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INFLUENCE OF CLINICAL CHARACTERISTICS ON MIR-93-5P GENE EXPRESSION IN BLADDER CANCER

Mundher Jabbar Al-Okhedi[1,2], Zubaida Adnan Al-ĵashamy[3], Duraid Taha Abdulkareem[4]
[1]Department of Medical Laboratories Techniques, Al-Maarif University College, Al-Anbar Governorate, Iraq, [2] Ministry of Education, AL -Anbar Education Directorate, Iraq, [3]Department of biology, College of Education of Pure and Applied Sciences, Tikrit university, Iraq, [4] Consultant urologist / Assistant professor of urology /Anbar university/college of medicine
mundher_alokhedi@yahoo.com , zubaida.biology@tu.edu.iq , duraid71@uoanbar.edu.iq

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Abstract:

Objective: This study's objective was the possibility of using tissue, blood, and urine derived miRNAs (miR-93-5p) as good indicators for detecting bladder cancer. And studying the effect of some clinical characteristics on the level of gene expression of miR-93-5p and determining the relationship between these characteristics and gene expression levels.

Methods: The study was conducted from November 2022 to May 2023 in Ghazi AL_ Hariri Hospital of the Medical City (Baghdad/ Iraq). We studied a total of 45 individuals (25 BC and 20 healthy controls), using RT-q PCR tests.

Results: Data indicate that both age and gender do not have effects on the gene expression level of miR-93-5p. The results suggest that different treatments (chemo and radiation), other diseases, and family history of the disease may affect gene expression in tissue, blood, and urine samples, but most differences are not strongly statistically significant based on the probability values provided. These results could indicate certain effects but need further investigation and study to confirm.

Conclusion: Data indicate that both age and gender do not have effects on the gene expression level of miR-93-5p. In addition, it has been shown that various treatments (chemical and radiation), other diseases (diabetes, diabetes with stroke, and prostate), and family history of the disease may affect the gene expression of miR-93-5p in tissue, blood, and urine samples. However, most of the differences are not statistically significant. It is also not possible to rely on the genetic aspect alone in such multifactorial diseases. There are non-genetic factors that influence and are involved in causing bladder cancer.

Keywords: Bladder Cancer, *miR-93-5p*.

INTRODUCTION

Three layers are characterized the bladder, the outer muscular layer, the underlying submucosa and a mucous layer of epithelial cells (urothelium). Recently, new bladder cancer was diagnosed. The type of cancer is located in the epithelial lining. The tumor cells grow from the submucosa into the muscular layer. Then, they attack the surrounding tissue to invade the lymph nodes and causes metastasis at the end (1).

One of the most prevalent urinary tract malignancy is the bladder cancer BC. Patients with BC are classified into two groups. About 75% of BC cases are non-muscle-invasive BC (NMIBC), while 25% are classed as muscle-invasive BC (MIBC). The main way of MIBC treatment is cystectomy, which has approximately 15% probability of repetition. This treatment happen a half-one and a half year after surgery (2).

Generally, BC is generated from the epithelium (urothelium)that covers the bladder's interior surface. One of the most common cancers which affects the bladder is urothelial cancer. BC with different histomorphological phenotypes was divulged (15-30% of cases) (3). These cases involve adenocarcinoma, small-cell-carcinoma and squamous cell-carcinoma. They could be diverted into glandular and squamous histologies. Different histology BCs are linked to various diseases, poor response to present therapies, and metastasis (4).

One of the small non- coding RNAs is MicroRNA (miRNAs). The RNAs are approximately 21-23 nucleotides in length. These RNAs serve as suppressors for the expression of genes via post transcriptional regulation. microRNAs contribute to different pathological and physiological processes (5), which include immune regulation, oncogenesis and embryo development. These types of RNAs were isolated from different biofluids such as urine, serum and plasma (6). The presence of miRNA withing these biofluids suggests a potential role of these miRNAs biomarkers of cancers (7).

MicroRNA-93-5p is a key miRNA involved in human cancer development. It is also referred to by different names, such as hsa-mir-93, MIR21, miR-93, MIRN93, and MIRN9. In humans, the miR-93 gene encoding premiR-93 is located on chromosome 7q22.1, and is expressed at high levels in various types of tumors (8).

