Pathol. Oncol. Res. (2014) 20:859–866
DOI 10.1007/s12253-014-9765-y

RESEARCH

Diagnostic Evaluation of Urinary Angiogenin (ANG) and Clusterin (CLU) as Biomarker for Bladder Cancer

Marwa I. Shabayek · Ola M. Sayed · Hanan A. Attaia · Heba A. Awida · Hamdy Abozeed

Received: 2 September 2013 / Accepted: 18 March 2014 / Published online: 3 April 2014

Abstract Bladder carcinoma is an important worldwide health problem. Both cystoscopy and urine cytology used in detecting bladder cancer suffer from drawbacks where cystoscopy is an invasive method and urine cytology shows low sensitivity in low grade tumors. This study validates easier and less time-consuming techniques to evaluate the value of combined use of angiogenin and clusterin in comparison and combination with voided urine cytology in the detection of bladder cancer patients. This study includes malignant (bladder cancer patients, $n=50$), benign ($n=20$) and healthy ($n=20$) groups. The studied groups were subjected to cystoscopic examination, detection of bilharzial antibodies, urine cytology, and estimation of urinary angiogenin and clusterin by ELISA. The overall sensitivity and specificity were 66 and 75 % for angiogenin, 70 and 82.5 % for clusterin and 46 and 80 % for voided urine cytology. Combined sensitivity of voided urine cytology with the two studied biomarkers was 88 % which is higher than the combined sensitivity of both markers alone (82 %) and that of the cytology with each marker (76 and 80 %) for angiogenin and clusterin respectively. In conclusion, combined use of the cytology with the studied biomarkers can improve the sensitivity for detecting bladder cancer, and may be very useful in monitoring the effectiveness of antiangiogenic and apoptotic therapies in bladder cancer.

M. I. Shabayek (✉) · H. A. Awida
Department of Biochemistry, Faculty of Pharmaceutical Sciences and Pharmaceutical Technologies, Future University, Cairo, Egypt
e-mail: biochemistry.fue@gmail.com

O. M. Sayed · H. A. Attaia
Department of Biochemistry, Faculty of Pharmacy, Azhar University, Cairo, Egypt

H. Abozeed
Urology Department, Faculty of Medicine, Azhar University, Cairo, Egypt

Keywords Bladder · Cancer · Cytology · Angiogenin · Clusterin · Sensitivity

Introduction

Bladder Cancer represents a global health problem. It is the seventh common human cancer. The American Cancer Society's estimates that, there would be 72,570 new cases of bladder cancer about 54,610 in men and 17,960 in women and 15,210 deaths from bladder cancer in 2013 [1]. In Egypt, Bladder cancer has been attributed to *Schistosoma* infection, a major risk factor for squamous cell carcinoma (SCC). Recently, transitional cell carcinoma (TCC) incidence has been increasing while SCC has declined [2] (Figs. 1 and 2).

Combination of cystoscopy and urine cytology is considered to be the "gold standard" for identification of bladder tumors [3]. However, Cystoscopy is the reference standard for diagnosis of bladder cancer patients. The procedure can be uncomfortable and can lead to problems in compliance [4]. Cytology of voided urine is the most established noninvasive method in the diagnosis and follow-up in patients with a history of bladder cancer and is used as an adjunct to cystoscopy [5]. Although it is a convenient noninvasive test, it has high sensitivity in high-grade tumors but low sensitivity in low-grade tumors [6]. Because of the low sensitivity of urine cytology, the invasive nature of cystoscopy, and the high cost, efforts have been put forth to find urinary biomarkers that would be noninvasive, simple, efficient, and objective and have high sensitivity and specificity [7].

Angiogenin (ANG) is a 14 KDa, non-glycosylated polypeptide so named for its ability to induce new blood vessel growth. Accumulating evidence indicates that the angiogenic activity of ANG is related to its ability in regulating ribosomal RNA (rRNA) transcription [8]. Angiogenin plays an important role in angiogenesis of urinary bladder cancer which

