Urinary exosomal microRNA-96 and microRNA-183 expression as potential biomarkers of bladder cancer

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Urinary exosomal microRNA-96 and microRNA-183 expression as potential biomarkers of bladder cancer

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Running head: Urinary exosomal microRNA-96 and microRNA-183 and bladder cancer

Abstract

Background: Because of low sensitivity and specificity of the currently available urine markers for bladder cancer (BC) detection and painful cystoscopy procedure, our study was aimed to evaluate expression of urinary exosomal microRNA (miR)-96 and miR-183 as probable non-invasive and accurate biomarkers for the diagnosis and follow up of BC.

Methods and results: Using quantitative real-time polymerase chain reaction; Expression of exosomal miR-96 and miR- 183 in the urine samples of 51 patients with BC, 21 patients with benign urinary bladder lesions and in 24 normal individuals as control group.

Our study results showed higher expressions of both miR-96 and miR-183 in urine of BC patients in comparison with control group ($P < 0.001$ for each). Analysis of receiver-operating characteristic curve demonstrated that each microRNA had good sensitivity and specificity for differentiating BC patients from non-BC patients (miR-96; 80.4% and 91.8%; and miR-183, 78.4% and 81.6% respectively) compared to cytology (37.3% and 100%). In addition, it was obvious that the sensitivity and specificity of combined miR-96 and miR-183 for the diagnosis of BC reached 88.2%% and 87.8%, respectively, which were higher than each one alone. We also found that expression of miR-96 and miR-183 with lymph node invasion, advancing grade, and pathological stage was significantly increased. After surgery, collected urine samples showed significantly lower expression of microRNA (miR-96: $P < 0.001$; and miR-183, $P = 0.002$).

Conclusion: In conclusion, miR-96 and miR-183 in urine are promising diagnostic tumor markers for BC; particularly, when they combined with each other or with urinary cytology.

Key Words: Bladder cancer, miRNA-96; miRNA-183; biochemical marker**.**

Introduction

Bladder cancer (BC) is one of the most common urogenital cancers [1]. BC is the 6th most common cancer in both genders and the 4th in males worldwide (incidence of 9.6 per 100,000 in male and 2.4 per 100,000 female) [1]. In 2018, more than 500,000 patients were diagnosed with BC and mortalities were about 200,000. North Africa had the highest mortality rate of bladder cancer with 4.4 per 100,000 patients [2].

At initial diagnosis, about 70% of patients have their cancer limited to the subepithelial connective tissue or the epithelium. BC has a high recurrence rate ranging from 50% to 70%. Moreover, 10 to 15% progress to muscle invasion over a period of five years. Lifelong surveillance in many cases is of high necessity [3].

Age as the major risk factor of BC, elderly are more commonly affected [4]. While smoking tobacco is the most firmly-established risk factor for BC development [5]. Aromatic amines, chlorinated hydrocarbons, and polycyclic aromatic hydrocarbons occupational exposure is the second most important risk factor for BC, representing 10% of all cases [6]. Ionizing radiation exposure is also linked with increased risk [6]. patients with bladder Schistosomiasis have an increased risk of BC, most commonly squamous cell carcinoma (SCC), Schistosomiasis is endemic in Egypt; inflammation is thought to play an important role in carcinogenesis associated with this parasite [7]. Strong epidemiologic evidence does not exist for a hereditary cause of most bladder cancers [8].

Cystoscopy and cytology are used for diagnosis and surveillance of bladder cancer. Although it is invasive, cystoscopy distinguishes most solid and papillary lesions. Urine cytology has acceptable specificity and sensitivity detecting high-grade bladder cancer, but very low sensitivity for detecting low-grade tumors, ranges only from 4% to 31% [9]. About 60% of high-

grade tumors are missed by cytology, however this might be an extrapolation of the results from series in which there were poor cytology standards [9].

MicroRNAs (miRNAs) are a class of small ribonucleic acid (RNAs), 20-25 nucleotides in length, that are important regulatory molecules within the cells of plants, animals, humans, and viruses [10]. Since their discovery in 2001, miRNAs have been found to regulate several cell processes by regulating gene expression of messenger RNA (mRNA) at the posttranscriptional level [11]. miRNAs are suggested to be involved in carcinogenesis due to their ability to inhibit translation of tumor suppressors and oncogenes. Recently, evidence demonstrated that miRNAs function in regulation of apoptosis, cellular proliferation, and differentiation. Depending on the role of their mRNA targets, miRNAs have been proposed to be functioning either as oncogens or as tumor suppressors [12].

Exosomes are membrane-bound vesicles that are released into body fluids such as serum, plasma, urine and saliva and carry miRNAs. Almost all classes of RNAs, including miRNA (exo-miRs), are loaded in exosomes. Mature miRNAs and pre-miRNAs, that undergoes exosome-associated miRNA processing are both present in exosomes. They are associated with exosome surface and appear in isolated exosome or packaged inside exosomes [13]. It has been reported that Intercellular transfer of exo-miRs elicit gene expression changes in the recipient cells [14].

Dysregulated miRNAs have been identified in several human cancers including bladder tumors through the use of several molecular techniques, and in some cases, may give a “tumor signature” that can be used for diagnostic purposes [15]. In contrast to long-chain mRNA, it is acceptable to assume that short mature miRNAs are more stable against nucleases degradation in urine due to the smaller size. Thus, novel, highly sensitive, and specific urine-based diagnostic

and/or prognostic tools are particularly attractive as urine is a promising and easily available source for molecular markers, including miRNAs [16]. Significantly, miR-96 and miR-183 are found in the same cluster; just a short distance (212 bp) on chromosome 7q32 separate them. Therefore, it is plausible that their expressions are synchronized for targeting the same genes [17]. Therefore, we aimed by this study to investigate the possible diagnostic and prognostic significance of urinary exosomal miR-96 and miR-183 in BC patients.

