g cr	152 IU/g cr
Sensitivity (%)	82.0	80.4	73.9	68.2
Specificity (%)	80.5	73.1	72.4	61.5

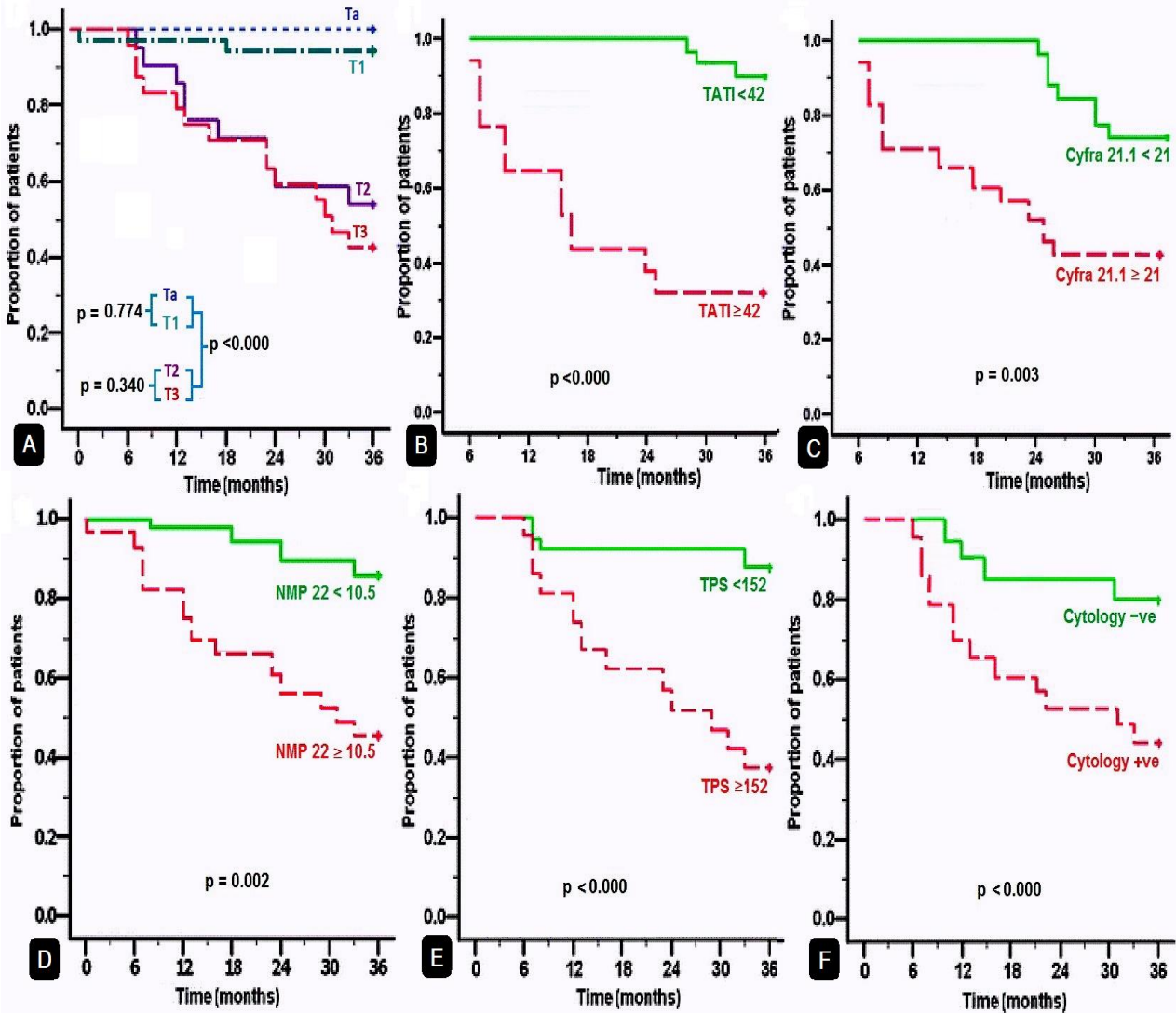


Figure 2: Kaplan-Meier Survival curves of all patients according to; A: stage; B: TATI; C: CYFRA 21.1; D: NMP 22; E: TPS; and F: cytology.

