

Comparative Study of NMP-22, Telomerase, and BTA in the Detection of Bladder Cancer

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ABSTRACT

Purpose: The diagnostic efficacy of Nuclear Matrix Protein-22 (NMP-22), bladder tumor antigen (BTA TRAK), and telomerase activity was evaluated in urine in a trial to assess their value in the detection of bladder cancer and to compare it to that of routine urine cytology.

Subjects and Methods: The study included 46 newly diagnosed bladder cancer patients, diagnosed by cystoscopy and histopathological typing, in addition to 20 patients with benign bladder lesions and 20 healthy age and sex matched volunteers as a control group. Fifty percent of the cancer patients (23/46) had proven bilharzial history. Most patients (27/46) had transitional cell carcinoma (TCC), 17/46 had squamous cell carcinoma (SCC), while only 2 patients had adenocarcinoma. A single freshly voided urine sample (\approx 100ml) was collected from each patient and control subject and aliquoted for each test. All assays were conducted according to the manufacturer's guidelines and the results were compared to those of urine cytology.

Results: The optimal cutoffs for NMP-22, BTA and telomerase activity as calculated by ROC curves were 12.1 U/ml, 78 U/ml, 0.48 (Ratio) respectively. The levels of the three parameters were significantly higher in the malignant group compared to either the benign group or normal controls, ($p < 0.001$) and the positive rates were also higher in the malignant group for all 3 parameters. The overall sensitivity of urine cytology, NMP-22, BTA and telomerase was 54.3%, 91.3%, 100% and 80.4% respectively. For bilharzial cancer bladder respective sensitivities were 69.6%, 95.6%, 100% and 73.9%, while for nonbilharzial cancer bladder the respective sensitivities were 39.1%, 87%, 100% and 87%. The overall specificities with urine cytology, NMP-22, BTA and telomerase was 100%, 87.5%, 92.5% and 95.0%, respectively. Combined sensitivity of voided urine cytology with one or more of the 3 biomarkers, or the use of these biomarkers in double or triple combinations gave higher positivity than each biomarker alone.

Conclusion: BTA showed the highest sensitivity in all the studied parameters in the bladder cancer group, bilharzial bladder cancer subgroup, and non bilharzial bladder subgroup, (100%), while the highest specificity was recorded with urine cytology (100%), followed by telomerase (95%), then BTA (92.5%), and lastly NMP-22 (87.5%). Use of markers in combination with cytology, or in a panel, improved the sensitivity, and specificity.

Key Words: Bladder cancer - Tumor markers - NMP-22 - BTA - Telomerase - Urine cytology.

INTRODUCTION

Bladder cancer is one of the most common urological cancers [1]. Carcinoma of the bladder is the most prevalent cancer in Egypt. At the National Cancer Institute (NCI), Cairo University, it constitutes 30.3% of all cancers [2].

Bladder cancer is usually diagnosed by urethrocytoscopy, which allows direct visualization of tumors and confirmation by biopsy and pathological analysis [3]. Nevertheless, voided urine cytology remains the method of choice for the noninvasive detection of bladder cancer, yet whilst it has a specificity of $>93\%$, its sensitivity is only 25-40%, especially for low-grade and T-stage tumors [4].

Nuclear Matrix Protein-22 (NMP-22) is a nuclear mitotic apparatus protein involved in the proper distribution of chromatin to daughter cells that is present in the nuclear matrix of all cell types and located in the mitotic spindle during mitosis [5]. In bladder cancer, NMP-22 is twice as sensitive as cytology in detecting early T-stage cancers, and up to 90% sensitive and 99% specific [6].

Bladder tumor antigen (BTA) has been identified as a human complement factor H related protein (hCFHrp), which is produced by bladder tumor cells in cell cultures and not by any other epithelial cell lines [7]. BTA is released into the urine of patients with bladder cancer as the tumor invades the stroma [5]. Initial reports indicated that BTA test had higher sensitivity [8] and lower specificity than cytology [9].

Telomerase is a ribonucleoprotein polymerase enzyme containing an integral RNA with a short template element that directs the *de novo* synthesis of telomeric repeats (TTAGGG) at the chromosomal ends, thus maintaining the length of telomeres and allowing immortal cells or tumor cells to continue [10]. Telomerase activity was found to be absent in most normal human somatic cells but present in over 90% of cancerous cells and in *in vitro*-immortalized cells [11]. Progressive telomere shortening is halted in cancer cells by the presence of telomerase enzyme, allowing cells to divide indefinitely [12]. The examination of telomerase activity in urine is helpful in the diagnosis of urothelial carcinoma and may be related to the differentiation degree of tumors [13].