Patients and methods:

Patients

A prospective multicenter case-control study included a total of 72 patients (including 21 patients suffering from urinary tract infection (UTI) and 51 newly diagnosed untreated Egyptian BC patients) and 28 healthy controls. The cancer bladder patients (mean age ± SD: 59.5±3.2 years; 42 were male and 9 were female) admitted to the Urology Departments, Faculty of Medicine, Zagazig and Ain Shams Universities Hospitals, Egypt. After approval from the Ethics Committees of Faculty of Medicine, Zagazig and Ain Shams Universities, all participants assigned informed written consent. Demographic data and medical history were obtained at presentation.

The diagnosis of BC was confirmed by several imaging techniques, including abdominal US, triphasic abdominal computed tomography with contrast and/or magnetic resonance imaging. Cystoscopy was done to all patients and all suspicious lesions or tumors were either biopsied or resected. The final diagnosis of BC was based on histopathologic examination. Tumors were staged according to TNM staging [18] and graded according to Yorukoglu et al [19]. Histopathologic examination of 35 patients (68.6 %) showed transitional cell carcinoma (27 bilharzial & 8 non-bilharzial), while that of 16 patients (31.4 %) showed squamous cell

carcinoma (11 bilharzial & 5 non-bilharzial). The number of patients in grade (G1) & (G2 and G3) were 42 and 9, respectively. The number of patients in stage (0–I) & (II–IV) were 22 and 29, respectively. A total of 6 (12%) patients had one or more lymph node metastasis and 3(5.9%) had distant metastasis.

The benign urinary bladder pathology; urinary tract infection (UTI), stone bladder, and benign prostatic hyperplasia patients group included 21 patients with (mean age ± SD: 58.0±2.2 years; 18 were male and 3 were female). A control group consisted of 28 healthy volunteers (mean age ± SD: 58.4±3.6 years; 23 male and 5 of them were female). Our controls and the benign urinary bladder pathology patients' groups were selected with similar age, gender, and smoking habits proportions to the cancer patients, and subjects were screened to ensure that they were within the normal range of all laboratory findings and had no history of cancer.

Sample Collection and Cytological Preparation

Voided urine samples and sera samples were obtained from all groups before beginning any treatment plan or surgery. Sera were used for testing schistosomiasis antibody. On the other hand, a total of 30 mL of voided urine samples was collected from patients before surgery, either radical cystectomy or transurethral resection of bladder tumor (TURBT). In BC patients who had undergone TURBT, additional urine specimens were also collected 4 weeks after the surgery. Urine sample was centrifuged at 3000 g for 30 minutes and at 13000 g for 5 minutes at 4◦C [20]. The pellet was used for cytologic examinations. The supernatant was used for exosomes isolation. In accordance with the protocol for the miRCURY™ Exosome Isolation Kit (Qiagen, Hilden, Germany), isolation of exosomes was done from 1.1 ml of urine supernatant dissolved on ice and centrifuged at 10,000 g (Eppendorf, Hamburg, Germany) at room temperature to remove cellular debris.

RNA Isolation

miRcute miRNA isolation kits (Tiangen biotech, Beijing, China) were used for the extraction of total miRNA from urinary exosomes according to the manufacturer's protocol. The purity of urinary RNA was confirmed by the value of optical density (OD) at 260 and 280 nm using a spectrometer (6320, Jenway LTD, UK), with acceptable RNA purity ranging from 1.9 to 2.1.

Reverse transcription of miRNA

We used miScript Reverse Transcription Kit (Qiagen GmbH, Hilden, Germany) for reverse transcription and polyadenylation of the miRNA to complementary DNA (cDNA). We centrifuged the mixture briefly and incubated it for 60 min at 37˚C after mixing with template RNA, miScript Reverse Transcriptase mix, 5X miScript buffer, and RNase-free water in a final volume of 20 µl. Samples were incubated for five minutes at 95˚C and placed on ice for the miScript Reverse Transcriptase mix inactivation.

Quantification of urinary exosomal miR-96 and miR-183 expression levels

Urinary levels of miR-96 and miR-183 expressions were quantified by real-time quantitative polymerase chain reaction (RT-QPCR) with a Stratagene™ Mx3005P. For amplification of miR-96 (MS00003360) and miR-183(MS00031507) primers were designed and purchased using miScript Primer Assay (Qiagen GmbH, Hilden, Germany). After dissolving the reagents and template cDNA, we carried out PCR reaction in a final volume of 20 µl containing 2 µl 10X miScript universal 8 reverse primer, 10 µl 2X QuantiTect SYBR-Green PCR master mix, 2 µl template cDNA.

2 µl 10 pmol forward specific stem-loop primer, and 4 µl RNase-free water. RT PCR conditions: 15 min at 95˚C (one cycle) for Initial activiation , followed by 40 cycles of 15 sec at 94˚C for denaturation, annealing for 30 sec at 55˚C and extension for 30 sec at 70˚C. We used SNORD68 as a control for the normalization of RT PCR results in miRNA quantification studies using the miScript PCR System. We analyzed the miR-96 and miR-183 expressions in parallel to SNORD68 gene and we normalized all data to SNORD68 expression. We acquired and analyzed Quantitative miRNA expression data using MxPro-Mx3005P version 3.00 (STRATAGENE), with the automatic threshold cycle (Ct) setting for adapting baseline and we determined relative expression using the Δ Ct method [21].

Statistical analysis

We analyzed the collected data using SPSS. We expressed categorical data as percentages and numbers while quantitative data as median & inter quartile range (IQR), and mean ± standard deviation (SD). We used ANOVA, Chi square test (X2), "Z" test, Goodness of fit test, paired “t” test, Mann Whitney U, Bonferroni adjusted Mann-Whitey U test, and Wilcoxon tests as tests of significance. We used Receiver operating characteristics curve for determination of cutoff values with optimum specificity, sensitivity, positive & negative predictive values (PPV & NPV). The accepted level of significance was stated at 0.05 ($P < 0.05$ was considered significant).

Results

General characteristics of the study subjects

The baseline characteristics of enrolled controls, benign urinary bladder lesions patients group and BC patients are listed in **Table 1**.