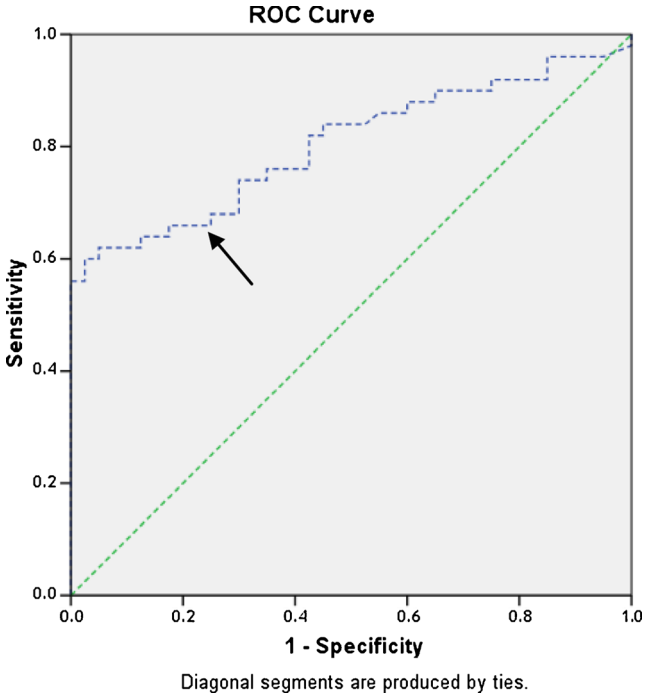


Fig. 1 ROC curve analysis for angiogenin (ANG). The *arrow* denotes a best cutoff point of 145 pg/ml at which sensitivity is 66 % and specificity is 75 %. Area under the curve is 0.803, and confidence limit is 0.712–0.894, $P<0.001$

initiates cell migration and aids in proliferation and differentiation of endothelial cells. Since it may have a role in the development and evolution of carcinomas, it is a particularly interesting molecule to study as a potential tumor marker and/or prognostic indicator for urinary bladder cancer [9].

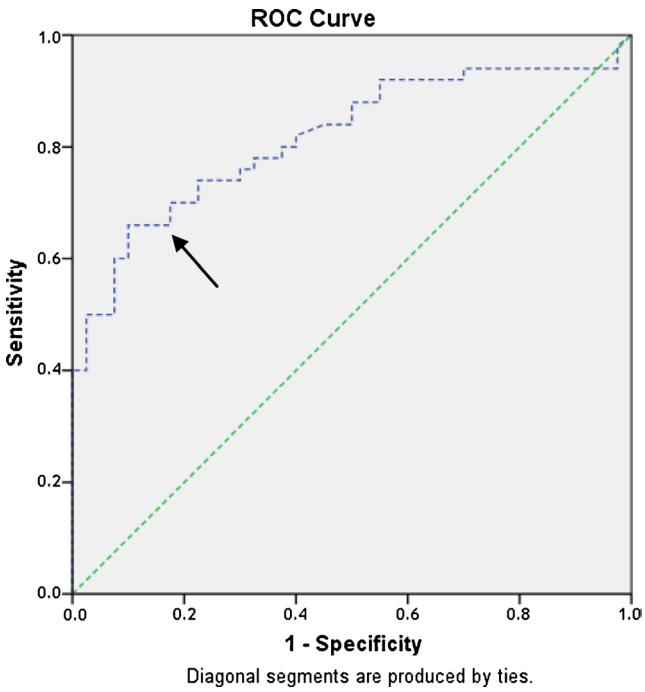


Fig 2 ROC curve analysis for clusterin (CLU). The *arrow* denotes a best cutoff point of 15 ng/ml at which sensitivity is 70 % and specificity is 82.5 %. Area under the curve is 0.817, and confidence limit is 0.729–0.905, $P<0.001$

Clusterin (CLU) is 80 KDa, a multifunctional secretory glycoprotein. Clusterin is found in all body fluids. Clusterin can modulate cell–cell and/or cell–matrix interactions, regulation of survival/apoptosis, tissue remodeling and tumor genesis [10]. Clusterin has a potential oncogenic role in the development and/or progression of several human cancers [11].

We supposed that the combined analysis of these two markers with different functional molecular targets could improve the general sensitivity and specificity of bladder cancer diagnosis in urine. In this study, we evaluated expression of angiogenin (ANG) and clusterin (CLU) in voided urine of patients with bladder cancer, benign urological disease and healthy volunteers. The value of combining use of the two biomarkers alone or with voided urine cytology was also evaluated.

Materials and Methods

Patient Database

Seventy samples were collected randomly from the national cancer institute in Egypt in the period of October 2011–October 2012 were included in the study after giving informed consent. The ethics committee and Internal Review Board of Future University approved this study. All patients provided morning voided urine sample (Approx. 50–100 ml) before cystoscopy. Of the 70 subjects, 50 were histological diagnosed as bladder cancer patients (mean age ± SD: 61.56±9.79; range: 44–91). Whereas the remaining 20 patients (mean age ± SD: 64.1±9.86; range: 36–80) were suffering from benign bladder lesions and benign prostate hyperplasia. All 70 subjects underwent cystoscopy as a reference standard method for bladder cancer detection and full histopathological examination was performed on suspicious lesions. Moreover, 20 healthy volunteers (mean age ± SD: 50.05±8.3; range: 35–65) were also included in this study as a control group. Tumor staging and grading was determined according to TNM and World Health Organization classification [12].