The aim of this work is to study the (NMP-22), the (BTA TRAK, quantitative test to estimate BTA) and telomerase activity in the urine of the patients with bladder cancer in a trial to assess their value in the detection of the tumors and to find a reliable non-invasive technique for the diagnosis of cancer bladder. Sensitivity and specificity of these tumor markers will be compared to conventional cytology in bladder cancer.

PATIENTS AND METHODS

Patients:

All the fresh urine samples (forty six) of newly diagnosed bladder cancer patients delivered to the Clinical Pathology Department of the National Cancer Institute (NCI), Cairo University, over a period of consecutive six months during the year (2004) were studied. Exclusion criteria that might lead to false positive results were haematuria, urinary tract infections and invasive techniques as cystoscopy and samples with these criteria were excluded. Also the urine samples of 20 patients with benign bladder conditions, and 20 apparently healthy age and

sex-matched volunteers were included as controls.

Methods:

Patients included in this study were subjected to careful history taking, clinical examination, routine radiological investigations, histopathological typing, examination of urine cytology, in addition to routine laboratory investigations.

Voided urine samples (100ml) of both controls and patients were collected; those of the patients were collected before cystoscopic examination. For NMP-22 a single void of urine was collected between midnight and noon (0:00 to 12:00 hours) and stabilized immediately by using Matritech NMP-22 Urine Collection Kit. The urine samples were aliquoted and kept at -20°C (for estimation of NMP-22 & BTA) and -80°C (for telomerase estimation) then subjected to the followings:

A- Estimation of BTA in urine by a quantitative enzyme immunoassay utilizing monoclonal antibodies to bind specifically to bladder tumor antigen in urine. The kit was supplied by Polmedco (Redmond, Washington).

B- Estimation of NMP-22 in urine by enzyme immunoassay which employed two monoclonal antibodies specific for the nuclear matrix protein NMP-22. The kit was supplied by Matritech (Cambridge, Massachusetts).

C- Estimation of telomerase activity in urine by PCR ELISA supplied by (Roche Molecular Biochemicals, Germany). In the first step, telomerase added telomeric repeats (TTAGGG) to the 3'-end of the biotinlabeled synthetic PI-TS-primer. These elongation products, as well as the internal standard (IS) included in the same reaction vessel, were amplified by PCR using the primers PI-TS and the anchor primer P2. PCR products derived from telomerase-mediated elongation products in the first step contained the telomerase-specific 6-nucleotide increments, while the internal standard (IS) generated a 216 bp PCR product. The PCR products were split into 2 aliquots, denatured and hybridized separately to digoxigenin-(DIG)-labeled detection probes specific for the telomeric repeats (p3-T) and for the internal standard (IS) (P3-Std), respectively. The resulting products were immobilized via the biotin label to a streptavidin-coated microtitre plate.

Immobilized amplicons were then detected with an antibody against digoxigenin that was conjugated to horseradish peroxidase (Anti-DIG-HRP) and the sensitive peroxidase substrate TMB [14]. Relative telomerase activity (RTA) within different samples in an experiment was obtained using the following formula:

$$RTA = \frac{(A_S - A_{S,O})/A_{S,IS}}{(A_{TS8} - A_{TS8,O})/A_{TS8,IS}} \times 100$$

A_S : Absorbance of sample.

$A_{S,O}$: Absorbance of heat-sample.

$A_{S,IS}$: Absorbance of internal standard (IS) of the sample.

A_{TS8} : Absorbance of control template (TS8).

$A_{TS8,O}$: Absorbance of lysis buffer.

$A_{TS8,IS}$: Absorbance of Internal standard (IS) of the Control template (TS8).