Expressions levels of urinary exosomal miR-96 and miR-183 in controls and patients

We compared the microRNAs expression levels in BC patients (n = 51) with that in non-BC individuals (n = 49), we found that the miR-96 and miR-183 expressions were significantly higher in BC than in non-BC (miR-96 median (range): 6.4(5.7 6.9) vs. 2.8(2.62- 2.9), P <0.001; and miR-183: 6.2 (3.9-6.9) vs. 3.05(2.62-3.6), P <0.001). The expression levels of miR-96 and miR-183 in BC were significantly higher than in controls (miR-96, median (range): 6.4(5.7-6.9) vs. 2.7(2.6-2.87), P < 0.0001; and miR-183: 6.2 (3.9-6.9) vs. 2.7(2.5-2.85), P < 0.0001). Also, there was an upregulation of miR-96 and miR- 183 expression in BC patients when compared with benign bladder lesions group (miR-96: 6.4(5.7-6.9) vs. 2.8 (2.65-2.9); and miR-183: 6.2 (3.9-6.9) vs. 3.5 (3.3-3.75), P < 0.0001 for each) (Fig. 1A, 1B). On the other hand, there were no significant differences in miR-96 and miR-183 expressions levels between patients with benign urinary bladder pathology and healthy controls (2.8 (2.65-2.9) vs. 2.7(2.6-2.87); 3.5 (3.3-3.75) vs. 2.7(2.5-2.85), respectively) (**Fig. 1A, 1B**).

Evaluation of miR-96 and miR-183 expression levels as a diagnostic marker for BC either alone or in combinations

The ROC curve analyses demonstrated that each microRNA had good sensitivity and specificity with optimal cut-off values for distinguishing BC patients from non BC patients as follows: miR-96, 80.4% (sensitivity), 91.8% (specificity), 4.35 (cut-off); and miR-183, 78.4% (sensitivity), 81.6% (specificity), 4.75 (cut-off) (Table 2). Both miR-96 and miR-183 had greater area under curve (AUC) = 0.85 (95%CI): 0.76-0.93; 0.83 (95%CI): 0.74-0.91) than that of cytology =0.69 (95%CI): 0.58-0.79) (**Fig. 2**).

The diagnostic performance was raised when we combined the miR-96 with urinary cytology data, AUC: 0.87, sensitivity: 82.4% and specificity: 91.8% (Table 2). Also, when we combined the miR-183 with urinary cytology, the AUC rose to 0.85, sensitivity 80.4% and specificity 91.8% (Table 2). In addition, we found that the sensitivity and specificity of combined miR-96 and miR-183 for the diagnosis of BC reached 88.2%% and 87.8%, respectively, which were higher than each one alone (**Table 2**).

The expression level of miR-96 and miR-183 in Bladder Cancer (BC) patients' group in relation to different features

We found significant increases in miR-96 and miR-183 expression with advancing tumor grade [G3] as compared to low-grade cancer [G1 or G2] (miR-96 median (range): 6.92 (6.3-7.1) vs. 6.25 (4.92-6.63), P=0.03; miR-183 median (range): 6.9 (6.6-13.8) vs. 6.1 (3.32- 6.9), P=0.004). The expression levels of the microRNAs were significantly lower in noninvasive tumors [stage 0-1]. The expression levels of the microRNAs were significantly lower in noninvasive tumors [stage 0-1] than in invasive tumors [stage 2-4] (miR-96: 6.25 (5.8-6.43) vs. 6.7 (6.3-6.9), P = 0.008; and miR-183: 6.4(6-6.9) vs. 6.9 (6.4-12.3), P = 0.02). Additionally, we found significant increases in miR-96 and miR-183 expression in relation to lymph node invasion (P = 0.03, P =0.01 respectively). In contrast, we found no significant differences between the miRNAs expressions and the age, gender of patients, tumor site, and presence of distant metastasis (**Table 3**).

Comparing expression of the miR-96 and miR-183 before and after surgery

Among the 39 patients who underwent radical surgery, (cystectomies and nephron uretectomies) only 27 were available for evaluating microRNAs expression; urine samples were collected at both pre- and post-surgery. The expression levels of the miRNA significantly decreased in the post-surgery urine compared with the pre-surgery urine (miR-96 median (range): 4(3.8-4.6) vs. 6.4(5.7-6.9), P<0.001; and miR-183: 3.9(3.5-5) vs. 6.2(3.9-6.9), P = 0.002) (**Fig. 3**).

Discussion

Recently, many studies have concluded that miRNAs are emerging as a new class of cancer biomarkers [22]. they have been identified in all types of body fluids, including plasma, saliva, urine, tears, peritoneal fluid [23]. Urine is a specilally desirable source of biomarkers for Bladder Cancer, and urinary miRNAs could be direct indicators of the urological condition [24]. miRNAs are commonly found within extracellular vesicles, and specifically exosomes, which are released from many different cell types [25]. Circulating or urinary miRNAs might be released from tumor cells in exosomes as a mechanism to communicate with recipient cells in the surrounding microenvironment [26]. At least cancer cells secrete ten-fold more exosomes than do normal cells [27]. Interestingly, the concentrations and composition of cell-free miRNAs differ in urine and serum [28], suggesting that urinary miRNAs probably originate from the urinary tract rather than from plasma filtrate [28]. In addition, urine is much more proper and less invasive to collect than blood, furthermore, miRNAs exist in urine in a stable form. Even after freezing and thawing for seven cycles or 72 h at room temperature, miRNA levels in the urine were largely unchangeable [29].

In our current study, there was a significant higher expression levels of miR-96 and miR-183 in Bladder Cancer group than in control group and benign bladder lesions group; but no significant differences between controls and benign lesions patients was observed. Therefore, these microRNAs can be promising diagnostic tumor markers to distinguish BC patients from non- BC subjects. Moreover, the sensitivity and/or specificity for diagnosing BC were higher when we combined the miR-96 with miR-183 detection findings or we combined each miRNA with cytology than we analyzed cytology results or each miRNA alone.

