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BLADDER CARCINOMA;

MICRO-RNAS: EXPRESSION OF MIR-145 IN PATIENTS, AS A NEW DIAGNOSTIC APPROACH

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ABSTRACT… Objectives: To determine the micro-RNA-145 expression in bladder cancer patients when compared with controls and clinicopathological parameters. **Study design:** A case control study. **Place of Study:** Samples were collected from Jinnah Postgraduate Medical Center Karachi and Civil Hospital Karachi. **Duration of Study:** January 2014 to December 2014. **Materials and Methods:** Total of 180 patients of bladder carcinoma were divided into three groups according to WHO/1998 classification system. PUNLMP in group-I, PUCLG in group-II, PUCHG in group-III were compared with 60 healthy controls in group-IV. **Results:** The results showed that miR-145 expression was decreased in patients with bladder cancer when compared with controls. Down regulation was more obvious in BC cases with increasing grade. A significant correlation was found between the expression of miR-145 with microscopic hematuria, cystoscopy and staging. Whereas no association was found with age, frequency, urgency, flank pain, gross hematuria, urine pH, and ultrasound. **Conclusion:** miR-145 is down regulated in BC patients with significant correlation with microscopic hematuria, cystoscopy and staging.

Key words: Bladder carcinoma, micro RNA, Hematuria.

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INTRODUCTION

All over the world, cancer is amongst the major health issues. In the United States of America, occurrences of Bladder Cancer (BC) are the highest in malignancies of the urinary tract. On a per-patient basis this is the costliest available treatment. Even though its occurrences are regular, incidents are morbid and of dreadful nature, the associated management costs and corresponding death rates are extremely high, it is not identified as a public health concern. To add fuel to fire, scientifically, it is grossly underfunded.[1,2] It is estimated that, in United States, 70,500 new cases are accounted due to bladder cancer while cancer related deaths would be approximately 14680 during year 2010. Bladder cancer rates are regarded as the fourth highest malignancy among men and ninth most obvious cause of deaths in men due to malignant growth in the bladder. Among the people who suffer from bladder cancer, the proportion of male to female is roughly 3 to 1.[2,3,4] Distribution of the disease between the male and female may show a different pattern and this is because of difference in exposure to carcinogenic agents, environmental toxins and other hormonal influences. In two decades counted to backward from 2008 till 1988, the number of BC cases detected per year in the United States has gone up more than 50% in which men's disease growth is 25% faster than that of women. In Russia during the five year time since 1999, it has reached to 5.3% and 12.5% respectively.[5]

The commonest pattern of bladder cancer i.e., urothelial carcinoma has got diverse genetic as well as phenotypic features. Many of these characteristics including chromosomal, genetic and epigenetic anomalies play vital role in the development and progression of tumor.[6] Many diagnostic tools were reported so far about cellular morphology, cell-surface antigens and soluble antigens (including survivin, NMP-22, BTA-Stat and BTA/TRAK group, HA-Hase and

BCLA-4) in respect of the tumor markers.[7]

The miRNA are present in all body fluids, interestingly these miRNA remain well preserved in formalin-fixed paraffin embedded tissue samples for years showing their remarkable stability. These miRNA were first reported in 1993 and since more than 1500 human miRNA have been registered in miRBase database.[8,9] miRNAs act as oncogenes or tumor suppressors.[10] Many researches showed the role of miRNAs in the etiology and pathogenesis of cancer.[11,12]

In patients, with or without BC, levels were measured by up-regulation or down regulation of miRNA. Few micro-RNAs were over expressed in patients with bladder tumor while others were down regulated showing inverse relationship with stage of the tumor like miR-145.

One of the targets of miR-145 was identified as FSCN1. Down-regulation of mature miR-145 expression is seen with some cancers and these cancers are esophageal, lung, gastric, breast, colorectal, prostate and bladder cancers.[13,14]

MATERIALS AND METHODS

A total of 240 serum samples were divided into; 60 diagnosed patients with papillary urothelial neoplasm of low malignant potential (PUNLMP) in group-I, 60 patients with papillary urothelial carcinoma low grade (PUCLG) in group-II, 60 patients with papillary urothelial carcinoma high grade (PUCHG) with or without muscle invasion in group-III and 60 healthy controls in group-IV. The sampling was carried out in Jinnah Postgraduate Medical Center Karachi and Civil Hospital Karachi during January 2014 to December 2014. Patients who were already treated for bladder cancer and patients with Squamous cell carcinoma, adenocarcinoma were excluded from the study.

The clinicopathological data including laboratory investigations and staging of bladder cancer patients were recorded in the well-designed Proforma. Total RNA extraction containing the micro RNA was done by TRIzol LS method. The serum samples stored at –40^{0}C were first thawed at room temperature for total RNA extraction which was followed by cDNA synthesis. Real time PCR was performed using the specifically designed primers of miRNA-145.