Sample Collection and Cytological Preparation

Blood and urine samples were collected and transported to the laboratory on ice within 2 h. Blood samples were centrifuged and sera were separated, and stored at −80 °C. Voided urine samples were collected and separated by centrifugation at 2,500–4,000 rpm for 15–20 min and filtered into urine supernatant and urine pellet. Then, supernatants were divided into aliquots and stored at −80 °C while pellets were preserved in a protease inhibitor cocktail and stored at −80 °C. Part of each pellet was applied on a slide, dried in air, fixed with 95 % ethanol, stained by Papanicolaou stain and sent to the

pathologist for cytology examination to detect malignant cells. Urine cytology was carried out in the cytopathology laboratory at oncology diagnostic unit by an expert pathologist.

Qualitative Determination of Schistosomiasis Antibodies

Bilharzial antibodies were measured in all patients' sera by using indirect haemagglutanation test IHA, Schistosomiasis FUMOUZE Kit, LEVALLOIS-PERRET CEDEX/FRANCE.

Enzyme-Linked Immunosorbent Assays for Angiogenin and Clusterin

The assay were conducted after several trials on different urine portions. This study validates urine supernatant as the efficient method for measuring angiogenin and clusterin. The levels of human Angiogenin (Cat # CSB-E04498h), and human Clusterin (Cat# CSB-E09121h) were measured in urine supernatant samples using enzyme-linked immunosorbent assays (ELISA).

Statistical Analysis

The threshold value for optimal sensitivity and specificity of angiogenin and clusterin were determined by a receiver operating characteristics (ROC) curve. The cutoff value for each marker that maximizes the sum of sensitivity and specificity was chosen to discriminate between malignant and non-malignant (benign and normal) groups. The sensitivity (percent positive cases of the 50 malignant cases), specificity (percent negative cases of the control group), positive predictive value (PPV), and negative predictive value (NPV) for angiogenin, clusterin and cytology were calculated using the 2×2 contingency table. The simultaneous evaluation of previous parameters was considered positive when any marker shows positive result and vice versa. The positivity rates were compared by chi-square test. The non-parametric Kruskal Wallis test was used for the statistical comparison of the variables between the different groups. The level of significance was determined to be $p<0.05$. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) (SPSS version 20, Chicago, IL, USA).

Results

Referring to the ROC curve, the best cutoff value for angiogenin was 145 pg/mg, whereas the area under the curve was 0.803. The best Cut off value for clusterin was 15 ng/mg. whereas the area under the curve was 0.817. As shown in (Table 1), angiogenin positivity rate (≥145 pg/mg) was detected in 66 % of the malignant group compared to 30 % in benign group and 20 % in healthy subjects at $P=0.000$. Clusterin positivity rate (≥15 ng/mg) was detected in 70 % of the malignant group compared to 30 % in benign group and 5 % in healthy subjects $P=0.000$. Urine cytology was detected in 46 % of the malignant group, 40 % in benign group and 0 % in healthy subjects. Another statistical method (Kruskal–Wallis test) was used and significant difference between malignant group and non malignant group was found for both angiogenin and clusterin ($P<0.001$) (Table 2). As shown in (Fig. 3) angiogenin has highest rank in the malignant group and clustrein has the highest rank in malignant group (Fig. 4)

Markers Positivity Rates in Relation to Different Clinicopathological Factors in Malignant Group

On comparing the positivity rates for angiogenin and clusterin in relation to different clinicopathological factors in the malignant group. Urinary angiogenin was proved to be directly related to bladder cancer grading and staging in which it showed significantly higher positivity rate in invasive stages (II–III) (100 %) than superficial stages (0–I) (43.33 %) at $P=0.000$. Moreover, it showed significantly higher positivity rate in grade III (100 %) than grade II (87.5 %) and grade I (0 %) at $P=0.000$. Smoking and bilharziasis were directly related to angiogenin positivity rate in which it significantly higher in smoker patients with bladder cancer (92.3 %) than non-smoker patients (37.5 %) at $P=0.000$. Also, it was significantly higher in patients with positive bilharzial antibody (78.26 %) than patients with negative bilharzial antibody (55.55 %). Urinary clusterin was proved to be directly related to bladder cancer grading and staging in which it showed significantly higher positivity rate in invasive stage (II–III) (95 %) than superficial stage (0–I) (53.33 %) at $P=0.016$. Moreover, it showed higher significant correlation in grade III (100 %) than grade II (70.83 %) and grade I (42.85 %) at $P=0.007$. Urinary clusterin positivity rate was significantly higher in non-smoker patients with bladder cancer (91.66 %) than smoker patients (50 %) at $P=0.019$ (Table 3).