MiR-93-5p has been observed in the clinical prognosis of cancer patients and has been shown to have a critical role in the incidence and development of several cancers. Patients with (BC) that have higher levels of miR-93-5p, which is thought to be a sensitive marker in BC. It was shown that miR-93-5p played a crucial role in the development of tumors, and that the degree of malignancy was significantly correlated with the up-expression of miR-93-5p in BC cells. The increased pathogenic grade during the development of cancer may be explained by this. For individuals with bladder cancer, the presence of miR-93-5p may be a significant clinical indication (9). These microRNAs are found on chromosome 19, As members of the chromosome 19 miRNA cluster (C19MC), The longest human miRNAs cluster discovered to date, C19MC is expressed by paternal imprinting (10).

MATERIALS AND METHODS

Patient sample collection was recruited from Ghaze Al_Hariri Hospital of Medical City (Baghdad/Iraq) between November 2022 and May 2023 as the set of discoveries used to find miRNA suitable for markers. The sample of the study was 45 (25 patients of BC and 20 controls). Written informed permission was acquired from every individual involved. The Ghaze Al-Hariri Hospital's ethical committee gave its approval for this study.

The samples of urine from each (BC) patient and controls were collected, complying with the criteria: Urine samples were taken from individuals who had antitumor treatments, including radiation, chemotherapy, or surgery. urine samples from controls were obtained from individuals who had medical examinations and did not exhibit any disease; none of these individuals had any indications of disease in other organs. Based on histological results, BC was diagnosed. After that 250 µl of urine was added to a 2 ml Eppendorf tube pre-filled with 750 µl Trizol, mixed well, and then kept at a temperature (-20) in preparation for the miRNA extraction process.

EXTRACTION OF MIRNA AND QRT-PCR

Extracted miRNA from all samples by using the EasyPure® miRNA Kit Reagent (TransGen, biotech. ER601-01) in compliance with the manufacturer's guidelines. Using the EasyScript One-Step, gDNA Removal, and cDNA Synthesis Super Mix Kit (Trans Gen, biotech. AE311-02), total miRNA was reverse transcribed to (cDNA). in compliance with the manufacturer's guidelines, the running was performed in a reaction volume of 20 µl. of total miRNA.

levels of the expression for (*miR 93-5p*) was performed by the qRT-PCR SYBR Green test to estimate the expression of the target gene.

Endogenous control gene U6 levels were amplified and utilized to normalize the *miR 516a-5p* and *miR 93-5p* genes levels. The primers used wereas follows : miR 93-5p (CAAAGTGCTGTTCGTGCAGGTAG), miRNA-uniR.P.1 (GCGAGCACAGAATTAATACGAC),miRU6F.P.(AGAGAAGATTAGCATGGCCCCT),anduniversalR.transcriptionp.(CAGGTCCA GTTTTTTTTTTTTTTTVN. The $2^{-\Delta\Delta Ct}$ method was used to calculate relative gene expression. (11).

DATA ANALYSIS

All the data were analyzed using the IBM SPSS Statistics program version 29 to determine the effect of various factors on study parameters. The Statistical analysis included a T-test and One-way ANOVA, the P-value, the mean and standard deviation (Mean ± SD) were utilized to compare means statistically. significance was chosen by using a chi-square test at probability value (< 0.001 and 0.05) was considered statistically significant. By using receiver operating characteristic (ROC) curves, potential miRNA or their combinations were estimated for their diagnostic accuracy. Estimate of CI in this study.

