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Comparison of urinary telomerase, CD44, and NMP22 assays for detection of bladder squamous cell carcinoma

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Abstract

Background: Squamous cell carcinoma (SCC) of the bladder is common in many regions around the world. Prognosis is very poor, as most cases are diagnosed at an advanced stage due to a lack of affordable and valid screening markers for this type of cancer. The diagnostic accuracy of urinary nuclear matrix protein-22 (NMP22), telomerase activity, and CD44 were evaluated in urine samples of patients with bladder SCC.

Materials and methods: We conducted a case-control study comprised of 60 consecutive newly diagnosed bladder SCC patients diagnosed by cystoscopy and histopathological examination, and controls were 60 outpatients with benign urologic conditions and healthy clinic visitors. Urine samples collected from each subject underwent testing for NMP22, telomerase activity, and CD44. Descriptive and correlational statistical analysis of cases and controls were carried out and receiver operating characteristic curve analysis was used to determine optimal cut-off points for the three assays.

Results: Area under the curve was calculated at 0.96, 0.93, and 0.62 for NMP22, telomerase, and CD44, respectively. Urine levels of NMP22 and telomerase activity were significantly higher in the SCC group compared to controls ($p < 0.001$). Urine CD44 levels were not significantly higher in the SCC group compared to controls ($p = 0.111$). The overall sensitivity of NMP22, telomerase, and CD44 was 96.7%, 87%, and 45%, respectively, while the specificity was 85%, 88.6%, and 86.7%, respectively.

Conclusions: Urinary telomerase activity, followed by NMP22 urine levels, showed high diagnostic yield and could hold potential promise as urinary biomarkers for the diagnosis of bladder SCC.

Keywords: Bladder tumor; CD44; NMP22; Squamous cell carcinoma; Telomerase; Tumor markers

1. Introduction

Bladder cancer (BC) is the sixth most prevalent cancer in both genders and the fourth most prevalent in males worldwide.[1] Urothelial carcinoma represents the most common histologic type of BC, accounting for 90% of all cases.[2] According to the Surveillance, Epidemiology, and End Results (SEER) report, in Western countries transitional cell carcinoma comprises 95.7% of bladder cancers.[3] Nonurothelial bladder tumors account for less than 10% of all global cases, including squamous cell carcinoma [SCC] (2%–5%), adenocarcinoma (0.5%–2%), and small cell carcinoma (<1%).[2] However, SCC is highly prevalent in certain regions of the world including the Middle East and East Africa, where it may account for up to 75% of BC cases.[4]

Risk factors for SCC of the bladder include tobacco exposure, occupational exposure from paint, dye, metal, and petroleum products, repetitive urinary tract infections (UTIs) and bladder irritants (including chemotherapy, such as cyclophosphamide).[5] Chronic irritation and keratinization in the urinary bladder wall resulting from indwelling catheters and chronic cystitis is the most common cause for bladder SCC in Western countries. These risk factors cause chronic inflammation, leading to metaplasia, dysplasia, and cancer.[6,7] SCC may develop following bilharziasis infection, also known as *schistosomiasis*, which is the most common cause of bladder SCC in Africa and the Middle East. Discovered in Egypt in 1851, bilharziasis is also known as *schistosomiasis* due to the role of the *Schistosoma* parasite, which has 3 major species, *S haematobium*, *S mansoni*, and *S japonicum*.[8] *Schistosomiasis* is endemic to 78 countries and is one of the most prevalent parasitic infestations worldwide, posing a major global health problem.[8] *S haematobium* is the species responsible for bladder symptoms and a risk factor for BC.[9]

Due to endemic bilharziasis infestation, BC is the second most common cancer among Egyptian males.[10] A successful bilharziasis control campaign over recent decades has reduced SCC incidence.[11] Nevertheless, the long-term consequences of bilharziasis are still apparent given the high prevalence of BC, which is a result of chronic inflammation of the bladder wall evoked by shedding of the parasite ova over 20 or more years.