After 36 months of follow up, these cut offs for all parameters were used to establish Kaplan-Meier survival curves according to disease stage, TATI, CYFRA 21.1, NMP 22, TPS and urine cytology. All parameters were significant factors for survival, as presented in figure 2. Stage was a significant variable in DFS ($p=0.000$), as patients with low stage showed higher survival (35 months) than patients with high stage (21 months). As for TATI, patients with initial TATI levels $<42 \mu\text{g/g}$ cr had significantly higher survival ($p=0.000$) than patients with TATI levels $\geq 42 \mu\text{g/g}$ cr (31.5 vs 17.3 months; respectively). Also patients with CYFRA 21.1 $<21 \mu\text{g/g}$ cr showed higher survival (29.8 months) than patients with CYFRA 21.1 $\geq 21 \mu\text{g/g}$ cr (19.4 months), and the difference was significant ($p=0.003$). The same was noted for NMP 22, where a significant difference ($p=0.002$) existed between patients with NMP 22 $<10.5 \text{ U/g}$ cr (32.1 months) and patients with NMP 22 $\geq 10.5 \text{ U/g}$ cr (23.3

months). Patients with TPS $<152 \text{ IU/g}$ cr showed a significantly higher survival (30.8 months) than patients with TPS $\geq 152 \text{ IU/g}$ cr (16.7 months) with $p=0.000$. A significant difference ($p=0.000$) also existed between patients with negative cytology (24.0 months) and patients with positive cytology (17.8 months).

Table 5 showed that the presence of more than one elevated marker strongly affected survival. Patients with 2 ($p=0.017$), 3 ($p<0.001$) or 4 ($p<0.001$) elevated markers had significantly lower survival rate compared to patients with no or 1 ($p=0.265$) elevated marker. Survival rate showed a gradual decrease as the number of elevated markers increased. The addition of cytology did not improve the diagnostic or prognostic performance of the makers under evaluation for bladder cancer. The addition of more than one marker did not add to the sensitivity and specificity of the diagnostic accuracy.

Table 5: Combined effects of the elevated urinary biochemical markers on disease-free survival of bladder cancer patients.

No. of elevated markers	No. of patients (%)	DFS rate	P1
0	6 (10.0 %)	83.3 %	-
1	19 (31.7 %)	78.9 %	0.265
2	16 (26.7 %)	56.3 %	0.017
3	12 (20.0 %)	25.0 %	<0.001
4	7 (11.6 %)	28.6 %	<0.001
P2		<0.001	

P1 significance when compared to 0.

P2 significance of trend among all groups.

DISCUSSION

Preliminary clinical diagnosis of bladder cancer relies predominantly on cystoscopy along with urine cytology. Cystoscopy is an uncomfortable and costly invasive procedure that may require anesthesia of the patient and entails the risk of urinary tract infection. The technique enables visualization of the bladder lining and biopsy of suspicious lesions for histopathological diagnosis and staging, but it often fails to detect in-situ neoplasm. Evaluation by cytology depends upon microscopic visualization of shed cancer cells in voided urine. The technique delivers good results in high-grade and high-stage tumors, but because Ta and T1 tumors shed very few cancer cells into the urine, the sensitivity of urine cytology for early-stage tumors is low, ranging only 20-40 %. Coupled with the fact that cytology is prone to inter-observer variation, it is understandably not considered as a standalone test for application in a potential bladder cancer clinical investigation.⁽²⁰⁾

After initial treatment of bladder cancer, patients are continuously surveilled by cystoscopy and cytology done routinely in order to achieve early detection of new tumor development. Consequently, the advent of non-invasive assays that could diminish the need for cystoscopy would be of extreme benefit to both patients and the healthcare system. For bladder cancer, the ease of access of urine samples is considered a principal advantage, as sample can be non-invasively obtained, avoiding patient discomfort and complications, ample amounts of sample could be obtained for analysis and repeat sampling is easily achievable.⁽²¹⁾

Thus far, no single marker was identified that can guide in the surveillance of bladder cancer and minimize the frequency of cystoscopy, but combining these markers may enhance their reliability; however, it may augment the costs.⁽²²⁾ In the current study a panel of four urinary markers (TATI, NMP 22, CYFRA 21.1 and TPS) were used in addition to urine cytology for detecting primary bladder cancer and predicting its recurrence.

In the current study, TATI levels in bladder cancer patients were found significantly higher than both benign and control levels. The ROC curve showed that TATI had an excellent diagnostic significance with an optimum cutoff value of 42 µg/g cr, sensitivity of 82.0% and specificity of 80.5%. Initially higher

TATI levels showed a significant association with higher stage and LN involvement. Concerning survival, patients with initial TATI<42 µg/g cr had a significantly better DFS than those with TATI≥42 µg/g cr.

Because of the discrepancy of results, our results were conforming to some studies and contradictory to others. In accordance with our results, Kelloniemi *et al.*⁽²³⁾ found that higher TATI levels in urine were associated with muscle-invasive disease at diagnosis and decreased survival in patients with superficial and invasive bladder cancer. Shariat *et al.*⁽²⁴⁾ and Patschan *et al.*,⁽²⁵⁾ reinforced this conclusion, demonstrating that patients with invasive tumor had greater TATI levels than those with superficial, non-invasive disease; however, no significant correlation was found with tumor grade. Another study exhibited that TATI was efficient in monitoring disease activity and response to treatment in patients with advanced and metastatic bladder cancer.⁽²⁶⁾ In contrast, Hotakainen *et al.*⁽²⁷⁾ have shown that TATI expression decreases with increasing tumor stage and grade. While Gkialas *et al.*,⁽²⁸⁾ found TATI to be a valuable urinary marker but was not correlated with tumor stage.