RESULTS AND DISCUSSION

Characteristics of pathological samples

This study included 25 individuals with bladder cancer and 20 uninfected individuals as a control group, and it was confirmed that none of them were infected with any of the diseases. We collected three samples from each infected individual (25 tissue samples, 25 blood samples, and 25 urine samples), and two samples from each control individual (20 blood samples, 20 urine samples), totaling 115 samples. (75 infected and 40 controls). Table 1 shows the clinical characteristics of some patients with bladder cancer included in this study. The ages ranged between (50-85) years, and the ages between (50-65) constituted 52.0% (13/25), while the ages older than (66-85) years were observed at a rate of 48.0% (12/25). . Regarding gender, the infection rate was higher in males, representing 64.0% (16/25), compared to females, which represented 36.0% (9/25). These results were identical to what was stated in the latest statistics of the Iraqi Ministry of Health (2022). This is consistent with what was indicated by Shariat et al. (2010) (13): that men are three to four times more likely to develop BC than women. It is also consistent with the findings of (14).

Table 1: The clinical characteristics of some patients with bladder cancer

Characteristics of patients cancer		Number	Percentage	Total
Age	50-65	13	52.0	25
	66-85	12	48.0	
Gender	male	16	64.0	25
	female	9	36.0	

The difference in the incidence and severity of diseases between the sexes may be related to several differences, including exposure to cancer, routes of entry, enzymatic treatment of environmental substances, and cellular and physiological responses. Bladder cancer is the fourth most common cancer in men after prostate, lung, and colorectal cancers, accounting for 6.6% of all cancer cases. However, BC affects men approximately three to four times more often than women (15).

There is no unified theory to explain the differential presentation and behavior of BC between the sexes. Overexposure to carcinogens such as tobacco and industrial chemicals in men has been suggested as an explanation. However, a previous study showed that the sex-related differential risks of developing colorectal cancer persisted even after controlling for these factors. Recently, several alternative hypotheses have been proposed that include genetic, anatomical, hormonal, social, and environmental factors (16).

One explanation for the differential behavior of BC between the sexes relates to sex steroids and their receptors. An epidemiological study showed that postmenopausal women have a greater risk of developing BC compared to premenopausal women (17). Animal studies have shown that the incidence of endogenous and chemically induced BC is significantly greater in male than in female rats, and treatment of male rats with androgen deprivation reduces the development of chemically induced BC. The androgen receptor (AR) has also been detected in normal bladder epithelium and in bladder tumors in men and women. Furthermore, experiments performed using AR antagonists, small interfering RNA against AR, and androgen deprivation suggest the importance of the AR signaling pathway in BC development. However, the mechanisms that regulate AR activity in BC cells remain unknown (18).

Age is now widely accepted as an important risk factor for developing BC. Although BC can occur at any age, it generally affects middle-aged and older people. BC is primarily a disease of the elderly. Due to the close association between age and the incidence of bladder cancer (19). This can be explained by the fact that older adults differ physiologically, psychologically, and socially from younger adults (20).

Relationship of gene expression level of miR-93-5p with age and gender of people with bladder cancer

Table (2) shows the increase in the fold expression level of the miR-93-5p molecule in tissue samples and urine samples of infected people depending on age, as ages (50-65) years increased at a rate of (1.4541, 1.9672) respectively for 13/25 samples, compared to ages (66-85) years, with a rate of (1.2945, 1.6492) on average, for a total of 12/25. While the fold expression level of the miR-93-5p molecule decreased in blood samples for ages (50-65) years, at a rate of (1.2270) for 13/25 samples, compared to ages (66-85) years, at a rate of (1.4738) for a total of 12/25. There was no significant difference in all samples depending on age ($P > 0.05$).As for gender, it was observed that the fold expression level of the miR-93-5p molecule was increased in tissue, blood, and urine samples in infected females, at a rate of (1.8357, 1.3666, 1.9609), respectively, for a total of 9/25 compared to infected males, at a rate of (1.1198, 1.3336, 1.7322), respectively, with a total of 16/25, with no significant difference in all samples depending on gender ($P > 0.05$).