In harmony with our results, overexpression of miR-96 and miR-183 has also been reported in human cancers such as urothelial, breast, lung, colon, liver, ovary, prostate, testis cancer and lymphoma [29-34]. Reports demonstrated that miR-96 and miR-183 were overexpressed in breast and endometrial cancers and simultaneously regulated the Fork head Box O subfamily of transcription factors (FOXO), which is a tumor suppressor gene promoting G1 cell arrest and cell death [30, 34]. Li et al found also that miR-183 directly regulated programmed cell death 4 (PDCD4), which is a proapoptotic molecule involved in TGF-β1-induced apoptosis in human hepatocellular carcinoma (HCC) cells [33]. Putting these results together implied that miR-96 and miR-183 are onco-micro-RNA of some human malignancies [35].

On contrary, Yu and colleagues demonstrated that miR-96 was downregulated in pancreatic cancers and plays as a tumor suppressor via KRAS gene regulation, one of the members of the RAS oncogene [36]. Notably, miR-183 was down-regulated especially in osteosarcoma, and inconsistently expressed in different breast tumor tissues and compared normal tissues [37-39]. Interestingly, 10 cases with urothelial cancer did not show a synchronized expression pattern of miR-96 and miR-183 although they are closely located. This

may be due to argonaute protein post-transcriptional modifications [34]. Thus, functional roles of these microRNA and their target genes may be different among malignancies.

Eissa et al found that urinary miRNA-96 in sediment samples is a good noninvasive diagnostic biomarker for BC with sensitivity and specificity 72.3 and 88.9 % respectively [40]. In another study, Eissa et al found that miRNA-96 in urine sediment was a good marker for screening BC patients with negative cytoscopy results with 76.6% sensitivity and 89.4 % specificity [41]. In the present study the sensitivity and specificity of urinary exosomal miRNA-96 were higher than Eissa's studies (80.4% and 91.8% respectively). This may be attributed to that miRNAs released from tumor cells in exosomes [25]. In the present study we found that miRNA-183 is a promising tumour marker for BC also with sensitivity and specificity 78.4% and 81.6% respectively. Interestingly, when we combined the miR-96 with miR-183 detection findings, the sensitivity for diagnosing BC rose to 88.2% and the specificity to 91.8%.

In the present study, miR-96 and miR-183 expressions in urine exosome were significantly related with tumor stage, grade, and presence of lymph node metastasis. While we did not find significant differences in both microRNAs expressions among the age, gender of patients, tumor site, and presence of distant metastasis. In addition, the expression of these miRNAs significantly decreased after radical surgery. In agreement with our results, Yamada et al [17] found significantly higher expression levels of miR-96 and miR-183 in the urine of UC patients (BC, renal pelvic and ureter UC) than in the case of healthy patients. This overexpression was correlated with tumor grade and pathological stage [17]. Additionally, Yamada et al [17] assumed that miR-183 might be useful as a staging marker but not as a diagnostic marker. They found more false positive cases in miR-183 detection compared with

miR-96 detection. Because the majority of their false positive cases were UTI patients, miR- 183 might be upregulated and function in UTI as well as UC [17], which disagrees with our results.

Wang et al [42] suggested that, miR-96 in particular may function as a promising diagnostic and/or prognostic marker in human UC, affecting the growth of bladder cancer cells by up-regulating IRS1 and MAP4K1 levels. Similarly, miR-183 was identified as a new molecular target involved in prostate cancers metastasis and it is conceivable that Ezrin might contribute to the regulation of metastasis by miR-183 [43].

The main limitation of our study is the small sample size. We concluded that miR 96 and miR-183 in urine exosome are promising tumor markers for BC and their expressions were correlated with clinicopathological features of BC. Also, miR-96 and miR-183 might be useful noninvasive diagnostic marker in combination with each other and/ or urinary cytology.

Declarations

Ethical approval: Written informed consent was obtained from all participants. The study was approved by the research ethical committee of our institutes. The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

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Author Contributions:

All the authors shared in:

1. Conception or design of the work.
2. Data collection.
3. Data analysis and interpretation.

4. Drafting the article.
5. Critical revision of the article.
6. Final approval of the version to be published.

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Code availability: none

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Table 1: Characteristics of the studied groups.

	Healthy controls (n =28)	Patients with benign bladder lesions (n =21)	Patients with bladder cancer (n =51)	Test of sig.	P
Age (years)	58.4±3.6	58.0±2.2	59.5±3.2	ANOVA=2.05	0.13
Sex,n (%)					
male	23(82.1)	18(85.7)	42(82.4)	X^2=0.14	0.93
female	5(17.9)	3(14.3)	9(17.6)		
Pathology,n(%)					
Transitional cell carcinoma (TCC)	---	--	35(68.6)	Z=2.87	0.004*
SCC	---	---	16(31.4)		
Smoking,n(%)					
Smokers	15(53.6)	11(52.4)	27(52.9)	X^2=0.01	0.99
Nonsmokers	13(46.4)	10(47.6)	24(47.1)		
Bilharziasis,n(%)					
Positive patients	0(0)	15(71.4)	38(74.5)	X^2=43.9	<0.001*
Negative patients	28(100)	6(28.6)	13(25.5)		
Stage,n (%)					
0–I	--	--	22(43.1)	Z=0.99	0.32
II–IV	--	--	29(56.9)		
Grade,n (%)					
G1,G2	--	--	42(82.3)	Z=6.06	<0.001*
G3	--	--	9(17.7)		
N stage					
N0	--	--	45(88.2)	Z=8.47	<0.001*
N1	--	--	6(11.8)		
M stage					
M0	--	--	48(94.1)	13.4	<0.001*
M1	--	--	3(5.9)		
Tumour site,n(%)					
Trigon	--	--	7(13.7)	Goodness of fit =10.7	0.005*
Lateral	--	--	26(51)		
Posterior	--	--	18(35.3)		
Cytology,n(%)					
Positive patients	--	--	19(37.3)	1.88	0.06
Negative patients	--	--	32(62.7)		