SCC is highly malignant, with an associated poor prognosis. Early detection improves prognosis, treatment outcomes, and quality of life. The 5-year survival rate for BC tumors is 97% if detected early while still limited to the mucosa, 71% for invasive tumors diagnosed at a localized stage, but only 35% and 5%, respectively, for regional and distant stage diseased[12,13] BC detected after metastatic spread to other organs means such patients will only

survive, on average, 12–18 months. Current clinical practice and guideline recommendations do not include early screening.

Flexible cystoscopy and cytology are standard diagnostic methods when evaluating BC patients or patients with symptoms suggestive of BC.[14] Cystoscopy has high diagnostic accuracy for low-grade disease, but is an invasive, uncomfortable, and relatively high-cost procedure. Associated costs and the lack of technical expertise required for cystoscopy limit its use in low-income African countries, and diagnostic accuracy may be reduced due to poor visualization because of uncommon locations of the cancer or microscopic disease.[15] Cytology represents a more affordable method for screening purposes and has good specificity, but its poor sensitivity and the associated risk of false negative results can lead to overlooking some existing cancers and thereby contribute to higher mortality.[15–17]

Testing for urinary biomarkers to screen for early stage BC before it becomes invasive is an alternative viable solution. If proven to be efficacious, these markers could be measured routinely in high-risk populations such as smokers, workers in the dye and rubber industry, as well as patients with chronic *schistosomiasis* infections in Africa. Fluorescence in situ hybridization technology is the most widely used testing method in urology clinics in the Western countries, but is expensive, at a cost of approximately $500 per sample, and cannot be performed in laboratories without fluorescent microscopes and specific filters needed for the test.

Most biomarker testing has been performed on transitional cell bladder carcinoma, which is the predominant type of BC in developed countries.[18] There are limited studies that have looked at the sensitivity and specificity of these biomarkers in detection of SCC BC which is the predominant type found in countries such as Egypt. In the last two decades, a few studies in the literature have evaluated molecular biomarkers in cases of SCC.[19,20] Nevertheless, most of these studies were retrospective with limited sample size and showed conflicting results, and they focused on the prognostic role of biomarkers rather than their role in diagnosis. An ideal screening biomarker for BC must be relatively inexpensive, reliable, and most importantly, highly sensitive and specific. The aim in this study was to validate the use of 3 of the more affordable currently available biomarkers, nuclear matrix protein-22 (NMP22), telomerase, and CD44, in urine samples from Egyptian BC patients diagnosed with SCC.

2. Materials and methods

This study was conducted with a cross-sectional convenience sample of consecutive patients referred to the Urology and Nephrology Center at Mansora University in Mansora, Egypt, who were newly diagnosed with squamous cell BC. All such patients were approached to participate in the study. Cases had to be newly diagnosed and urine samples collected prior to surgical intervention. BC diagnosis was confirmed by either positive cystoscopy or cytology. BC type and staging was confirmed by histopathology of the tumor. Consent forms in Arabic were provided to participants after the study was explained to them. Patients who agreed to participate, after written informed consent, provided a urine sample to be tested using the 3 assays described below. Sixty patients were recruited for the study and 60 controls were recruited from among individuals accompanying patients to the outpatient clinic and outpatients with noncancer related conditions. Ethical approval for the study was obtained from the University of California San Diego and Mansoora University.

BC case subjects were asked about their medical and surgical history and current symptoms. Urine culture and cytology for cancer cells, infection, and red blood cells was carried out (Table 1). Radiologic imaging tests for tumor radiological staging were conducted,

Table 1

Characteristics of bladder cancer cases.

Characteristic	Cases (n=60)
Age, mean (SD), yr	58.4 (8.3)
Sex, n (%)	
Male	45 (75)
Female	15 (25)
History of schistosomiasis, n (%)	45 (75)
Bladder mass on cystoscopy, n (%)	41 (68.3)
Urine cytology positive, n (%)	42 (70)
Cystectomy, n (%)	50 (84.6)
Hematuria	43/59 (73%)
Microscopic urinalysis, n (%)	
Normal	3 (5)
Hematuria	12 (20)
Pyuria	3 (5)
Hematuria and pyuria	42 (70)
Smoking history, n (%)	
Smoker	29 (48.3)
Nonsmoker	31 (51.7)
Histopathological grade, n (%)	
Grade I	29 (48.3)
Grade II	18 (30)
Grade III	13 (21.7)
Histopathological stage, n (%)	
Stage I	10 (16.7)
Stage II	25 (41.7)
Stage III	14 (23.3)
Stage IV	11 (18.3)
Bone metastasis, n (%)	1 (1.7)
Lymph node involvement, n (%)	13 (21.7)