Statistical Analysis:

Data management and analysis were performed using Statistical Analysis Systems (Sigma Stat). The graphs were done using Microsoft Word. Numerical data were summarized using means and standard deviations, also the median and interquartile range. Categorical data were summarized as percentages. Kruskal Wallis non-parametric analysis of variance was used to compare medians of the 3 independent groups. Cases and controls with respect to numeric variables were done using the Mann-Whitney nonparametric *t*-test. Spearman's correlation coefficient was used to measure the strength of association between two numeric variables [15]. All *p*-values were two sided. *p*-values ≤ 0.05 were considered significant. Sensitivity, specificity and diagnostic accuracy were the validity measures used for testing the studied parameters as screening tools for diagnosis of bladder cancer. The different cut offs were chosen using the receiver operating characteristic (ROC). The ROC curve was constructed by plotting sensitivity versus 1-specificity. The curve was drawn through points that represent different decision cut off levels [16].

RESULTS

Bladder cancer patients were 33 males and 13 females with ages ranging from 23 to 77 years, while patients with the benign bladder lesions were 16 males and 4 females; 11 had

non-specific cystitis, 6 bilharzial cystitis and 3 patients had bladder stones. Their ages ranged from 42 to 78 years.

Bladder Cancer Group was Classified as Follows:

1- According to Pathology:

a- Transitional Cell Carcinoma (TCC):

This subgroup included 27 patients (8 females and 19 males). According to the T-stage of bladder cancer, one patient was T1, 8 were T2, 16 were T3, one was T4 and one was TX. According to the grade, one patient was grade 1, 17 grade 2, and 9 grade 3. Ten out of 27 patients (37%) had history of bilharziasis.

b- Squamous Cell Carcinoma (SCC):

This subgroup included 17 patients (5 females and 12 males). According to the T-stage of bladder cancer, two were T1, 7 were T2 and 8 were T3. According to grade, four patients were grade 1, 12 patient grade 2, and 1 was grade 3. Thirteen out of 17 patients (76%) had history of bilharziasis.

c- Adenocarcinoma:

This subgroup included 2 male patients. According to the T-stage and grade of bladder cancer, one was T2 and the other was T3, while both were grade 2. Neither had bilharziasis.

2- According to T-Stage:

3 out of 46 patients (6.5%) were T-stage I, 16 (34.8%) were T-stage II, 25 (54.3%) were T-stage III, while both T-stage IV and T-stage X included only 1 patient (2.2%) each.

3- According to Grade:

5 out of 46 patients (10.9%) were grade 1, 30 (65.2%) were grade 2, while 11 (23.9%) were grade 3.

4- According to Bilharzial Origin:

23 out of 46 patients had history of bilharziasis (50%), while the other 23 (50%) did not.

The median NMP-22, BTA and RTA values were statistically significantly higher in the bladder cancer group when compared to the corresponding median of both the benign bladder lesions group and the control group (*p*-value < 0.001) each. The median NMP-22 and BTA values were higher in the benign bladder lesions

group when compared to the corresponding median of control group (Table 1).

When comparing the studied tumour markers in different pathologic bladder cancer subgroups (SCC and TCC) and in relation to tumour grades and T-stages, no significant difference could be detected.

Comparative analysis of NMP-22, BTA, and RTA levels between bilharzial and non-bilharzial bladder cancer group and benign group showed a significant difference between benign and cancer groups for all the three markers (p -value ≤ 0.001), while there was no significant difference between bilharzial and non-bilharzial cases whether subgroups of benign bladder conditions group or of bladder cancer group (p -value = 0.176, 0.270 and 0.672 for NMP-22, BTA, and RTA, respectively) and the difference between the groups was the same for bilharzial and non-bilharzial subgroups (p -value = 0.226, 0.924, and 0.894 for NMP-22, BTA, and RTA, respectively) (Table 2).

Table (3) shows the diagnostic performance of tumor markers and cytology in bladder cancer patients. The cutoff values of NMP-22, BTA and RTA as shown by ROC curve, were 12.1U/ml, 78U/ml and 0.48, respectively, and areas under the curve were 0.971 (excellent), 1.000 (excellent) and 0.902 (excellent), respectively.

When comparing the diagnostic performance of tumor markers and cytology in bilharzial and non-bilharzial cancer bladder patients (Table 4), BTA showed sensitivity of (100%) in bilhar-

zial and non-bilharzial groups, NMP-22 (95.6%, and 87%, respectively), and RTA (73.9% and 87%, respectively). The cytology showed sensitivity of (69.6% and 39.1%, respectively).

Fig. (1) shows the diagnostic performance of all the studied parameters in double combinations (either abnormal). In all combinations, the either abnormal combination gave better sensitivities than both abnormal combinations.