In the current study, CYFRA 21.1 urinary levels in bladder cancer patients were statistically higher than that in both benign and control levels. Regarding diagnostic value, CYFRA 21.1 had an excellent diagnostic significance with an optimum cutoff value of 21 µg/g cr, sensitivity of 80.4% and specificity of 73.1%. Initially higher CYFRA 21.1 levels were not associated with clinicopathological features. As for survival, patients with initial urinary CYFRA 21.1 level <21 µg/g cr had a significantly better DFS than those with CYFRA 21.1≥21 µg/g cr.

Several studies and meta-analyses have reported that bladder cancer patients had higher CYFRA 21.1 levels than healthy subjects.^(9,10, 28-31) This result suggested that CYFRA 21.1 levels are a competent biomarker for diagnosing bladder cancer. In addition, CYFRA 21.1 values in patients having various stages of bladder cancer were not significantly different. Thus, it is necessary to develop additional urinary biomarkers for the differential diagnosis of stage I, II and III bladder cancer.^(9,10, 28-31) However, contradictory results have been reported specially that its application as a surveillance tool for superficial bladder carcinoma was not very useful, because urinary assessment of CYFRA 21.1 showed low sensitivity for recurrent disease.^(32,33) Discrepancy of data reported is most probably attributed to

various methods of detection and different cutoff levels used. To our knowledge, the prognostic power of preoperative evaluation of CYFRA 21.1 in urine has not been reported previously.

Our results indicated that NMP-22 levels in urine of bladder cancer patients were significantly higher than that in both benign and control levels. As a diagnostic marker, NMP-22 had a good diagnostic significance with an optimum cutoff value of 10.5 U/g cr, sensitivity of 73.9% and specificity of 72.4%. Initially higher NMP-22 levels was not significantly associated with tumor pathology of patients, but it showed only a significant association with urine cytology. Concerning survival, patients with initial NMP-22 < 10.5 U/g cr had significantly better DFS than those with NMP-22 ≥ 10.5 U/g cr.

NMP-22 is expressed specifically in urinary tract transitional cells.⁽³⁴⁾ Typically, patients with TCC are expected to have higher concentration of NMP-22 in their urine than patients with SCC, but that was not the case in the current study, where no significant difference was noted in NMP-22 urinary levels between TCC and SCC. Although previous reports all demonstrated the validity of NMP-22 as a diagnostic bladder cancer test, the sensitivities and specificities varied widely depending on the cutoffs used in each study. Sensitivities ranged from 47% to 100% and specificities ranged from 55% to 98%.^(11,34-35) A meta-analysis of 24 studies⁽³⁶⁾ showed that at a cutoff value of 10U/ml the sensitivity of NMP-22 test was 71% and the specificity was 80%, these data were in consensus with our results.

The current results showed that urinary TPS levels in bladder cancer patients were significantly higher than that in both benign and control levels. For diagnosis, ROC curve showed that TPS had a good diagnostic significance and at the optimum cutoff value of 152 IU/g cr, sensitivity was 68.2% and specificity was 61.5%. Most of previous reports demonstrated the diagnostic potential of TPS for bladder cancer, the sensitivities and specificities varied widely depending on the cutoffs used in each study. Sensitivities ranged from 34.6% to 73.1% and specificities ranged from 50% to 88.4%.^(12,37-38)

Interestingly, initially higher TPS levels were significantly associated with most of the clinicopathological parameters as higher tumor size, grade, stage and lymph node involvement. Contradictory results were reported regarding correlation with clinicopathological features where some reported positive correlation with some clinicopathological parameters^(37,39), others found no correlation with any of them.⁽³⁸⁾ As regard survival, our patients with initial TPS < 152 IU/g cr had significantly better survival than those with TPS ≥ 152 IU/g cr.

Most published studies used serial measurements of urinary markers to follow up bladder cancer patients, but very few studies directly investigated the association between urinary levels of biomarker at presentation and outcome, with fewer studies investigated if urinary biomarkers may provide prognostic information over and above that provided by typical clinicopathological factors.

The most significant finding of this study was that combined analysis of multiple urinary tumor markers had a much higher potential value in terms of prognostic importance. Bladder cancer patients with higher levels of any of the four studied urinary markers had significantly worse survival, however, when they were combined, DFS decreased as the number of elevated markers increased (p < 0.0001). The most significant drawback of using one of the biochemical markers was that its

sensitivity and specificity were not high enough in addition to the overlap with normal or benign levels, but our results suggested that the sensitivity of these markers can be improved significantly if they are used in combination as a panel of markers.

In conclusion, in bladder cancer patients, initial urinary levels of TATI, CYFRA 21.1, NMP-22 and TPS at presentation were significant diagnostic markers with acceptable sensitivities and specificities, and their higher levels were significantly associated with decreased survival. When these four markers were used together, DFS decreased as the number of elevated markers increased.

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