Overall Sensitivity, Specificity, PPV and NPV of Each of the Investigated Bladder Cancer Markers Either Alone or in Combinations Were Estimated

Sensitivity, specificity, PPV and NPP at the best cutoff for angiogenin (≥145 pg/mg protein) were (66 %, 75 %, 76.74 % and 63.82 % respectively) and (70 %, 82.5 %, 83.33 %, and 68.75 % respectively) at the best cutoff for clusterin (≥15 ng/mg protein). The sensitivity and NPV were raised to reach (82 and 75 % respectively) when angiogenin was combined with clusterin while; the specificity and PPV were decreased to (67.5 and 67.5 % respectively). When angiogenin was combined with clusterin and urine cytology, the sensitivity and NPV were increased to (88 and 78.57 % respectively) while,

Table 1 Comparison of angiogenin (ANG), clusterin (CLU), and urine cytology among normal, benign, and malignant group using chi-square test

Urine markers	Malignant group (n=50) (%)	Benign group (n=20) (%)	Normal group (n=20) (%)	Chi-square X^2	P value
Angiogenin					
No. of positive cases (≥145 pg/ml)	33 (66 %)	6 (30 %)	4 (20 %)	15.373	0.000
No. of negative cases (<145 pg/ml)	17 (34 %)	14 (70 %)	16 (80 %)		
Clusterin (ng/ml)					
No. of positive cases (≥15 ng/ml)	35 (70 %)	6 (30 %)	1 (5 %)	27.121	0.000
No. of negative cases (<15 ng/ml)	15 (30 %)	14 (70 %)	19 (95 %)		
Cytology					
No. of positive cases	23 (46 %)	8 (40 %)	0 (0 %)	13.739	0.001
No. of negative cases	27 (54 %)	12 (60 %)	16(100 %)		

Significance is at P<0.05

the specificity and PPV were decreased to (55 and 70.96 % respectively) (Table 4).

Discussion

Bladder cancer is a heterogeneous disease with unpredictable clinical course.

The two predominant histological types are transitional cell carcinoma (TCC) and squamous cell carcinoma (SCC). Cigarette smoking, and chronic infection with Schistosoma haematobium have been established as majour risk factors for bladder cancer [13].

With the government's efforts to eradicate S. haematobium and treat infected individuals over the past 3 decades plus results in a shift from SCC to TCC. However, the incidence of bladder cancer in Egypt has not decreased; and more commonly diagnosed in men [14].

Twenty-six percent bladder cancer patients included in the present study were associated with bilharziasis. Salem and Mahfouz found that from 2001 to 2010, the incidence of associated bilharziasis decreased from 80 to 50 %. A significant increased occurred in transitional cell carcinoma from 20 to 66 %, with a significant decrease in squamous cell carcinoma from 73 to 25 % [15]. In the present study, TCC constituted 86 % of cases, whereas, SCC constituted 14 %.

The incidence of bladder cancer increases with age. our study corroborates previously published data of Ahmedin et al. as approximately 80 % of newly diagnosed cases in both men and women occur in people aged 60 years and older [16].

In the current study, males constituted 76 % while female constituted 24 % of the cases. The male predominance in our study reflects the difference in the lifestyle of both genders in our country, with men more exposed to schistosomal infection, smoking, and occupational hazards known to cause bladder cancer.

Males consistently show a higher incidence of bladder cancer than females throughout the world. In the United States, bladder cancer is the fourth most commonly diagnosed malignancy in men and the eighth most commonly diagnosed malignancy in women [17].

The present study improved the sensitivity of in the diagnosis of bladder carcinoma, through measuring angiogenin and clusterin in supernatant of voided urine sample by ELISA. Different combinations of the two markers and urine cytology were tried to achieve the highest sensitivity and specificity. In the current study, cytology results revealed a sensitivity of 46 % and a specificity of 80 % between the malignant and the nonmalignant groups.