The best either abnormal double combinations were, cytology with BTA at cutoff (78U/ml) with sensitivity and specificity of (100% and 92.5%) respectively. NMP-22 (12.1U/ml) with BTA (78U/ml) with sensitivity and specificity of (100% and 90%, respectively), BTA (78U/ml) with RTA (0.48) with sensitivity and specificity of (100% and 87.5%, respectively). Also NMP-22 (12.1U/ml) with RTA (0.48) with sensitivity and specificity of (97.8% and 85%, respectively). The best both abnormal double combination was for NMP-22 (12.1U/ml), and BTA (78U/ml) with sensitivity and specificity of (91.3% and 95%, respectively).

The diagnostic performance of all the studied parameters in triple combinations (One and all abnormal) showed that one abnormal parameter gave better sensitivities and lower specificities than all abnormal parameters in all mentioned triple combinations Table (5).

On evaluating the correlation between tumor markers, there was a significant correlation between NMP-22 and BTA ($r=0.80925$), between NMP-22 and RTA ($r=0.60676$) and between BTA and RTA ($r=0.60917$).

Table (1): Comparative study of the tumor markers (NMP-22, BTA and RTA) between control, benign bladder lesion and bladder cancer groups.

Variable	Control (N° = 20)	Benign (N° = 20)	Bladder (N° = 46)	<i>p</i> -value
NMP-22 (Unit/ml)	0.6 (0.425-0.8)	0.8 (0.5-13.5)	117.5 (47.5-271.25)	<0.001*
BTA (Unit/ml)	1.9 (0.85-5.375)	33.3 (0.8-49)	1917 (1425-2160)	<0.001*
RTA (Ratio)	0.17 (0.076-0.319)	0.15 (0.049-0.353)	6.41 (0.58-31.93)	<0.001*

Values are medians and interquartile ranges in parentheses.

*Significant.

NMP-22: Nuclear Matrix Protein-22.

BTA : Bladder tumor antigen.

RTA : Relative telomerase activity.

Table (2): Comparative analysis of NMP-22, BTA, and RTA levels between bilharzial and non-bilharzial bladder cancer and benign groups.

Bilharziasis	NMP-22 (Unit/ml)		BTA (Unit/ml)		RTA (Ratio)	
	Benign conditions	Bladder cancer	Benign conditions	Bladder cancer	Benign conditions	Bladder cancer
Negative	0.85 (0.65-10.9)	101 (40-245)	22.8 (0.78-56.3)	1915 (1320-2060)	0.18 (0.05-0.38)	4.9 (0.68-31.36)
Positive	0.7 (0.50-21)	155 (57-540)	38 (1.6-3.375)	1920 (1620-2215)	0.14 (0.06-0.34)	13.1 (0.38-35.8)
Analysis of variance						
Effect	<i>p</i> -value	Significance	<i>p</i> -value	Significance	<i>p</i> -value	Significance
Group	<0.001*	Yes	<0.001*	Yes	<0.001*	Yes
Bilharziasis	0.176	No	0.27	No	0.672	No
Group*Bilharziasis interaction	0.226	No	0.924	No	0.894	No

Values are medians and interquartile ranges in parentheses.

*Significant.

NMP-22: Nuclear Matrix Protein-22.

BTA : Bladder tumor antigen.

RTA : Relative telomerase activity.

Table (3): Diagnostic performance of tumor markers and cytology in bladder cancer patients.

Variable	Cutoff Value	Sensitivity (%)	95% confidence interval (sensitivity)	Specificity (%)	95% confidence interval (specificity)
NMP-22 (Unit/ml)	12.1 (Unit/ml)	91.3	(78.3-97.2)	87.5	(72.4-95.3)
BTA (Unit/ml)	78 (Unit/ml)	100	(90.4-100)	92.5	(78.5-98)
RTA (Ratio)	0.48 (Ratio)	80.4	(65.6-90.1)	95	(81.8-99.1)
Cytology		54.3	(39.1-68.8)	100	(89.1-100)

NMP-22: Nuclear Matrix Protein-22.

BTA : Bladder tumor antigen.

RTA : Relative telomerase activity.

Table (4): Diagnostic performance of tumor markers and cytology in bilharzial and Non-bilharzial bladder cancer.