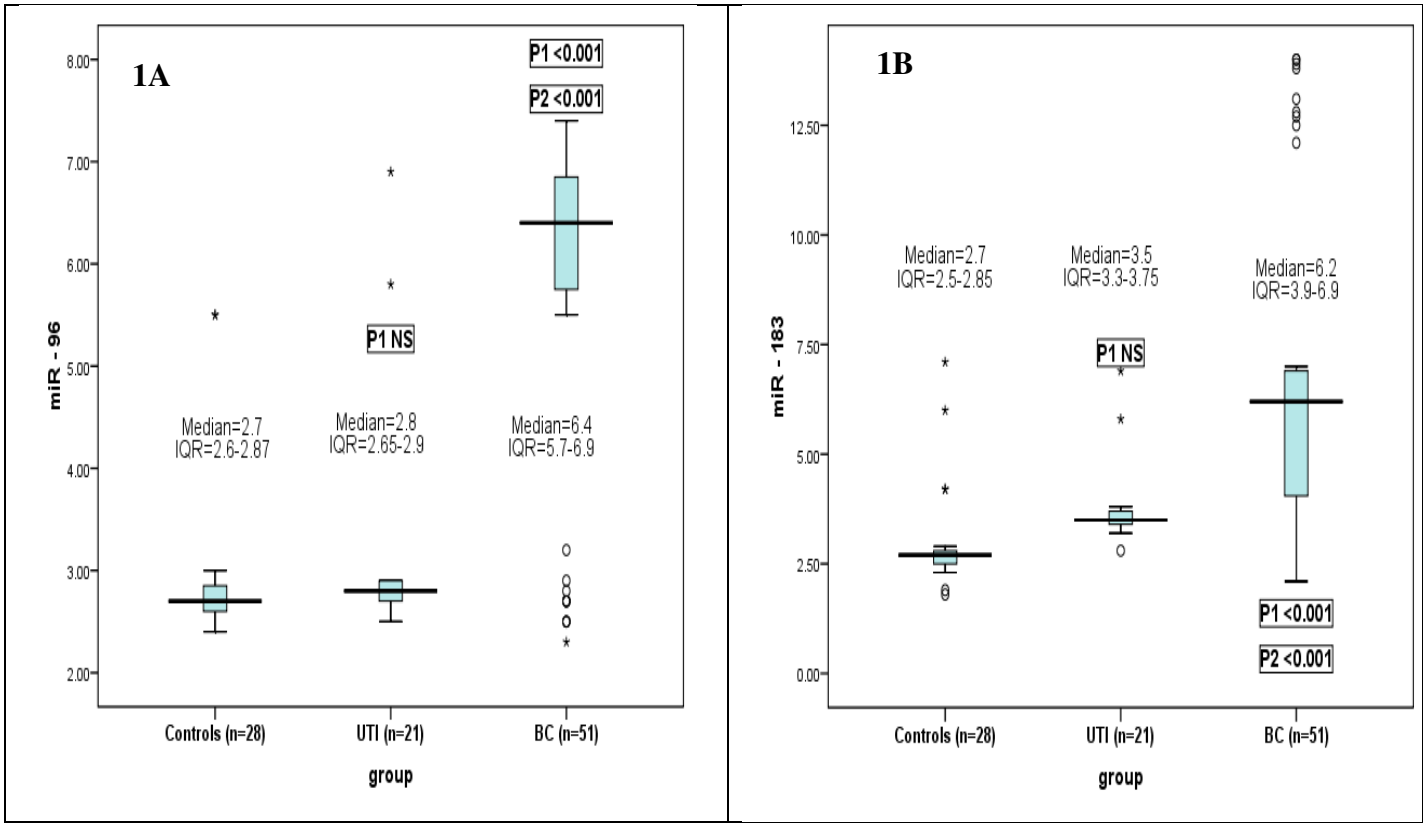

Figure 1 : Relative expression of urinary exosomal miR-96 (1A) and miR-183(1B) in controls and patients. P1→ Comparison with the controls. P2→Comparison with UTI group. Statistical significance was determined by Bonferroni adjusted Mann–Whitey *U* test (adjusted P value=0.017).

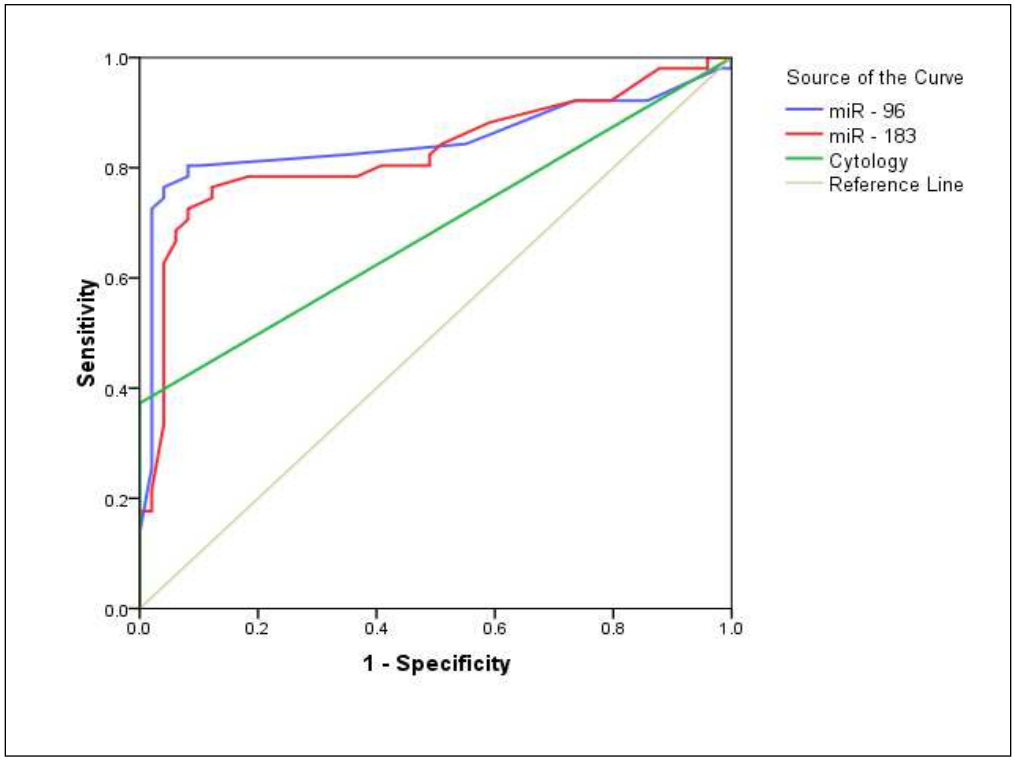

Figure 2**:** ROC curve analysis of miR-96 , miR-183 and cytology in diagnosis of BC

Table 2: Overall sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of all investigated bladder cancer markers and their combinations.

