

Expression of miR-100 as a novel ancillary non-invasive biomarker for early detection of bladder carcinoma

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Abstract

Objective: To determine the expression of microribonucleic acid-100 in bladder cancer patients and to evaluate its association with clinicopathological parameters.

Methods: This cross-sectional case-control study was conducted at Jinnah Postgraduate Medical Centre Karachi and Civil Hospital Karachi from January to December 2014. Serum samples from patients having bladder carcinoma were collected, and divided into three equal groups. All the study participants were briefed about the study objectives, its outcomes, sample collection technique and their written consent was obtained. Serum samples of bladder carcinoma and healthy control subjects were divided into four equal groups. Group I included patients with papillary urothelial carcinoma of low malignant potential, group II had patients with papillary urothelial carcinoma low grade, and group III had patients with papillary urothelial carcinoma high grade. Group IV comprised healthy controls. From the serum samples, total ribonucleic acid were extracted, followed by micro ribonucleic acid extraction. The complementary deoxyribonucleic acid was synthesised and samples were further processed for real time polymerase chain reaction using the specifically designed primers of micro ribonucleic acid-100. Statistical analysis was done by SPSS 21.

Results: There were 240 subjects with 60(25%) in each of the four groups. Micro ribonucleic acid-100 expression was decreased in all the three patient groups compared to the control group. A significant correlation was found between the expression of micro ribonucleic acid-100 with microscopic haematuria, cytology, cystoscopy and staging ($p < 0.05$ each). No correlation was found between the expression and age, gender, gross haematuria and smoking ($p > 0.05$ each).

Conclusion: Micro ribonucleic acid-100 was down-regulated in urothelial cancer patients with significant correlation with microscopic haematuria, cytology, cystoscopy and staging.

Keywords: Bladder carcinoma, Micro RNA, Cystoscopy, Staging. (JPMA 68: 759; 2018)

Introduction

Bladder cancer stands in the top 10 malignant tumours of the world and second most frequent amongst urological cancers.¹ Mortality rate of bladder carcinoma remains very high and surgical techniques, adjuvant therapies and other advanced treatments have not been fruitful.² Invasive investigations are not routinely practised until and unless when gross haematuria or other similar features are present. Bladder tumours can be diagnosed and monitored by presently available methods including cystoscopy, ultrasound and contrast urography but all these techniques are uncomfortable and invasive.^{3,4} Despite the simplicity and specificity of urine cytology it is less sensitive in early detection of the well-differentiated neoplasms.^{5,6} The identification of simple, easily approachable and non-invasive marker for the detection of bladder carcinoma is the key factor. Up-regulated or

down-regulated micro ribonucleic acids (miRNAs) have been found in many types of cancers. Hyperfunction or hypofunction of miRNA do contribute in the cancer development.⁷⁻⁹

Urothelial carcinoma is considered to be the most frequently occurring pathological type of bladder tumour. Identification of new diagnostic and prognostic markers by means of miRNAs can be helpful, which are differentially expressed between normal tissue type and urothelial carcinoma.¹⁰⁻¹²

The current study was planned to determine the expression of miR-100 in bladder cancer patients.

Patients and Methods

This cross sectional case-control study was conducted at Jinnah Postgraduate Medical Centre (JPMC), Karachi, and Civil Hospital, Karachi, from January to December 2014. Serum samples from patients having bladder carcinoma were collected after approval from Isra University research and ethics committee. All subjects were briefed about the study and written consent was obtained from each of them.

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The sample size was calculated using the formula at confidence level ($Z1 - \alpha/2$) of 95%. Probability (p) was assumed at 4% and 5% margin of error was considered for the particular statistic. Bladder carcinoma patients of either gender were included in the study, while patients with metastatic bladder carcinoma, and those who were already treated for bladder cancer as well as patients with other types of bladder cancers were excluded.

Serum samples of the selected patients and healthy subjects were collected and divided into four equal groups. Group I comprised patients with papillary urothelial carcinoma of low malignant potential (PUCIMP), Group II with papillary urothelial carcinoma low grade (PUCIG), and Group III with papillary urothelial carcinoma high grade (PUCHG). The classification were in line with 1998 World Health organisation [WHO] / International Society of Urological Pathology (ISUP) consensus classification system.¹³ Group IV had healthy controls.