and histopathological classification of tumor stage and grade were carried out using flexible cystoscopy. Transurethral resection of visible bladder tumors or cystectomy were documented for the cases. Urine samples from both cases and controls were collected and stored at –80°C before being analyzed using ready-made kits for each of the tests.

2.1. Laboratory analysis

The assay kits were shipped in dry ice to the laboratory of the Urology and Nephrology Center at Mansora University in Mansora, Egypt, for analysis in accordance with assay guidelines provided by the manufacturers. CD44, a nonkinase transmembrane glycoprotein overexpressed in several cell types including cancer stem cells, was detected using cell lysates prepared from urine pellets as follows. Urine samples were centrifuged at $4000 \times g$ for 2 minutes and the supernatant that included a protease inhibitor was removed leaving the pellet behind. The urine pellets were washed in ice cold saline and homogenized on ice in a lysis buffer. The homogenate was incubated on ice for 30 minutes, then filtered and centrifuged at $20,000 \times g$ for 20 minutes at 4°C. The resulting supernatants (lysates) were frozen at –80°C until used for measurement of CD44. Human CD44 was measured using an ELISA quantitative kit (Bender MedSystems) according to the manufacturer's instructions. Briefly, a monoclonal antibody specific for CD44 was used to capture CD44. Biotinylated anti-CD44, streptavidin-horseradish peroxidase, and substrate solution 3,3',5,5'-tetramethyl benzidine were then added. The enzyme–substrate reaction was stopped by adding $2N\ H_2SO_4$. Color intensity was measured at 450 nm using an ELISA plate reader. A standard curve was plotted in Excel to determine CD44 concentrations, expressed in ng/mL. Protein concentration in the cell lysates

produced from urine pellets was measured by Bradford's method using bovine serum albumin as a calibrator.[20] CD44 concentration was then expressed as ng/mg protein.

Telomerase levels were measured using a Telo TAGGG telomerase PCR ELISA PLUS kit (Roche Diagnostics, Mannheim, Germany) in urine samples from 46 cases and 34 controls. There were not enough kits to conduct analysis on the remaining samples and cases and controls were randomly selected for this test. To control for differences in urine concentration, urine protein was determined by the Bradford method. Relative telomerase activity was measured by a telomeric repeat amplification protocol assay, as first described by Kim et al.[22] The telomerase mediated elongation products were subsequently amplified by PCR to allow highly sensitive detection of telomerase activity. A 216bp homologous standard (internal standard) provided in the kit allowed the clear detection of Taq DNA polymerase inhibitors.[23] Each sample (5 µL) was heat inactivated by incubation at 85°C for 10 minutes before telomeric repeat amplification protocol assay in order to inactivate telomerase protein to produce negative controls. PCR was performed using a Hybaid PCR thermocycler (Thermo Electron, Waltham, MA), including primer elongation for 1 cycle at 25°C for 10–30 minutes, telomerase inactivation for 1 cycle at 94°C for 5 minutes, amplification (denaturation) for 1–30 cycles at 94°C for 30 seconds, annealing at 50°C for 30 seconds, polymerization at 72°C for 90 seconds, and final extension at 72°C for 10 minutes.

To determine the NMP22 levels, an NMP22 assay kit (Matritech; Newton, MA) was used, which targets mitotic protein fragments expressed in BC. Nuclear matrix proteins make up the framework of a cell's nucleus and play a role in gene expression. NMP22 localizes with the spindle poles during mitosis and regulates chromatid and daughter cell separation. This protein is present in significantly higher amounts in BC cells than in normal cells. The NMP22 assay is a quantitative enzyme immunoassay using 2 monoclonal antibodies to detect the protein. Specimens were tested on a lateral immunochromatographic strip with 2 separate antibodies, 1 capture and 1 reporter.