Marker	Cutoff value	Sens%	Spec%	PPV%	NPV%	Accuracy %	AUC	95%CI	*P*
miR-96	≥4.35	80.4%	91.8%	91.1%	81.8%	84.8%	0.85	0.76-0.93	<0.001*
miR-183	≥4.75	78.4%	81.6%	81.6%	78.4%	82.7%	0.83	0.74-0.91	<0.001*
Cytology	+ve	37.3%	100%	100%	39.5%	68.6%	0.69	0.58-0.79	0.001*
Combined miR-96 and miR-183	+ve	88.2%	87.8%	88.2%	87.8%	88%	0.88	0.81-0.95	<0.001*
Combined miR-96 and cytology	+ve	82.4%	91.8%	91.3%	83.3%	87.1%	0.87	0.79-0.95	<0.001*
Combined miR-183 and cytology	+ve	80.4%	91.8%	91.1%	81.8%	85.1%	0.85	0.78-0.94	<0.001*

Compared to non BC patients

Table 3: *The relative expressions of miR-96 and miR-183 in BC patients group in relation to different clinicopathological features*

Variable		miR-96		**P**	miR-183		**P**
		Median	IQR		Median	IQR	
Gender	Male	6.5	6.07-7.02	0.87	6.6	6.1-7.03	0.84
	Female	6.3	6.05-6.95		6.4	6.2-7.05	
Site†	Trigon	6.3	5.3-6.4		6.4	6.2-6.9	
	Lateral	6.5	6.17-6.9	0.22	6.6	6.1-12.55	0.91
	Posterior	6.45	5.85-6.9		6.7	6.1-7.05	
Stage	0-1	6.25	5.8-6.43	0.008*	6.4	6-6.9	0.02*
	2-4	6.7	6.3-6.9		6.9	6.4-12.3	
N stage	N0	6.4	6-6.9	0.031*	6.5	6.1-6.9	0.012*
	N1	7	6.42-7.2		13.85	6.47-14	
M stage	M0	6.4	6.02-6.9	0.33	6.5	6.1-6.97	0.14
	M1	6.8	6.2-7.2		13.8	6.2-14	
grade	G1,2	6.25	4.92-6.63	0.034*	6.1	3.32-6.9	0.004*
	G3	6.92	6.3-7.1		6.9	6.6-13.8	

Statistical significance was determined by Mann–Whitey U test.

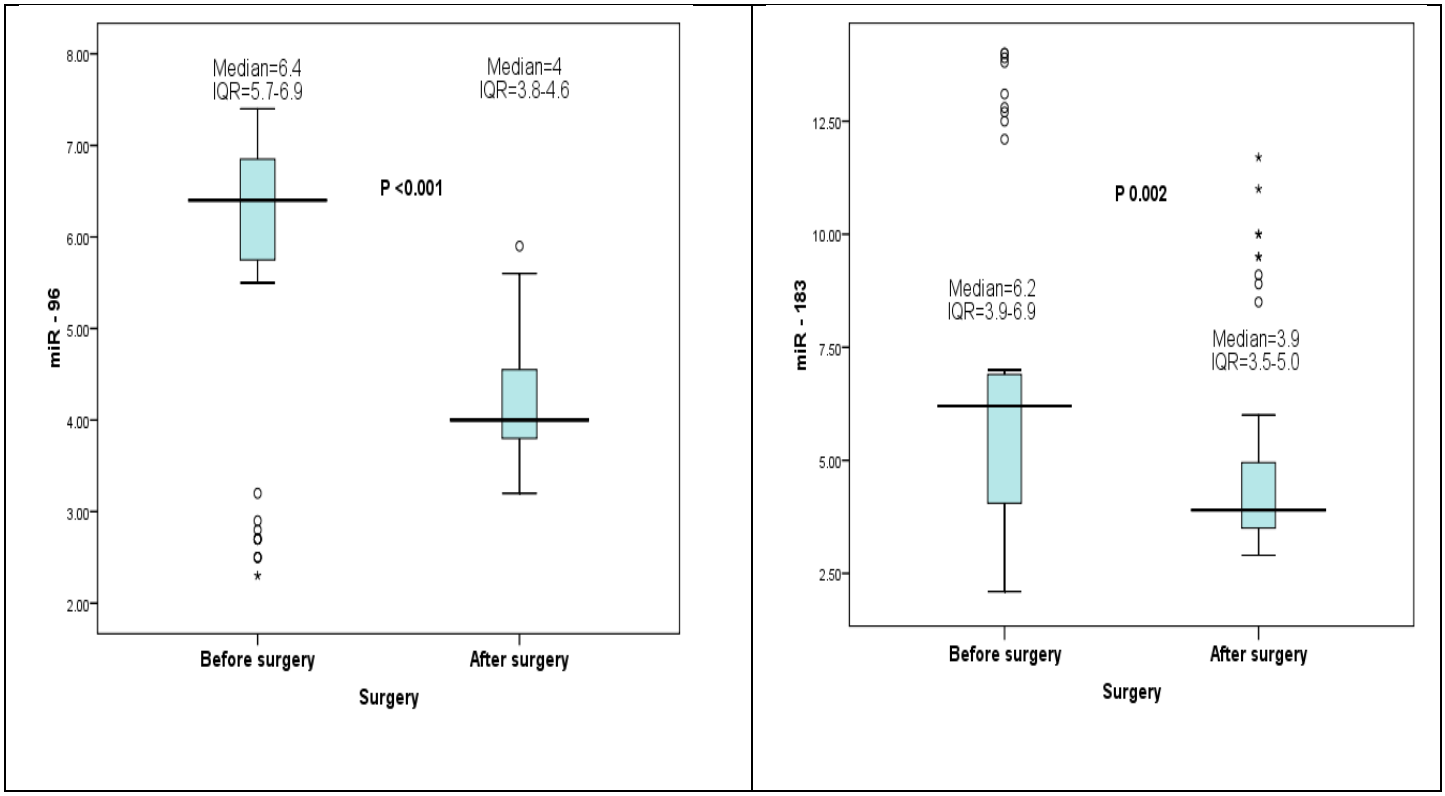

Figure 3: Comparing relative expressions of the miR-96 and miR-183 before and after surgery. Statistical significance was determined by Wilcoxon test.