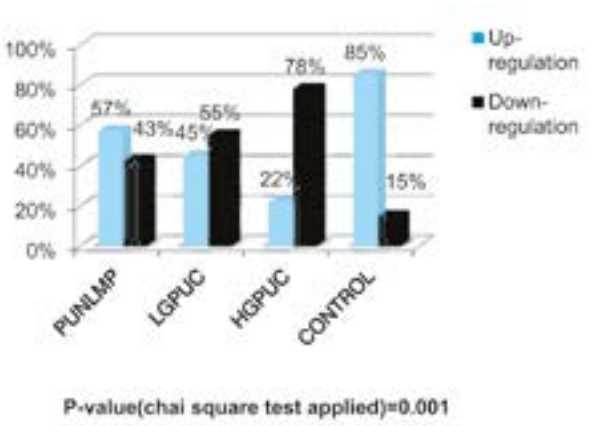
miR-145 primer sequence

5'-GTCCAGTTTTCCCAGGAATCCCT- 3'

The normality of the data for different variable was checked with Chi-square test. Continuous variables for mean±SD and categorical variables for frequency and percentage were calculated. Sigmoid curves for expression of miRNA were taken by Bio-Rad CFX software whereas graph with Microsoft excel. The data was analyzed on SPSS version 21.0. A p-value ≤ 0.05 was taken as statistically significant.

RESULTS:

Regarding the expression of miR-145 in healthy control group-IV, out of total 60 individuals, 51(85%) individuals showed up-regulation while in group-I patients down regulation was noted in 26(43.3%) having PUNLMP. However; miR-145 was down regulated with the advancing grade of the patients with PUCLG and PUCHG with 33(55.0%) and 47(78.3%) cases respectively. The overall P-value regarding the expression of miR-145 was found to be 0.001 which is highly significant (shown in graph- I and figure-1).



Graph-I: Expression of miR-145 in bladder cancer patients and controls (n=240)

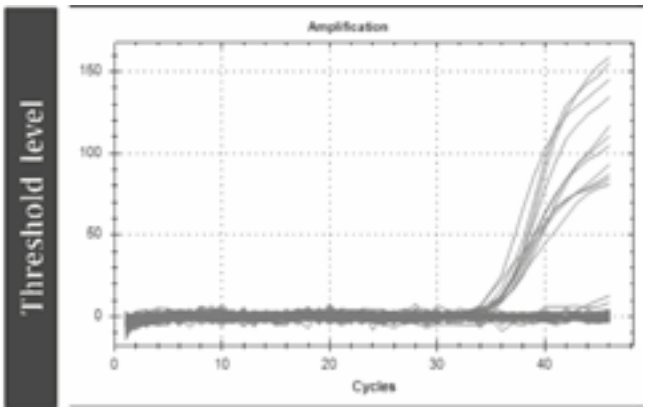


Figure-I: Expression of miR-145 in bladder cancer patients and controls. Straight lines below the threshold level showing down regulation in bladder cancer patients and sigmoid curves showing up-regulation in healthy controls

Regarding the demographic correlation of the patients with bladder carcinoma gender was compared with expression of miR-145. Regarding the comparison with expression of miR-145, up-regulation was seen in 61(39.4%) and down regulation in 94(60.6%) male patients whereas 12(48.0%) and 13(52.0%) female patients showed up-regulation and down regulation respectively. The P- value was found to be 0.16 which is non-significant (shown in table-I).

Regarding the demographic correlation of the patients with bladder carcinoma, age wise distribution of the patients was compared with expression of miR-145. Regarding the comparison with expression of miR-145, up-regulation was seen in 5(33.3%) patients between 30-40 years, 10(38.5%) patients between 41-50 years, 44(46.3%) patients between 51-60 years, 10(32.3%) patients between 61-70 years and 5(38.5%) patients above the age of 70 years. The down regulation was seen in 10(66.7%) patients between 30-40 years, 16(61.5%) patients between 41-50 years, 51(53.7%) patients between 51-60 years, 21(67.7%) patients between 61-70 years and 8(61.5%) patients above the age of 70 years. The P- value was found to be 0.63 which is non-significant (shown in table-I).