According to WHO tumour, node and metastases (TNM) staging system, patients were categorised into Ta, T1, T2a, T2b, T3 and T4 stages and these were also compared with miR-100 expression.¹⁴

Complete demographic data, including name, age, history of current illness, clinical features and other laboratory findings, such as urine detailed report (D/R), ultrasound and biopsy were recorded in well-designed

proforma I and proforma II respectively. The miRNA was extracted from total RNA by TRIzol LS method then complementary deoxyribonucleic acid (cDNA) was synthesised. Real time polymerase chain reaction (PCR) was performed using the specifically designed primers of miRNA-100 as below.

5'-AACCCGTAGATCCGAACCTGTG-3'

SPSS 21.0 was used for data analysis. Chi square test was applied to determine significance of different variables and mean±standard deviation (SD), frequency and percentage were calculated. Correlation between the miRNA expression and other data was checked by univariate analysis. Expression of miRNA was represented through sigmoid curves using Bio-Rad's CFX software.

Results

There were 240 subjects with 60(25%) in each of the four groups. Of the 180 diagnosed patients in the first three groups, 155(86%) were males and 25(14%) were females.

Expression of miR-100 showed up-regulation in 50(83.3%) subjects in the control group. In contrast, 23(38.3%) subjects in Group I, 35(58.3%) in Group II and 45(75.0%) in Group III showed down-regulation ($p<0.001$) (Figure 1-2).

Regarding correlation, miR-100down-regulation was seen in 92(59.4%) male patients followed by and 11(44.0%) female patients. Odds ratio (OR) with 95% confidence level (CI) showed no correlation with gender ($p=0.15$).

Table-1: Correlation of miR-100 expression with demographic and clinicopathological characteristics in Bladder Cancer patients (n=180).

Variable	miR-100		P value	Univariate analysis	
	High	Low		Odds Ratio	(95% CI)
Gender					
Male (n=155)	63(40.6%)	92(59.4%)	0.15	0.895	0.693 - 2.358
Female (n=25)	14(56.0%)	11(44.0%)			
Age					
30-40 years (n=15)	5(33.3%)	10(66.7%)	0.29	0.714	0.534-1.914
41-50 years (n=26)	10(38.5%)	16(61.5%)	0.34	0.871	0.492-2.371
51-60 years (n=95)	43(45.3%)	52(54.7%)	0.09	1.037	0.861-3.037
61-70 years (n=31)	13(41.9%)	18(58.1%)	0.21	0.887	0.661-2.187
> 70 years (n=13)	6(46.2%)	7(53.8%)			
Smoking					
Absent (n=47)	21(44.7%)	26(55.3%)	0.08	0.900	0.461-1.760
Present (n=133)	56(42.1%)	77(57.9%)			
Gross haematuria					
Absent (n=91)	41(45.1%)	50(54.9%)	0.23	0.828	0.459-2.496
Present (n=89)	36(40.0%)	53(59.6%)			
Microscopic haematuria					
Absent (n=57)	35(61.4%)	22(38.6%)	0.001	1.326	0.770-2.625
Present (n=123)	42(34.1%)	81(65.9%)			

miR-100: micro ribonucleic acid-100.

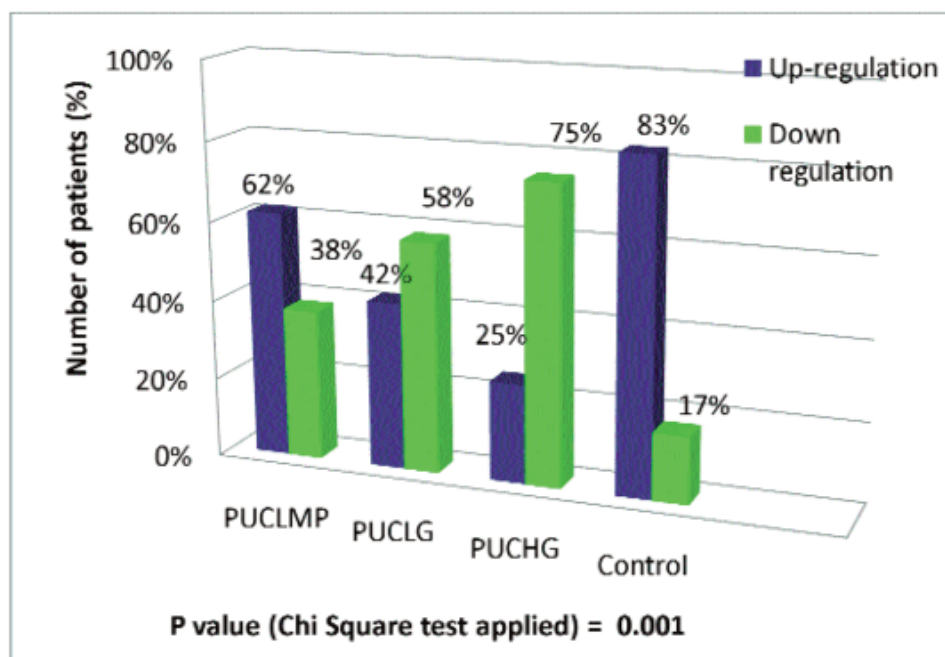
Table-2: Correlation of miR-100 expression with demographic and clinicopathological characteristics in Bladder Cancer patients (n=180).