Figures

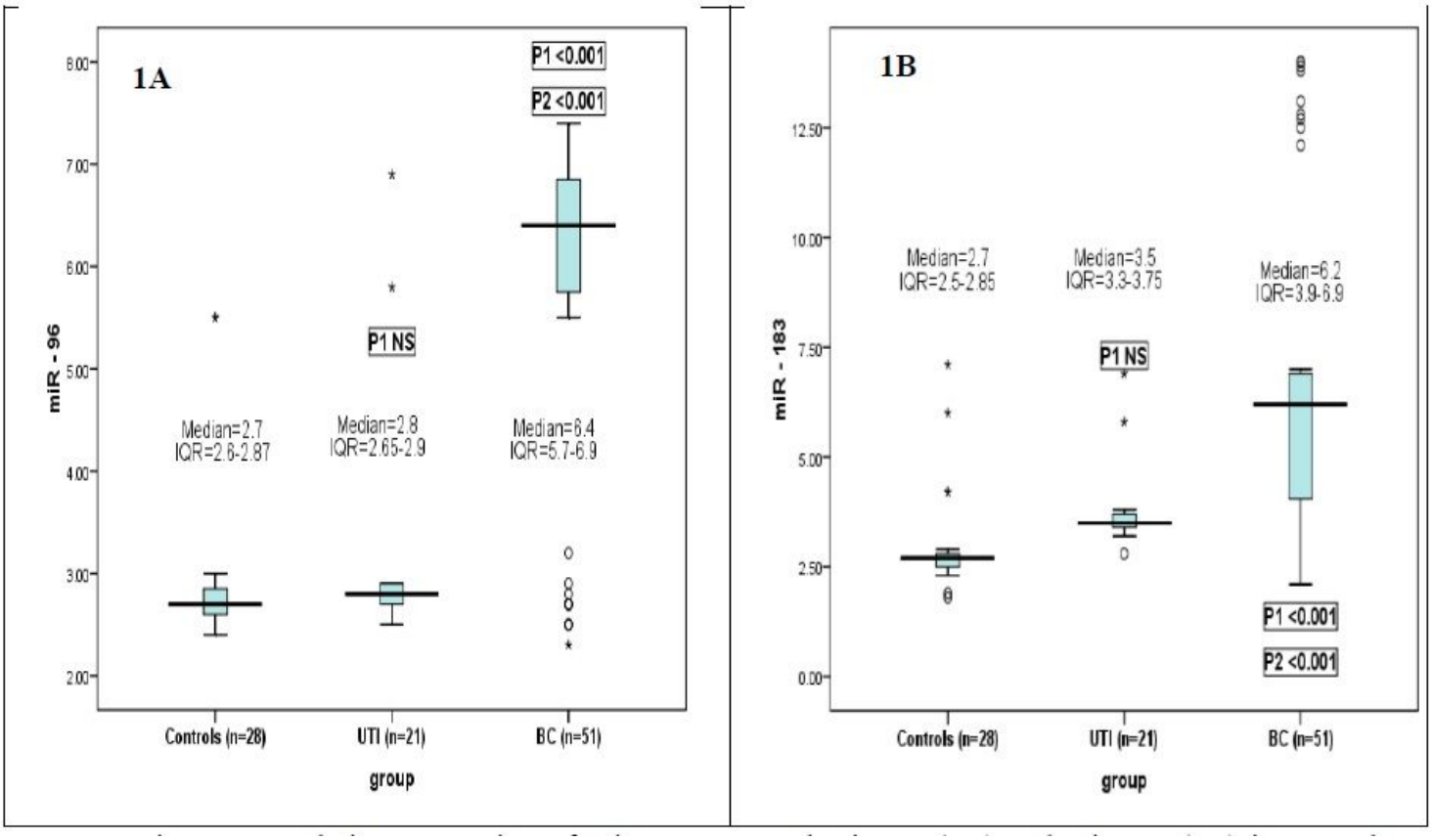

Figure 1

Relative expression of urinary exosomal miR-96 (1A) and miR-183(1B) in controls and patients. P1 Comparison with the controls. P2 Comparison with UTI group. Statistical significance was determined by Bonferroni adjusted Mann–Whitey U test (adjusted P value=0.017).