Table 2 Comparison of angiogenin (ANG) and clusterin (CLU), among normal, benign, and malignant group using Kruskal–Wallis test

Urine markers	Malignant group (n=50)	Benign group (n=20)	Normal group (n=20)	Chi-square X^2	P value
Angiogenin (pg/ml)					
Mean rank	57.63	32.20	28.48	24.460	0.000
Median	307.45	45.46	27.46		
Range	1233.86	289.58	196.16		
Clusterin (ng/ml)					
Mean rank	58.17	35.23	24.10	28.278	0.000
Median	38.19	10.535	5.22		
Range	748.75	56.88	29.57		

Significance is at P<0.05

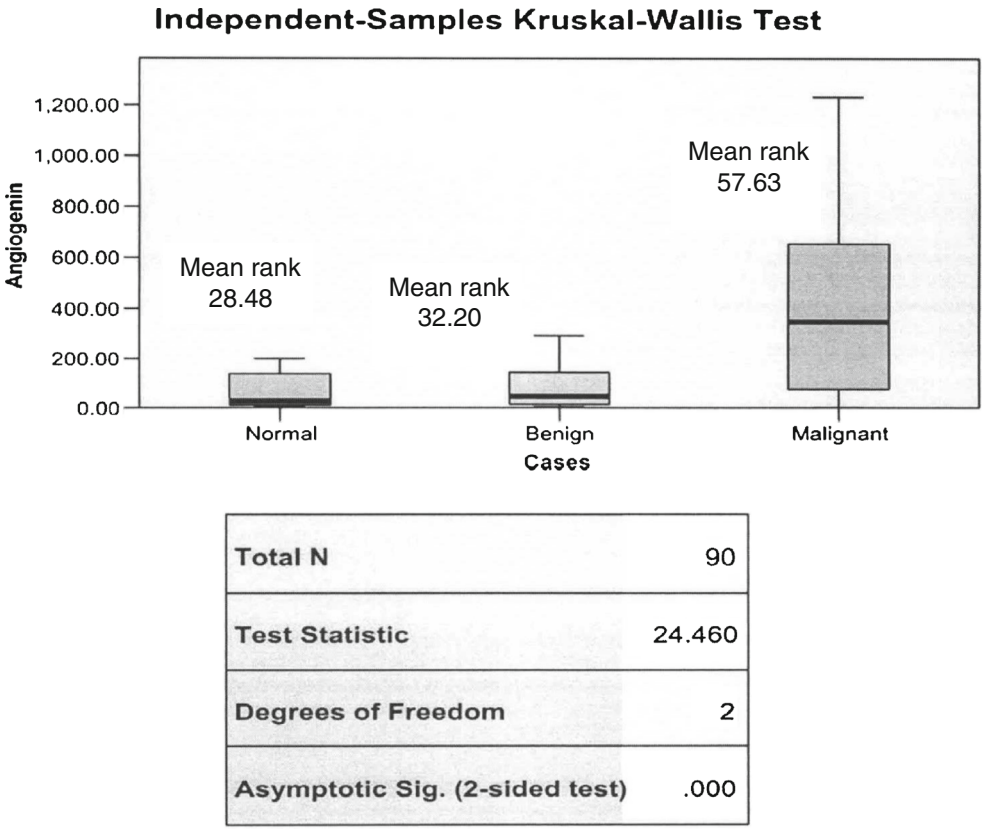


Fig 3 Post hoc analysis after Kruskal–Wallis for angioigenin (ANG) shows significant difference between groups with $p=0.000$

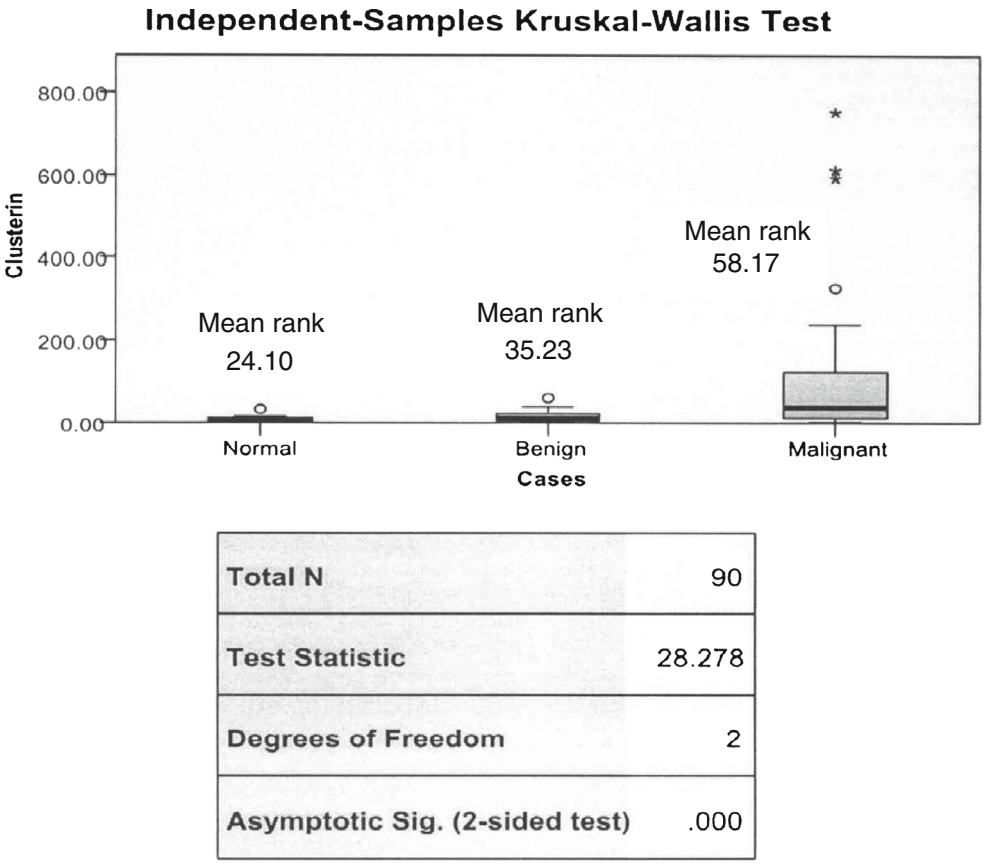
Angiogenin binds to a cell-surface actin, and these complex results in plasmin generation, which directly degrades the extracellular matrix facilitating cell migration and invasion [18]. When we compared the correlation between the positivity rate of urinary angiogenin and the different clinicopathological factors in the malignant group, Eissa et al. Found that angiogenin was highly expressed in patients with bladder SCC vs bladder TCC (76 vs 73 %) [9]. In our study angiogenin was also highly expressed in SCC vs TCC of bladder cancer (100 vs 60.46 %) with significance vale ($p=0.041$).