Variable	Bilharziasis (N=23)				Nonbilharziasis (N=23)			
	Cytology	NMP-22 (Unit/ml)	BTA (Unit/ml)	RTA (Ratio)	Cytology	NMP-22 (Unit/ml)	BTA (Unit/ml)	RTA (Ratio)
Sensitivity%	69.6	95.6	100	73.9	39.1	87	100	87
95% confidence interval	(47-86)	(76-99.8)	(82-100)	(51.3-88.9)	(20.4-61.2)	(65.4-96.6)	(82.2-100)	(65.4-96.6)

NMP-22: Nuclear Matrix Protein-22.

BTA : Bladder tumor antigen.

RTA : Relative telomerase activity.

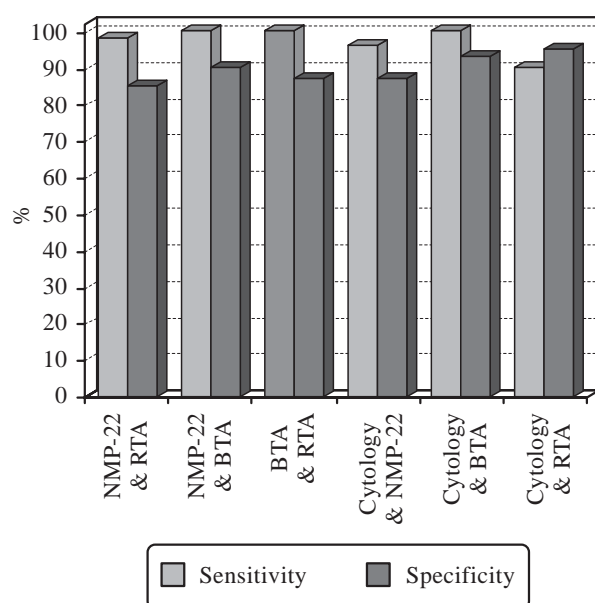
Table (5): Diagnostic performance of (NMP-22, BTA and RTA), (NMP-22, BTA & cytology) and (NMP-22, RTA and cytology), (BTA, RTA and cytology) in triple combinations (One and all abnormal).

Variable	NMP-22 & BTA & RTA		NMP-22 & cytology BTA		NMP-22 & BTA & RTA		NMP-22 & cytology BTA	
	One abnormal	All abnormal	One abnormal	All abnormal	One abnormal	All abnormal	One abnormal	All abnormal
	Cutoff NMP-22 (12.1U/ml), BTA (78U/ml), RTA (0.48)	Cutoff NMP-22 (12.1U/ml), BTA (78U/ml), RTA (0.48)	Cutoff NMP-22 (12.1U/ml), BTA (78U/ml)	Cutoff NMP-22 (12.1U/ml), BTA (78U/ml)	Cutoff NMP-22 (12.1 U/ml), RTA(0.48)	Cutoff NMP-22 (12.1 U/ml), RTA(0.48)	Cutoff BTA (78U/ml), RTA (0.48)	Cutoff BTA (78 U/ml), RTA (0.48)
Sens%	100	73.9	100	50	97.8	41.3	100	45.7
Spec%	82.5	100	85	100	85	100	87.5	100

NMP-22: Nuclear Matrix Protein-22.

BTA: Bladder tumor antigen.

RTA: Relative telomerase activity.



NMP-22: Nuclear Matrix Protein-22
 BTA : Bladder tumor antigen
 RTA : Relative telomerase activity

Fig. (1): Diagnostic performance of tumor markers and cytology in double combination (either abnormal).

DISCUSSION

Carcinoma of the bladder is the most prevalent cancer in Egypt and in most African countries [2]. Diagnosis is usually done at a late T-stage when therapy is rarely curative. The need for early detection must be emphasized [17].

In this study, NMP-22, BTA and RTA were estimated in the urine of patients with bladder cancer in a trial to assess their value in the detection of the tumour, and to find a reliable non-invasive technique for the diagnosis of bladder cancer. They all showed significantly higher values in bladder cancer patients than in benign bladder conditions and control group.

In case of NMP-22, results of this study are in agreement with other researchers [18,19], who reported significantly higher levels in bladder cancer than in benign bladder conditions and controls, but their studies comprised only patients with transitional cell carcinoma of the bladder. Also, Lekili et al. [20] found the urinary NMP-22 levels in bladder cancer patients to be significantly higher than control group. They attributed this to the elevation and release of nuclear mitotic apparatus protein from tumor cells in detectable levels, sometimes more than 25-fold greater than normal cells, perhaps due to cell death [21,22].