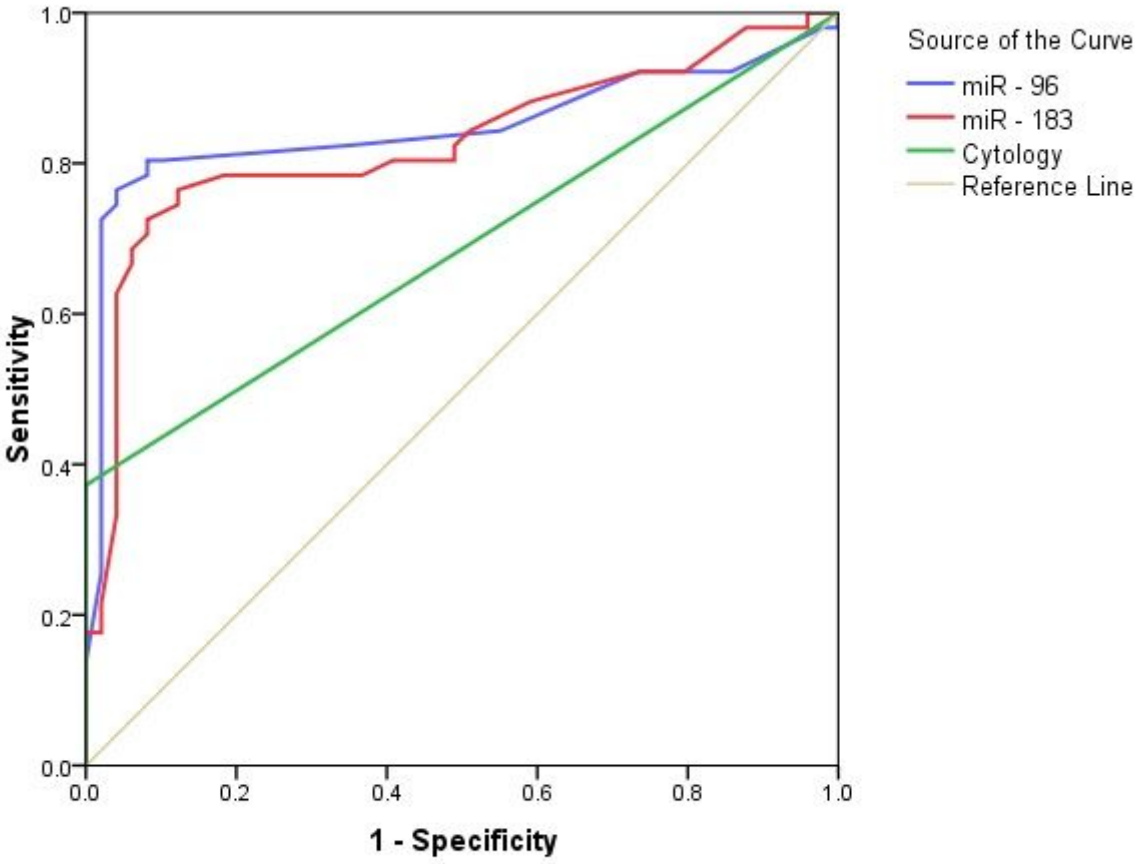

Figure 2

ROC curve analysis of miR-96 , miR-183 and cytology in diagnosis of BC

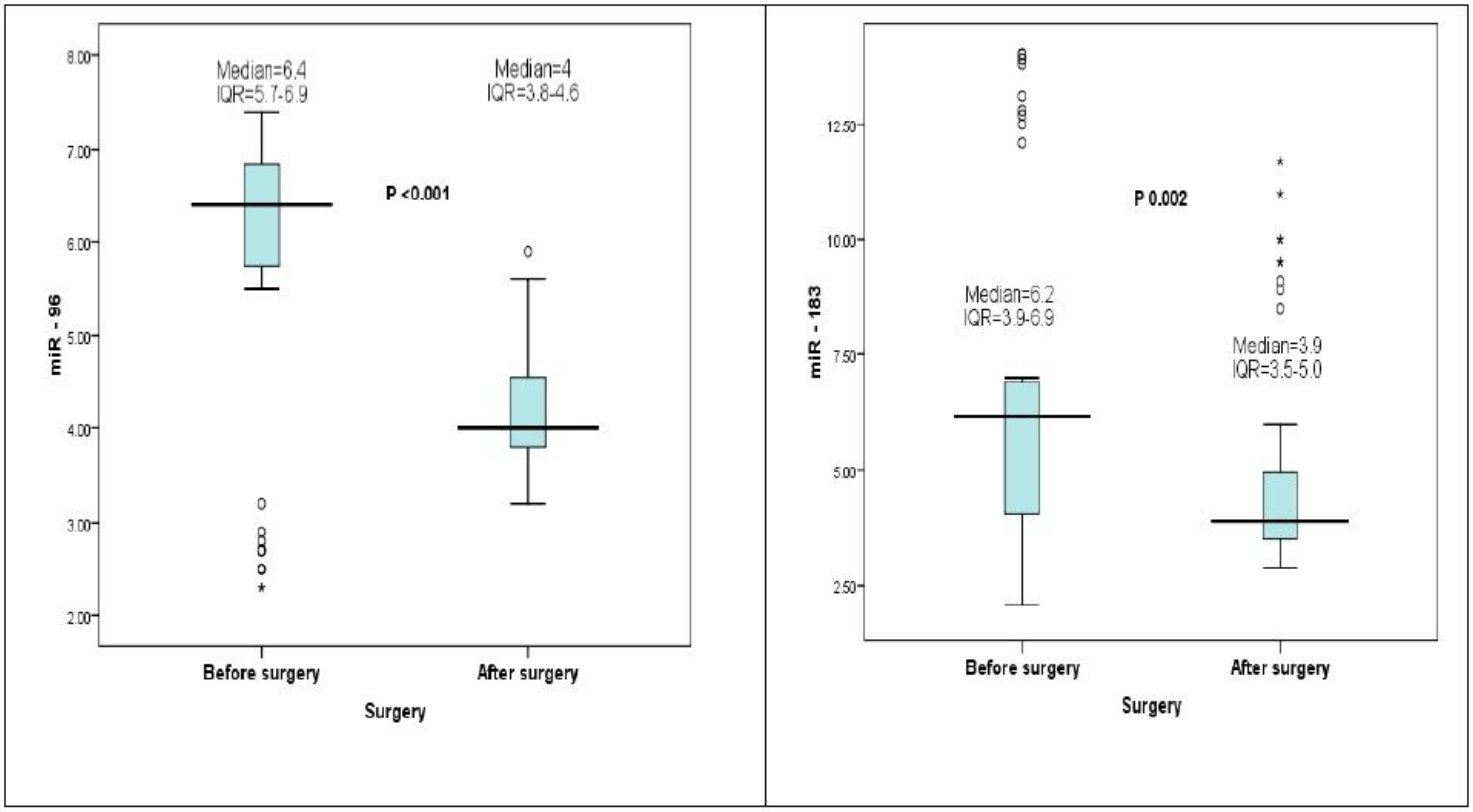

Figure 3

Comparing relative expressions of the miR-96 and miR-183 before and after surgery. Statistical significance was determined by Wilcoxon test.