Variable	miR-100		P value	Univariate analysis	
	High	Low		Odds Ratio	(95% CI)
Cytology					
Atypical cells not seen (n=116)	65(56.0%)	51(44.0%)	0.001	1.281	0.68-2.375
Atypical cells seen (n=64)	12(18.8%)	52(81.2%)			
Cystoscopy					
Un diagnosed (n=29)	23(79.3%)	6(20.7%)	0.13	0.719	0.414-1.184
Polypoidal (n=42)	22(52.4%)	20(47.6%)	0.01	1.067	0.481-2.249
Solitary (n=38)	15(39.5%)	23(60.5%)	0.001	1.312	0.901-2.429
Multifocal (n=27)	14(51.9%)	13(48.1%)	0.05	1.068	0.736-3.174
Exophytic (n=44)	3(6.8%)	41(93.2%)			
Staging					
Ta (n=69)	36(52.2%)	33(47.8%)	0.31	0.606	0.301-1.584
T1 (n=41)	21(51.2%)	20(48.8%)	0.10	0.938	0.443-2.462
T2a (n=10)	4(40.0%)	6(60.0%)	0.60	0.500	0.137-2.683
T2b (n=44)	10(22.7%)	34(77.3%)	0.02	1.278	0.790-3.883
T3 (n=12)	6(50.0%)	6(50.0%)	0.26	0.838	0.619-3.011
T4 (n=4)	0(0.0%)	4(100.0%)			

There were 26(14%) subjects in the 41-50 year age bracket and 16(61.5%) 16/26 of them had down-regulation ($p<0.09$).

Regarding correlation of expression of miR-100 and gross haematuria, down-regulation was seen in 53(59.6%)

patients and in 50(54.9%) patients without gross haematuria. In terms of microscopic haematuria, down-regulation was seen in 81(65.9%) patients ($p<0.001$). In relation to smokers, down-regulation was seen in 77(57.9%) smokers and in 26(55.3%) non-smokers ($p<0.28$) (Table-1).



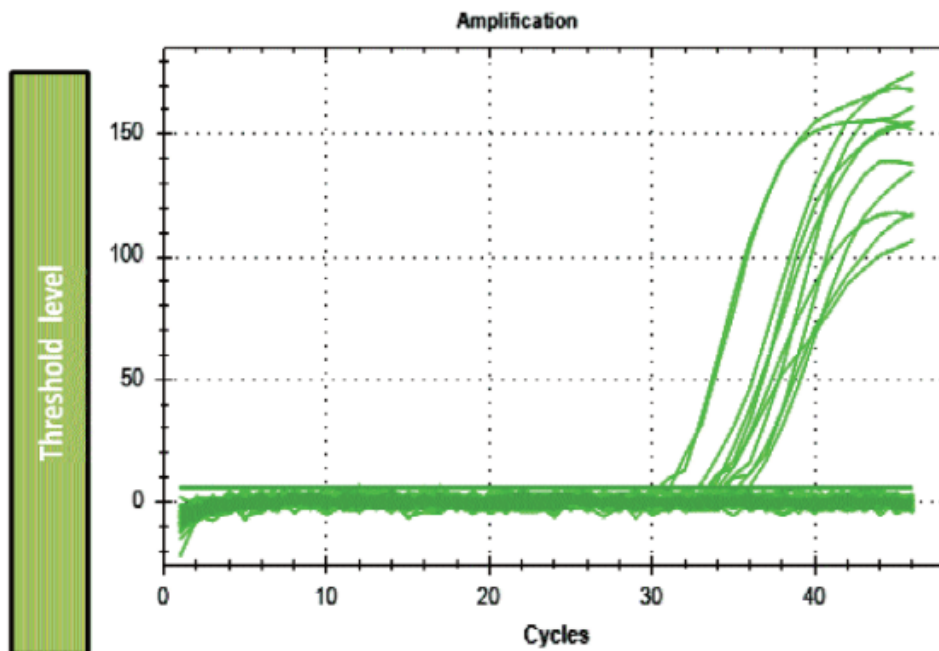
PUCLMP: Papillary Urothelial Carcinoma of Low Malignant Potential
 PUCLG: Papillary Urothelial Carcinoma Low Grade
 PUCHG: Papillary Urothelial Carcinoma High Grade

Figure-1: Expression of miR-100 in bladder cancer patients and controls (n=240).