Urquidi et al. analyzed the supernatant of voided urine sample from a cohort of 127 consisted of 64 subjects with active urinary bladder cancer and 63 subjects without active bladder cancer, history of bladder cancer, gross hematuria, urolithiasis or urinary tract infection. The median urinary angiogenin levels in bladder cancer patients vs. benign subjects were 410.98 vs. 44.58 pg/ml respectively. Urinary angiogenin had a respectable diagnostic capability; sensitivity of 67 %, and specificity of 68 % [4]. In the current study, we were able to confirm these results with median urinary angiogenin levels of 307.45 pg/mg vs.45.46 pg/mg in cancer vs. benign subjects, respectively. Urinary angiogenin had a respectable diagnostic capability; sensitivity of 66 %, and specificity of 75 %.

Angiogenesis plays a central role in both local tumor growth and distant metastasis. Angiogenin appears to be a crucial stimulant for the angiogenic process to allow tumor growth beyond a few millimetres as well as the development of metastasis [19]. This was confirmed in our study, angiogenin was highly expressed in invasive stage (II–III) (100 %) than superficial stage (0–I) (43.33 %) at $P<0.05$. Moreover, angiogenin was highly expressed in grade III (100 %) than grade II (87.5 %) and grade I (0 %) at $P<0.05$.

There are two different but related clusterin protein isoforms, a secreted or cytoplasmic clusterin sCLU as well as an non-glycosylated form nuclear clusterin nCLU [20]. It is now accepted that the primary function of clusterin in distinct genetic backgrounds of cancer cells is antiapoptotic [21]. This antiapoptotic activity of clusterin may account for the genesis and biologically aggressive behavior of several cancer cells [22].

Stejskal and Fiala have examined clusterin concentrations of the urine in 43 patients with bladder cancer by using ELISA test and compared them with 50 patients with benign urological diseases. They found that urine clusterin were significantly higher in individuals with bladder cancer with sensitivity of 49 % and specificity of 92 %. They concluded that urine clusterin could be



Total N	90
Test Statistic	28.278
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.000

1. The test statistic is adjusted for ties.

Fig 4 Post hoc analysis after Kruskal–Wallis for clusterin (CLU) shows significant difference between groups with $p=0.000$

the possible laboratory marker of bladder cancer [23]. Hazzaa et al. used ELISA to measure the concentration of clusterin urine and serum. Clusterin was observed to increase from nontumor control to superficial low grade TCC to invasive high grade carcinoma in both urine and serum ($p<0.001$). The sensitivity and specificity of urine clusterin as a tumor marker for TCC of the bladder was found to be 87.1 and 96.7 % respectively [10]. In the current study, we confirmed these results. The sensitivity and specificity of urine clusterin as a tumor marker for bladder was found to be 70 and 82.5 % respectively.