The expression of miR-145 up-regulation was seen in 20(42.6%) patients without history of smoking and 54(40.6%) smoker patients, whereas down regulation was seen in 27(57.4%) nonsmokers and 79(59.4%) smoker patients. The P- value was found to be 0.47 which is non-significant (shown in table-I).

	miR-145		Chi Square test
Variable	**High**	**Low**	**P value**
Gender			
Male	61(39.4%)	94(60.6%)	0.16
Female	13(52.0%)	12(48.0%)	
Age			
30-40 years	5(33.3%)	10(66.7%)	0.63
41-50 years	10(38.5%)	16(61.5%)	
51-60 years	44(46.3%)	51(53.7%)	
61-70 years	10(32.3%)	21(67.7%)	
> 70 years	5(38.5%)	8(61.5%)	
Smoking			
Absent	20(42.6%)	27(57.4%)	0.47
Present	54(40.6%)	79(59.4%)	
Hematuria (Gross)			
Absent	36(39.6%)	55(60.4%)	0.39
Present	38(42.7%)	51(57.3%)	
Hematuria (Microscopic)			
Absent	33(57.9%)	24(42.1%)	0.002
Present	41(33.3%)	82(66.7%)	
Cytology			
Atypical cells not seen	64(52.2%)	52(44.8%)	0.001
Atypical cells seen	10(15.6%)	54(84.4%)	
Cystoscopy			
Un-diagnosed	23(79.3%)	6(20.7%)	0.001
Polypoidal	21(50.0%)	21(50.0%)	
Solitary	15(39.5%)	23(60.5%)	
Multifocal	12(44.4%)	15(55.6%)	
Exophytic	3(6.8%)	41(93.2%)	
Staging			
Ta	35(50.7%)	34(49.3%)	0.006
T1	22(53.7%)	19(46.3%)	
T2a	4(40.0%)	6(60.0%)	
T2b	9(20.5%)	35(79.5%)	
T3	4(33.3%)	8(66.7%)	
T4	0(0.0%)	4(100.0%)	

Table-I. Correlation of miR-145 expression in bladder cancer patients with clinicopathological parameters (n=240)

Regarding the laboratory findings in the urine D/R; gross hematuria and microscopic hematuria was assessed and compared with the expression of miR-145 in the bladder cancer patients. EExpression of miR-145 in the bladder cancer patients with absence of gross hematuria showed up-regulation in 36(39.6%) patients and in 38(42.7%) patients with presence of gross hematuria. The down-regulation without gross hematuria was in 55(60.4%) and in 51(57.3%) patients with the presence of hematuria under gross examination. The P-value was found to be 0.39 which is non-significant (shown in table-I).

Similarly expression of miR-145, 33(57.9%) patients showed up-regulation with absence of microscopic hematuria and in 41(33.3%) patients with microscopic hematuria. Down-regulation with the absence of hematuria was in 24(42.1%) patients and 82(66.7%) patients with the presence of hematuria under microscopic examination. The highly significant finding was noted with P-value of 0.002 (shown in table-IV-13).

Similarly the expression of miR-145 up-regulation was seen in 64(52.2%) patients and down regulation in 52(44.8%) patients without atypical cells on cytology, whereas up-regulation was seen in 10(15.6%) patients and down regulation in 54(84.4%) patients with atypical cells on cytology. The P- value was found to be 0.001 which is highly significant (shown in table-I).

Regarding the laboratory findings in the cystoscopy, patients had polypoidal, solitary, multifocal, exophytic growth or some of the patients were missed on cystoscopy. These findings were compared with expression of miR-145. Regarding the comparison with expression of mir-145, up-regulation was seen in 21(50.0%) cases of polypoidal, 15(39.5%) cases of solitary growth, 12(44.4%) cases of multifocal growth, 3(6.8%) cases of exophytic growth and 23(79.3%) undiagnosed patients on cystoscopy, whereas down regulation was seen in 21(50.0%) cases of polypoidal, 23(60.5%) cases of solitary growth, 15(55.6%) cases of multifocal growth, 41(93.2%) cases of exophytic growth and 6(20.7%) undiagnosed patients on cystoscopy. The P- value was found to be 0.001, which is highly significant (shown in table-I).

Regarding the clinical staging according to WHO TNM classification system patients were divided into Ta, T1, T2a, T2b, T3 and T4 stages. These findings were compared with expression of miR-145. Regarding the comparison with expression of miR-145, up-regulation was seen in 35(50.7%) cases of Ta stage, 22(53.7%) cases of T1 stage, 4(40.0%) cases of T2a stage, 9(20.5%) cases of T2b stage, 4(33.3%) cases of T3 stage and 0(0.0%) cases of T4 stage, whereas down regulation was seen in 34(49.3%) cases of Ta stage, 19(46.3%) cases of T1 stage, 6(60.0%) cases of T2a stage, 35(79.5%) cases of T2b stage, 8(66.7%) cases of T3 stage and 4(100%) cases of T4 stage. The P- value was found to be 0.006, which is highly significant (shown in table-I).

On univariate analysis miR-145 showed significant correlation with males (p-value=0.03), hematuria (p-value=0.002), cytology (p-value=0.001), cystoscopy (p-value=0.001) and staging (p-value=0.03) with Odds ratio showing higher risk of developing BC. Whereas no association was found with age, frequency, urgency, flank pain, gross hematuria, urine pH, and ultrasound.