Expression of miR-100 down-regulation was seen in 51(44.0%) patients without atypical cells on cytology, and in 52(81.2%) patients with atypical cells on cytology ($p<0.001$).

Also, the expression of miR-100 down-regulation was seen in 20(47.6%) patients with polypoidal growth, 23(60.5%) with solitary growth, 13(48.1%) with multifocal growth, 41(93.2%) with exophytic growth and 6(20.7%) undiagnosed patients on cystoscopy ($p<0.001$).

According to TNM staging, down-regulation was seen in 33(47.8%) patients of Ta stage, 20(48.8%) of T1, 6(60%) T2a, 34(77.3%) T2b, 6(50%) T3, and 4(100%) patients of T4 stage ($p<0.02$) (Table-2).



*Straight lines below the threshold level showing down regulation in patients with Bladder cancer and sigmoid curves showing up-regulation in healthy controls.

Figure-2: Down-regulation* of miR-100 in bladder carcinoma patients in comparison to the healthy controls.

Discussion

Bladder cancer is a heterogeneous disease with a variable natural history. Despite improving the methods of diagnosis and treatment, it continues to be the cause of a high toll in morbidities and mortalities.¹⁵ For better management and good prognosis, early detection improved reproducible evaluating criteria for grading of bladder cancers. For the diagnosis several classification schemes with revision and refinements have been reported in the literature. The current study was conducted to enable early diagnosis with a non-invasive method of bladder carcinoma for better prognosis.

A total 180 diagnosed cases of urothelial carcinoma and 60 controls were selected for the assessment of bladder cancer in patients on the basis of demographic data, clinical findings, laboratory profile, clinical staging with expression of miR-100 by using real time PCR.

The Expression of miR-100 showed down-regulation in 38% patients in Group I, 58% in Group II and 75% in Group III. In healthy subjects in Group IV, 83% individuals showed up-regulation. Previously, several studies have reported decreased expression of miR-100 in many of the cancers, including childhood tumours of livers, serous cell adenocarcinoma and bladder tumours.^{16,17} Similar to our findings, down-regulation of miR-100 was found to be the

most common dysregulation in bladder carcinoma.¹⁸ Another study revealed significantly lower expression of miR-100 in tissue from bladder cancer in comparison with the normal bladder tissues.¹⁶ Also, the high-grade bladder tumours showed strikingly lower expression of miR-100. Thus this miR acts as an important biomarker with prognostic significance.¹⁶ Regarding the comparison of demographic correlation of the gender and age with bladder carcinoma no significant correlation was seen with the down-regulation of miR-100 with gender and age. One of the earlier studies reported that down regulation of miR-100 showed no significant relationship with gender.¹⁹ Some other earlier researches have also reported no association of expression of miR-100 with age.²⁰⁻²²

The current study also compared smoking, gross and microscopic haematuria in patients of bladder carcinoma with expression of miR-100. No significant correlation with smoking and gross haematuria was seen, while strong association was seen with microscopic haematuria.

In the present study regarding the correlation of urine cytology and cystoscopy examination, expression of miR-100 was also compared with bladder carcinoma and it was found that the down regulated miR-100 showed further decrease expression in patients with atypical cells on urine cytology and with exophytic growth on cystoscopy.

In the present study regarding the correlation between staging and expression of miR-100 in patients with bladder carcinoma, it was found that down regulation was seen with advanced stage of tumour. In accordance to our findings decreased expression of miR-100 was correlated with increasing stage of the tumour from Ta to T2 and above.¹⁶ In a study down regulation of miR-100 was shown to be associated with more morbid clinicopathological features such as tumour recurrence and bad prognosis.¹⁶ In contrast with these findings in patients with PUNLMP, down regulation of miR-100 was characteristically found.¹⁷

Conclusion

Expression of miR-100 was down-regulated when compared with controls in bladder cancer patients. Decreased expression was more obvious with increasing stage of tumour. In univariate analysis, miR-100 showed significant correlation with microscopic haematuria, cytology, cystoscopy and staging with odd ratio showing higher risk of developing bladder cancer. Whereas, no correlation was found between miR-100 expression and age, gender, gross haematuria or smoking.

Disclaimer: The study is part of the PhD project completed at Isra University, Hyderabad, Pakistan.

Conflicts of Interest: None.

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