2.2. Statistical analyses

Statistical analysis was conducted using SAS software (Version 9.1.3, 2006, SAS Institute, Inc., Cary, NC). The chi–square test was used for categorical variables (such as gender) and t-test was used for continuous variables (such as age). Results of categorical variables are reported in terms of frequency and percentages, and the means, standard deviations, medians and inter-quartile ranges are reported for the biomarkers. Because of the nonparametric distribution of biomarker values, the median-two sample test was used to determine the significance of differences in biomarkers between cases and controls. For multivariate analysis, logistic regression analysis adjusting for age (continuous), and gender (male, female) of participants was used, with case or control status set as the dependent variable and the biomarkers (continuous) as independent predictors.

Specificity was calculated by dividing the number of true negatives (tested negative by the test and the cystoscopy) by the total number of negatives (that includes false positives). The positive predictive value (PPV) of a test calculated by dividing up the number of true positives by all those who tested positive by the test (including false positives). The negative predictive value (NPV) of a test was calculated as the true negatives divided by all those who tested negative by the test (including false negatives). Sensitivity and specificity levels resulting from the continuous variation of cut–off points for each of the biomarkers were represented as receiver operating characteristic

(ROC) curves calculated from the whole range of results observed. The Y axis represents the sensitivity or fraction of true positives (defined as the probability of obtaining a positive result when the individual has the disease) as calculated in the group of patients. On the X axis is the fraction of false positives or "1-specificity." A test that discriminates perfectly, with no overlapping of results from the two populations, has a ROC curve that passes through the top left-hand corner, where S and Sp have maximum values (S and Sp = 1). Qualitatively, the closer the ROC curve is to the top lefthand corner, the higher the overall accuracy of the test. Quantitatively, the area below the ROC curve is an overall measurement of the accuracy of a diagnostic test. The ROC was created using a SAS Macro procedure.[24] The area under the curve (AUC) quantifies the discriminatory ability of a test. An area of 1.0 corresponds to an ideal test since it achieves both 100% sensitivity and 100% specificity. On the other extreme, an area of 0.5 means that the test is no better than random classification.

3. Results

The mean (SD) age of cases and controls was 58.4 (8.3) and 40.8 (13.1) years, respectively (p < 0.001). Males represented 75% (n = 45) of cases and 45% (n = 27) of controls (p < 0.001). Not surprisingly given the strong association between schistosomiasis and squamous BC, a majority (75%, n = 45) of cases reported a history of schistosomiasis. Microscopic urine analysis demonstrated normal results in only 3 cases (5%) and pyuria in 3 cases (5%), while the remaining 54 cases (90%) all had microscopic hematuria. Regarding tumor grade and stage, the largest proportion of cases (>41%) had lower grade tumors (grade I or stage II tumors). Only 1 case had bone metastases and 13 (21%) had lymph node involvement. Among controls, 20% (n = 12) had UTIs, 30% (n = 18) had urinary tract stones, and 50% (n = 30) were healthy individuals.

Table 2 shows results of the 3 biomarkers among cases compared to controls. Both NMP22 and telomerase levels were significantly higher among cases compared to controls, but no such difference was found for CD44. NMP22 and telomerase levels were both approximately 10 times higher among cases compared to controls. Unlike telomerase, NMP22 levels among controls with UTI were 2–3 times higher (13U/mL) than healthy controls or those who only had urinary tract stones. None of the 3 biomarkers, telomerase (p = 0.208), CD44 (p = 0.840), or NMP22 (p = 0.347), were found to have significantly different levels according to stage of cancer. Higher histology stages of 3 and 4 were associated with higher mean biomarker levels, but this difference did not reach statistical significance regarding reported history of schistosomiasis infection, telomerase levels were significantly higher among those with schistosomiasis (p < 0.001), but no such difference was found in terms of CD44 (p = 0.768) or NMP22 levels (p = 0.375).