Recent data indicate that progression towards high-grade and metastatic carcinoma leads to elevated clusterin levels and altered intracellular distribution of nuclear clusterin. Thus, the function of clusterin in tumors may be related to a pattern shift in its isoform production [24]. This was confirmed in the present study, we found that the mean expression level of clusterin in SCC specimens was higher than that in TCC (100 and 65.11 %) but it was not significant with p value 0.061. Based on the semi-quantitative analysis of clusterin levels, we found that the clusterin expression level correlated significantly with pathologic stage i.e., over expression of clusterin was more frequently detected in invasive stages (II and III) when compared to that in superficial stages (0 and I) with (95 and 53.33 %) respectively with $p0.016$. Moreover, we found that the clusterin expression level correlated significantly with tumor grade. It was higher in grade (III) (100 %) than grade (I and II) (42.85 and 70.83 %) respectively with $p=0.007$. These results were in agreement with many studies which have also documented that increased expression of clusterin was involved in the development and progression of several types of carcinomas. Similarly, Kruger et al. found that clusterin may be used, in addition to conventional and other immunohistochemical prognostic factors, as a supplementary tool to provide more prognostic information in patients undergoing cystectomy for muscle-invasive bladder cancer [25].

A direct comparison between urine cytology, angiogenin, and clusterin showed that clusterin had the highest sensitivity (70 %), and the highest specificity (82.5 %). Combination of urine cytology with angiogenin increases the sensitivity to (76 %), combination of urine cytology and clusterin increases the sensitivity to (80 %), and the combination of urine angiogenin and clusterin increases the sensitivity to

Table 3 Positivity rate for angiogenin (ANG) and clusterin (CLU) in relation to different clinicopathological factors in the malignant group

Parameter	Total no. of patients	No. of angiogenin positive patients (%)	No. of clusterin positive patients (%)
Pathological type			
SCC	7	7 (100 %)	7 (100 %)
TCC	43	26 (60.46 %)	28 (65.11 %)
P value		0.041	0.062 NS
Grade			
I	14	0 (0 %)	6 (42.85 %)
II	24	21 (87.5)	17 (70.83 %)
III	12	12 (100 %)	12 (100 %)
P value		0.000	0.007
Stage			
0	14	2 (14.28 %)	7 (50 %)
I	16	11(68.75 %)	9 (56.25 %)
II	10	10(100 %)	10(100 %)
III	10	10(100 %)	9 (90 %)
P value		0.000	0.016
Smoking			
Smoker	26	24 (92.3 %)	13 (50 %)
Non smoker	24	9 (37.5 %)	22 (91.66 %)
P value		0.000	0.019
Bil			
Negative bilharzial antibodies	37	21 (56.75 %)	26 (70.27 %)
Positive bilharzial antibodies	13	12 (92.3 %)	9 (69.23 %)
P value		0.02	0.994 NS
Cytology			
Negative cytology	27	15 (55.55 %)	17 (62.96 %)
Positive cytology	23	18 (78.26 %)	18 (78.26 %)
P value		0.091 NS	0.239 NS
Gender			
Male	38	21 (55.26 %)	26 (68.42 %)
Female	12	12 (100 %)	9 (75 %)
P value		0.004	0.665 NS

Significance is at $P<0.05$

TCC transitional cell carcinoma, *SCC* squamous cell carcinoma, *NS* non-significant

(82 %). Combined use of the three urine markers improved the sensitivity up to (88 %) at the expense of specificity (55 %).

In conclusion, urinary angiogenin and clusterin can be considered as potentially useful markers in detection of bladder cancer with two different molecular mechanisms as non invasive biomarkers where their combination gives high sensitivity. Moreover, combining the gold standard cytology with the previous markers gives the highest sensitivity and NPV. However, large

Table 4 Overall sensitivity, specificity, PPV, and NPV of all investigated bladder cancer markers and their combinations

Parameter	Sensitivity	Specificity	PPV	NPV
ANG	66 %	75 %	76.74 %	63.82 %
CLU	70 %	82.5 %	83.33 %	68.75 %
Cytology	46 %	80 %	74.19 %	54.23 %
ANG + CLU	82 %	67.5 %	75.92 %	75 %
ANG + cytology	76 %	62.5 %	71.69 %	67.56 %
CLU + cytology	80 %	65 %	74.07 %	72.22 %
ANG + CLU + cytology	88 %	55 %	70.96 %	78.57 %

multicentre studies should be carried out to prove the usefulness of these marker combinations.