Similar to the present study, significantly elevated BTA levels were reported in bladder cancer by Khaled et al. [17]; Priolo et al. [23]; Babjuk et al. [24] and Bassi et al. [25], in comparison to benign bladder lesions and controls.

Telomerase activity was found in this study to be significantly higher in bladder cancer than in benign bladder lesions and control group. Other reports agree with these findings [26,27].

Some researchers view telomerase activity as an important non-invasive diagnostic tool to detect bladder cancer, even in patients with negative or non-assessable urine cytology and with low-grade and early T-stage lesions [28]. Others regard telomerase activity as non-specific for malignancy and may be detected in many non-malignant pathological conditions [29].

In the present study, evaluation of the diagnostic performance of the studied tumor markers was done. The cutoff values were determined by ROC curve. The cutoff values used were 12.1U/ml for NMP-22, 78U/ml for BTA and 0.48 for RTA.

BTA showed the highest sensitivity of 100%, followed by telomerase (95%), then NMP-22 (91.3%). Urine cytology showed a sensitivity of 54.3%. The highest specificity was that of urine cytology (100%). This was followed by telomerase (95%), then BTA (92.5%) and lastly NMP-22 with a specificity of 87.5%.

Although a large number of studies concerning NMP-22 and BTA have been published, there is no agreement on the optimal cutoff level. In contrast, cutoffs have ranged from 3.6-27 U/ml for NMP-22 and from 14-60U/ml for BTA TRAK, depending on the optimum sensitivity and specificity of ROC curve in each study [17,23,30].

BTA, in this study, showed the highest sensitivity of 100%, with specificity of 92.5% at a cutoff of 78U/ml.

Many studies have been published regarding BTA TRAK in bladder cancer patients, resulting in a wide range of sensitivities and specificities. Khaled et al. [17], using a cutoff of 60U/ml, reported a sensitivity of 94% and a specificity of 66%. The sensitivities and specificities reported by Babjuk et al. [24] were 87.1% and 74.4%, respectively at a cutoff of 18U/ml.

Priolo et al. [23], compared the diagnostic performance of BTA with cytology. At a cutoff of 34U/ml, BTA showed a sensitivity of 63% and specificity of 71% while cytology showed a sensitivity of 68.3% and a specificity of 73.4%. The authors decided that cytology was diagnostically better. In a similar study, Fernandez-Gomez et al. [30], also compared BTA with cytology results, but favored the use of BTA. At a cutoff of 14U/ml, BTA gave a sensitivity

of 61.2% with a specificity of 68.4% while cytology showed a sensitivity of 41.1% with a specificity of 97.3%. They favored the use of BTA.

In this study, NMP-22 showed a sensitivity of 91.3% with a specificity of 87.5% at a cutoff of 12.1U/ml.

Gutierrez Banos et al. [31], reported that with a cutoff of 6U/ml, sensitivity of NMP-22 was 84.2% and specificity was 86.5% while when the cutoff was raised to 10U/ml, the sensitivity dropped to 76.3% and the specificity was higher (90.5%).

The sensitivity and specificity of NMP-22 were found to be 81% and 87%, respectively, by Saad et al. [32], while they were 91.7% and 72.1%, respectively, by Su et al. [33] at cutoff of 13U/ml.

Diagnostic performance of RTA in this study showed a high specificity of 95%, with sensitivity of 80.4%, at a cutoff of 0.48.

Few and mostly qualitative or semiquantitative data on the possible differences of telomerase activity in cancer cells and exfoliated cells have been reported [34]. Accordingly, in most cases, no cutoffs were stated in these studies.

Results of most studies on RTA are quite close to our results. In bladder cancer patients, Cheng et al. [26] reported a sensitivity and specificity of 82% and 91%, respectively and Abdel-Salam et al. [35], at a cutoff of 0.2, reported a sensitivity of 47.4% for bilharzial bladder cancer detection. Sensitivity and specificity reported by Saad et al. [32], were 84% and 93%, respectively, by Eissa et al. [27], 83% and 86.3%, respectively, while Sanchini et al. [28], reported high sensitivity (from 75% to 93%) and specificity (72% to 92%) for telomerase in detection of bladder cancer.