The sensitivity of cystoscopy among cases in this study was 68.3%, and the sensitivity of cytology was 70%. These 2 tests are considered the gold or commonly used standards for diagnosis of BC. Based on ROC analysis, the optimal cut–off value was 15 U/mL for NMP22, 1.8 for telomerase, and 20ng/mL for CD44. As shown in Table 3, NMP22 and telomerase demonstrated higher sensitivity, PPV, and NPV as compared to CD44. Specificity was comparable for the 3 biomarkers. In terms of overall validity, NMP22 had the highest AUC (0.96), followed by telomerase (0.93), whereas the AUC for CD44 was substantially lower (0.62), as shown in Figure 1. When NMP22 and telomerase were combined as predictors of BC, no appreciable change in AUC was found (0.97).

Finally, results are shown in Table 4 of univariate and multivariate logistic regression analysis of the biomarkers to predict BC

Table 2

Urine biomarker levels detected among cases and controls.

	Total, median (interquartile range)	Cases, median (interquartile range)	Controls, median (interquartile range)-*Median according to the type of control*	p^a
NMP22, U/mL	14.86 (2.7–406.3) (n = 120)	39.63 (10.1–480.3) (n = 60)	4.43 (2.38–25.65) (n = 60) • Urinary tract: 13 • Stone: 5.9 • Healthy: 3.79	$p < 0.0001$
Telomerase	2.10 (0.25–50.6) (n = 80)	5.23 (1.11–57.2) (n = 46)	0.63 (0.03–7.14) (n = 34) • Urinary tract: 0.85 • Stone: 0.67 • Healthy: 0.40	$p < 0.0001$
CD44, ng/mL	20.38 (7.7–87.0) (n = 120)	22.93 (7.78–108.8) (n = 60)	18.67 (6.19–50.89) (n = 60) • Urinary tract: 18.28 • Stone: 23.8 • Healthy: 15.92	$p = 0.111$

[a]Kruskal Wallis test of significance for the median-two sample test. *p* value is for difference between cases and controls.

while adjusting for confounding variables of age and gender between cases and controls (Table 4). The odds ratio to predict BC was highest for telomerase at 1.76 (95% CI, 1.17–2.65), followed by NMP22 at 1.14 (95% CI, 1.06–1.21), and CD44 at 1.02 (95% CI, 1.00–1.05).

4. Discussion

In this study, we found urine telomerase levels to have the highest predictive value as a screening biomarker for squamous cell BC, followed by NMP22. CD44 did not demonstrate adequate predictive value for use as a screening biomarker for squamous cell BC in this study in Egypt. Existing biomarkers used for transitional cell BC recurrence were valid for squamous cell BC detection. Furthermore, telomerase and NMP22 demonstrated better validity in this study than cytology and cystoscopy. Currently, diagnosis of squamous cell BC usually occurs at a late stage when curative therapy is rarely possible; thus, the need for early detection must be emphasized. Therefore, there is a need for a sensitive urine–based biomarker that can help physicians determine which patients require further diagnostic testing and evaluation for cancer.

Early detection can lead to higher BC survival rates. The bladder's ability to accommodate tumor growth can mask symptoms such as pressure or nearby organ displacement, resulting in delayed diagnosis and high mortality. While routine screening in the general population is not feasible due to the relatively low prevalence of BC globally, in certain areas of Africa where schistosomiasis is highly prevalent, regular screening in individuals at high risk for BC is warranted. Those with a history of schistosomiasis infection for more than 10 years who are over 40 years of age and smoke cigarettes should be a priority for screening.

NMP22 is a nuclear matrix protein involved in the distribution of chromatin during mitosis.[25] It is generally elevated in BC but can be present in normal urothelial cells. In patients presenting with microscopic hematuria, use of the NMP22 test demonstrated a higher sensitivity for detection of early stage transitional BC (70%) in comparison with cytology alone (27%).[25] Its performance in the diagnosis of transitional BC varies with tumor grade, with a lower sensitivity in the detection of low-grade lesions.[25,26] The presence of NMP22 in normal urothelial cells reduces its specificity as a diagnostic test, and false-positive results may occur. The NMP22 test has a lower specificity (80%) in comparison with cytology (96%).[25] In a follow-up study of BC patients, NMP22 demonstrated low sensitivity (33%), nonetheless higher than that of cytology (25%), but NMP22 specificity (76%) was lower than that of cytology (97%).[25] In our study, the sensitivity, specificity, and PPV of urine NMP22 levels for detection of SCC were 96.7%, 85%, and 86.6%, respectively.