References

1. American Cancer Society (2013) Cancer facts and figures 2013. American Cancer Society, Atlanta
2. Zaghloul S (2012) Bladder cancer and schistosomiasis. J Egypt Natl Cancer Inst 24:151–159
3. Eissa S, Shabayek M, Ismail M, Abd-Elateef A (2010) Diagnostic evaluation of apoptosis inhibitory gene and tissue inhibitor matrix metalloproteinase-2 in patients with bladder cancer. IUBMB Life 62(5):394–399
4. Urquidi V, Goodison S, Kim J, Chang M, Dai Y, Rosser CJ (2012) Vascular enothelail growth factor, carbonic anhydrase 9, and angiogenin as urinary biomarkers for bladder cancer detection. Urology 79(5):1185.e1–1185.e6
5. Mitra A (2010) Urine cytologic analysis: special techniques for bladder cancer detection. In: Connection. 169–177
6. Yafi A, Brimo F, Auger M, Aprikian A, Tanguay S, Kassouf W (2013) Is the performance of urinary cytology as high as reported historically? A contemporary analysis in the detection and surveillance of bladder cancer. Urol Oncol Semin Orig Investig 32(1):27.e1–6.
7. Budman L, Kassouf W, Steinberg JR (2008) Biomarkers for detection and surveillance of bladder cancer. Can Urol Assoc J 2:212–221
8. Li S, Hu G (2010) Angiogenin-mediated rRNA transcription in cancer and neurodegeneration. Int J Biochem Mol Biol 1(1):26–35
9. Eissa S, Swellam M, Labib RA, El-Zayat T, El Ahmady O (2009) A panel of angiogenic factors for early bladder cancer detection: enzyme immunoassay and Western blot. J Urol 181(3):1353–1360
10. Hazzaa M, Elashry M, Afifi K (2010) Clusterin as a diagnostic and prognostic marker for transitional cell carcinoma of the bladder. Pathol Oncol Res 16(1):101–109
11. Miyake H, Hara I, Gleave M, Eto H (2004) Protection of androgen dependent human prostate cancer cells from oxidative stressinduced DNA damage by overexpression of clusterin and its modulation by androgen. Prostate 61:318–323
12. American Joint Committee on Cancer (2010) AJCC cancer staging manual. Urinary bladder, 7th edn. Springer, New York, pp 497–502
13. Felix AS, Soliman AS, Khaled H, Zaghloul MS, Banerjee M, El-Baradie M (2008) The changing patterns of bladder cancer in Egypt over the past 26 years. Cancer Causes Control 19(4): 421–429
14. Zheng YL, Amr S, Saleh DA, Dash C, Ezzat S, Mikhail NN, Gouda I, Loay I, Hifnawy T, Abdel-Hamid M, Khaled H, Wolpert B, Abdel-Aziz MA, Loffredo CA (2012) Urinary bladder cancer risk factors in Egypt: a multicenter case–control study. Cancer Epidemiol Biomark Prev 21(3):537–546
15. Salem HK, Mahfouz S (2012) Changing patterns (age, incidence, and pathologic types) of schistosoma-associated bladder cancer in Egypt in the past decade. Urology 79(2):379–383
16. Ahmedin J, Ram CT, Taylor M, Asma G, Alicia S, Elizabeth W, Eric JF, Michael JT (2004) American Cancer Society. Bladder Cancer J Clin 96:54–56
17. Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, Kassouf W, Kiemeney LA, La Vecchia C, Shariat S, Lotan Y (2013) Epidemiology and risk factors of urothelial bladder cancer. Eur Urol 63(2):234–241
18. Gho YS et al (2002) Antiplasmin activity of a peptide that binds to the receptor-binding site of angiogenin. J Biol Chem 277(12):9690–9694
19. Yang Y, Sun M, Wang L, Jiao B (2013) HIFs, angiogenesis, and cancer. J Cell Biochem 114:967–974
20. Wong P, Ulyanova T, Organisciak D, Bennett S, Lakins J, Arnold JM, Kutty RK, Tenniswood M, vanVeen T, Darrow RM, Chader G (2001) Expression of multiple forms of clusterin during light-induced retinal degeneration. Curr Eye Res 23:157–165
21. Trougakso I, So A, Jansen B, Gleave ME, Gonos ES (2004) Silencing expression of the clusterin/apolipoprotein J gene in human cancer cells using small interfering RNA induces spontaneous apoptosis, reduced growth ability, and cell sensitization to genotoxic and oxidative stress. Cancer Res 64:1834–1842
22. Leskov K, Klokov D, Li J, Boothman DA (2003) Synthesis and functional analyses of nuclear clusterin, a cell death protein. J Biol Chem 278:11590–11600
23. Stejskal D, Fiala R (2006) Evaluation of serum and urine clusterin as a potential tumor marker for urinary bladder cancer. Neoplasma 53(4):343–346
24. Kim N, Yoo J, Han J (2012) Human nuclear clusterin mediates apoptosis by interacting with bcl-xl through c-terminal coiled coil domain. J Cell Physiol 227:1157–1167
25. Krüger S, Mahnken A, Kausch I, Feller AC (2006) Value of clusterin immunoreactivity as a predictive factor in muscle-invasive urothelial bladder carcinoma. Urology 67(1):105–109