The specificity of the telomerase assay was comparable to that of cytology, in a study by Ramakumar et al. [36], which compared cytology, NMP-22, BTA stat (qualitative test for the detection of the presence or absence of BTA), FDP, telomerase, chemiluminescent hemoglobin and hemoglobin dipstick results in bladder cancer patients and controls. Telomerase had the best result of 70% sensitivity and 99% specificity.

In this study, NMP-22, BTA, and RTA were all significantly higher in the bilharzial bladder cancer group than in the bilharzial benign bladder conditions. In agreement with these results, BTA was reported to be extremely sensitive in the detection of bilharzial bladder cancer in the Egyptian population. Positive BTA results were also observed in patients with active bilharziasis [17].

In this study, when comparing the sensitivities of cytology and the assayed tumor markers in bilharzial and non-bilharzial bladder cancer subgroups the sensitivities of cytology and NMP-22 were higher in bilharzial (69.6% and 95.6% respectively) than non-bilharzial cases (39.1% and 87% respectively). On the other hand, telomerase activity showed a higher sensitivity of 87% in non bilharzial bladder cancer than bilharzial subgroups (73.9%).

The results of a study by Eissa et al. [27], were different. They reported that the sensitivity of telomerase was significantly higher in bilharzial bladder cancer patients: 88% in urine and 89% in bladder wash, compared to non - bilharzial cancer patients: 50% in urine and 62.5% in bladder wash. Also Abdel-Salam et al. [35], reported that RTA was increased in bilharzial bladder cancer (47.4%), although to a lesser degree than that reported for TCC in the western world. This could be explained by different biological behavior or different assay methods.

Double and triple combinations of the assayed markers and cytology gave better sensitivity than each parameter individually. The either abnormal double combination gave better results than both abnormal, and the one abnormal gave better results than all abnormal combinations. The best either abnormal double combinations in this study were for BTA with cytology, BTA with NMP-22 and BTA with RTA. Slightly inferior diagnostic performance was observed for the combinations NMP-22 with RTA, NMP-22 with cytology and cytology with telomerase. The both abnormal double combination of NMP-22 and BTA also gave very good results. The best diagnostic performance for the one abnormal triple combinations was observed with the combinations BTA, RTA and cytology, then NMP-22, cytology and BTA. Lastly comes NMP-22, BTA and RTA.

The overall sensitivities of the triple marker combinations in this study were similar to those of the double marker combinations. This emphasizes previous reports that the results of these tumor markers overlap [37]. This may be explained by the strong correlation found in our study between NMP-22 and BTA and between each of NMP-22 and telomerase, and BTA and telomerase.

The use of BTA TRAK in combination with urinary cytology was recommended in the management of bladder cancer patients by Gibanel et al. [38]. Also Ramakumar et al. [36], recommended the use of the combination of cytology with telomerase in bladder cancer patients and Fedriga et al. [39], added that this combination gave a sensitivity of 90%, versus 78% for telomerase alone.

Although NMP-22 and urine cytology gave similar positivity rates in a study by Kurokawa et al. [40], they suggested that a combination of both can be more useful than either used alone for monitoring bladder cancer. Su et al. [33], also recommended this combination and considered it the most complete way to avoid unnecessary cystoscopic surveillance.

Combination of NMP-22 with TRAP assay for telomerase, especially for well-differentiated and superficial tumors, was found to give significantly better detection than voided urine cytology. Researchers reported that the results of NMP-22 and relative telomerase activity yielded sensitivity values comparable with cystoscopy [32].

Conclusion:

We can conclude that all the tumor markers in our study estimated in urine had higher sensitivity than routine urinary cytology, but had lower specificity. The sensitivity and specificity improved when the parameters were used in combination with cytology or in a panel. It can be concluded that BTA TRAK, NMP-22 and telomerase all have enough potential for future clinical use, and although BTA showed slightly better diagnostic performance individually than NMP-22 and telomerase, it is difficult to select a single marker for detection of bladder cancer. Detection of markers in urine in combination with urine cytology or in a panel is recommended as they are easy, non-invasive and sensitive enough to diagnose cancer bladder. It can also

be concluded that the higher sensitivity of NMP-22 in bilharzial than non-bilharzial bladder cancer make it a potentially useful tool for screening patients with bilharziasis at risk for malignancy.

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