Telomeres are the structures that cap the ends of chromosomes, playing an essential role in the maintenance of intracellular eukaryotic chromosome stability by specifically binding to structural proteins. Of note, more than 90% of human cancers are telomerase positive, whereas most normal tissues or benign tumors contain low or undetectable telomerase activity.[21] Testing for telomerase in exfoliated cells collected from bladder washings could be a promising tool for the diagnosis and management of BC. Some studies have reported telomerase activity in BC significantly higher than in benign bladder lesions and controls.[26,27] Our results agree with these findings. The specificity to detect BC by a telomerase assay was comparable to that of cytology in a study by Ramakumar et al.[28]; telomerase demonstrated 70% sensitivity and 99% specificity. The sensitivity, specificity, and PPV of the telomerase assay used in our study were 87%, 88.6%, and 90.9%, respectively.

Table 3

Biomarker assay test characteristics using optimal cut–off values derived from ROC curves.

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
NMP22 (n = 120)	96.7	85	86.6	96.2
Telomerase (n = 80)	87	88.6	90.9	83.7
CD44 (n = 120)	45	86.7	77.1	61.2

ROC = receiver operating characteristic; NMP22 = nuclear matrix protein-22.
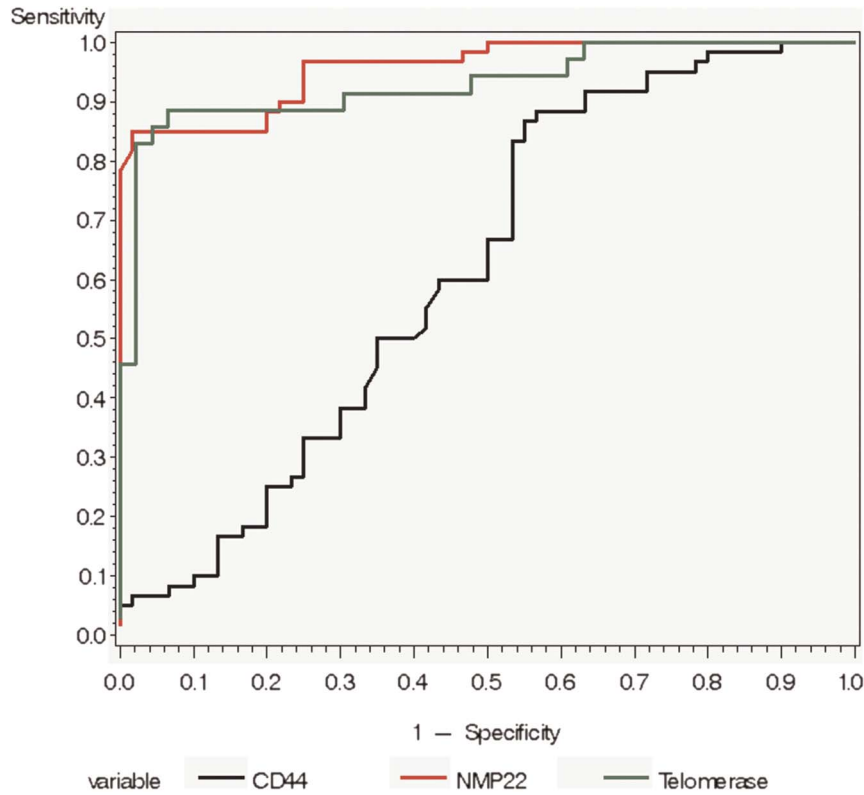
Figure 1. Receiver operating characteristic curves for urinary CD44, NMP22, and telomerase, to detect squamous cell bladder cancer. NMP22 = nuclear matrix protein-22.