DISCUSSION

Bladder cancer is still the most common malignant tumor. The annual death rate from this disease is significant and every year there is an increase in its incidence globally.[3,15] Worldwide about 3.2% of the total tumors are tumors of urinary bladder.[16] In both gender maximum number of patients affected with bladder cancers are observed in North America and Europe. Moreover males are the main victims as reported for Belgium and females in Hungary and ratio of male to female incidence is about 4:1.[5,15,17,18] The origin of bladder cancer is multifocal and the most common type is urothelial carcinoma. In another study 72% and 28% cases of urothelial carcinoma were reported in men and women respectively.[19] Improvements in early detection have made reproducible grading criteria for better management and good

prognosis.[20]

In the present study the serum samples of urothelial carcinoma patients and the age and sex matched healthy controls were than reconfirmed for the expression of miR-145 using real time PCR technique.

Regarding the expression of miR-145, it showed down regulation in 43% patients with PUNLMP, 55% patients with LGPUC and 78% patients with HGPUC. However in healthy control group-IV, 85% individuals showed up-regulation. In accordance to our findings Chiyomaru et al[13], Liu et al[14] reported down regulation of miR-145 in many of the tumors including adeno carcinoma of stomach and colon, tumors of breast and bladder carcinoma. Similarly Sachdeva et al[21] reported down regulation of miR-145 in ovarian and pituitary tumors along with bladder carcinoma. Letelier *et al*[22] in a study described the genomic location of miR-145 on chromosome 5. Ostenfeld et al[23] reported the role of miR-145 in carcinogenesis by its interaction as tumor suppressor gene in variety of tumors including bladder tumors. Also the ectopic production of miR-145 resulted in increased cell death in urothelial carcinoma of bladder. In accordance to our findings Ichimi et al[24] studied panel of micro RNA including miR-145 and found it's down regulation in bladder tumors. Decreased expression of miR-145 and miR-133a in bladder carcinoma is also described by Chiyomaru et al13. Similarly Pignot et al25 reported strong association of down regulation of miR-145 with development of bladder carcinoma.

Another parameter in the present study was the comparison of demographic, clinical and laboratory parameters with the expression level of micro RNAs. It was found that the down regulated miR145 showed no any significant relationship with age, smoking and gross hematuria. Also consistent to our findings regarding the association of miR-145 with gender distribution no any significant difference was seen in male and female patients.2[6] In another study it was revealed that median value for association of gender with miR-145 was non-significant in patients with esophageal squamous cell carcinoma (ESCC). Similarly down regulation of miR145 in patients with ESCC showed no any significant association with patients above and below 55 years of age[27] which supports our findings. In accordance to our findings Mohamed and colleagues[26] compared the association of smokers and nonsmokers with expression of miR-145 and reported no any relationship. Regarding the comparison of microscopic hematuria in patients with bladder carcinoma with expression of miR-145, it was found that the expression of miR-145 showed significant association with microscopic hematuria. Similarly expression of miR-145 was further decreased with atypical cells in urine cytology and in cystoscopy with exophytic growth.

Another parameter is the comparison of miR-145 expression with staging in patients with bladder carcinoma. In accordance to our results miR-145 expression was decreased with increasing stage of the tumor.[28] In a study it was found that dysregulation of miR-145 is not influenced by the histological type of urinary bladder tumors. Also the patients with tumor histology of well differentiated carcinoma and patients with tumor histology of moderately differentiated carcinoma have non-significant difference.[26] In a study conducted on esophageal squamous cell carcinoma over expression of miR-145 was significantly related with recurrence of the tumor.[29]

CONCLUSION

Expression of miR-145 was down-regulated when compared with controls in bladder cancer patients. Decreased expression was more obvious with increasing stage of tumor. On univariate analysis miR-145 showed significant correlation with males (p-value=0.03), hematuria (p-value=0.002), cytology (p-value=0.001), cystoscopy (p-value=0.001) and staging (p-value=0.03) with OR showing higher risk of developing BC. Whereas no association was found with age, frequency, urgency, flank pain, gross hematuria, urine pH, and ultrasound.

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PREVIOUS RELATED STUDY

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"Don't wait for the PERFECT MOMENT take the moment and make it PERFECT."

Unknown

AUTHORSHIP AND CONTRIBUTION DECLARATION

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2	Prof. Dr. M. Ahmed Azmi	Obtained funding, Analysis and interpretation Critical revision of the article	
3	Dr. Muhammad Hanif	Critical revision of the article, Statistical analysis	
4	Dr. Amin Fahim	Writing the article, Data collection, Statistical analysis	