The use of telomerase and NMP22 could facilitate early detection of squamous cell BC in less developed countries with limited access to cystoscopy. Several studies have confirmed that telomerase is an appropriate marker for bladder TCC and SCC.[29–31] Before carrying out routine tests for these biomarkers in the population, the assays should be further refined and made more sensitive. Future studies should collect information about other factors that might influence these biomarkers. Out of a panel of 14 biomarkers tested, five of them [cyclooxygenase-2 (COX-2), p53, Bax, fibroblast growth factor-2 (FGF-2) and epidermal growth factor receptor] were significantly correlated with oncologic outcomes, in particular disease recurrence and BC-specific mortality.[20] Previous studies did not examine the value of urinary biomarkers in the diagnosis of bladder SCC.

Limitations of the study include a small budget that prevented inclusion of other available biomarkers, such as the bladder tumor antigen quantitative biomarker. Another limitation was that lower number of cases and controls were tested for telomerase, though this was at random and unlikely to impact the results. Patients with hematuria were not excluded from the study since this is the most common presenting symptom for initial referral to the center. Only 1 person did not have hematuria or pyuria and only 3 had only pyuria, therefore one cannot compare the influence of hematuria or pyuria in this population. As previously observed by others, pyuria from UTI were associated with higher NMP22 levels among controls.[31] Furthermore, the influence of hematuria on the biomarkers used in this study has been docu- mented.[15,32] However, since all cases, except one, had hematuria, there should be no differential error in the results of the biomarkers based on having a symptom of hematuria or not. Another study design limitation of an unmatched convenience sample is the difference in age and

Table 4

Univariate and multivariate logistic regression analyses of biomarkers as independent predictors for squamous cell bladder cancer.

	Univariate OR (95% CI)	*p*	Multivariate OR (95% CI)	*p*
NMP22, U/mL	1.15 (1.09–1.22)	<0.001	1.14 (1.06–1.21)	<0.001
Sex (male vs. female)			6.19 (1.32–28.97)	0.021
Age (yr)			1.12 (1.04–1.20)	0.002
Telomerase	2.02 (1.36–2.99)	<0.001	1.76 (1.17–2.65)	0.007
Sex (male vs. female)			14.35 (2.12–96.99)	0.006
Age (yr)			1.11 (1.03–1.19)	0.005
CD44, ng/mL	1.03 (1.01–1.05)	0.005	1.02 (1.00–1.05)	0.054
Sex (male vs. female)			3.04 (1.04–8.87)	0.042
Age (yr)			1.16 (1.10–1.23)	<0.001

OR = odds ratio; CI = confidence interval; NMP22 = nuclear matrix protein-22.

gender between cases and controls. We therefore adjusted for these variables in the final logistic regression model. However, an advantage of having different heterogeneous group of controls, with some being healthy, others with UTI, and others with renal stones, was that it was possible to detect differences in biomarkers among controls without cancer, such as the increased levels of NMP22 with UTI. These data will help inform future research and practice using these biomarkers, specifically the confounding effect of UTI on measurement of NMP22 for BC detection.

5. Conclusions

In conclusion, compared to the more expensive and lab equipment requirements of the fluorescence in situ hybridization test, NMP22 and telomere ELISA tests are cost-effective assays in detecting squamous cell BC with high sensitivity. NMP22 levels are influenced by UTI, leading to a potential for false positive results and poor specificity in the general population. Therefore, telomerase appears to be the most appropriate marker to be developed for early detection of SCC, in order to facilitate reduction in associated mortality in these countries with SCC such as Egypt, the Middle East, and Africa.

Acknowledgments

Statement of ethics

Ethical approval for the study was obtained from the University of California San Diego and Mansoora University. All patients provided written informed consent for participation and publication of this study. All procedures performed in study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest statement

The authors declare no conflicts of interest.

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Author contributions

WKA conceptualized the idea and study design and drafted the manuscript; AA carried out the recruitment and lab data analysis; AE contributed to data analysis and drafting the manuscript; HA participated in drafting the manuscript and interpretation of results; AS participated in the design, planned and had oversight of the data collection and interpretation of results.

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