Table 2: Shows the relationship of gene expression level of miR-93-5p with age and gender of people with bladder cancer

Groups 93			N	Mean	Std. Deviation	Std. Error of Mean	p-value
Tissue	gender	male	16	1.1198	0.7692	0.1923	P>0.05 NS
		female	9	1.8357	1.0581	0.3526	
	Age	50-65	13	1.4541	1.1783	0.3268	P>0.05 NS
		66-85	12	1.2945	0.6365	0.1837	
Blood	gender	male	16	1.3336	0.8970	0.2242	P>0.05 NS
		female	9	1.3666	0.9115	0.3038	
	Age	50-65	13	1.2270	0.4238	0.1175	P>0.05 NS
		66-85	12	1.4738	1.2131	0.3501	
Urine	gender	male	16	1.7322	0.7898	0.1974	P>0.05 NS
		female	9	1.9609	0.6877	0.2292	
	Age	50-65	13	1.9672	0.692	0.1921	P>0.05 NS
		66-85	12	1.6492	0.8006	0.2311	

The effect of chemotherapy and radiation therapy and the interaction of some other diseases and family history on the level of gene expression of miR-93-5p for people with bladder cancer.

The results indicate that miR-93-5p gene expression is higher in people who did not receive chemotherapy in all samples (tissue, blood, and urine), and the average gene expression values were (1.6000, 1.5042, 1.8939),

respectively, compared to those who received chemotherapy (0.9820, 1.0634, 1.6737), respectively. This may indicate that chemotherapy reduces the gene expression of this miR-93-5p, with no significant difference in gene expression between patients who received chemotherapy and those who did not receive chemotherapy. At p-value = (0.1, 0.2, 0.4), which indicates that the difference is not statistically significant. As for radiotherapy, the results indicate that gene expression is lower in patients who received radiotherapy. The average gene expression values in tissue, blood, and urine samples were (1.1968, 1.2314, and 1.8063), respectively, compared to people who did not receive radiotherapy (1.4227, 1.3740, and 1.8167), respectively. Although there were slight differences in expression between people who did not receive radiotherapy and people who received radiotherapy, these differences were not statistically significant, as the p-value was (0.6, 0.7, 0.9). The results also showed that there were no statistically significant differences in gene expression for miR-93-5p between patients who had a family history of the disease and those who did not have a family history, although people with a family history had higher gene expression results (1.4386, 1.4409, 1.8372), compared to people with no family history (1.3431, 1.3481, 1.8019). It is noted that the gene expression of miR-93-5p in the three samples (tissue, blood, urine) of people who have a family history of the disease is slightly higher than that of people who do not have a family history. These small differences may be an indication that people with a family history of bladder cancer may have genetic factors that make them more susceptible to increased miR-93-5p gene expression associated with this disease. These differences may help us understand the effect of genetic factors on gene expression and how the body responds to disease. This information can be useful in developing prevention and treatment strategies based on the patient's family history.

The results of miR-93-5p gene expression in people who had other diseases such as diabetes, diabetes with stroke, and prostate had a significantly lower mean compared to people who did not have other diseases. The average gene expression in tissue samples was (0.8986, 0.8507, 0.8568), respectively, and in blood samples (1.0962, 1.1258, 1.0528), and in urine samples it was (1.4981, 1.3368, 1.8289). Compared to people who do not have other diseases, the average gene expression values in each of the tissue, blood, and urine samples were 1.8395, 1.6482, and 1.9869, respectively. It is clear from these results that people with other diseases show significantly lower levels of miR-93-5p gene expression compared to people without other diseases, in all sample types (tissue, blood, and urine), with no significant differences at p-value = (0.1, 0.6, 0.4).

The results suggest that different treatments (chemo and radiation), other diseases, and family history of the disease may affect gene expression in tissue, blood, and urine samples, but most differences are not strongly statistically significant based on the probability values provided. These results could indicate certain effects but need further investigation and study to confirm. Note Table (3).

Table 3: Shows the effect of some clinical characteristics on the gene expression level of miR-93-5p

Sample	Clinical characteristics		No	Mean	SD.	SE.	p-value
Tissue	Chemotherapy	No	16	1.6000	1.04743	.26186	0.1
		Yes	9	.9820	.51570	17190	
	Radiotherapy	No	20	1.4227	1.01916	.22789	0.6
		Yes	5	1.1968	.45960	.20554	
	Family history	No	17	1.3431	.58694	.14674	0.8
		Yes	8	1.4386	1.39481	.46494	
	Other disease	diabetic	6	.8986	.65160	.29140	0.1
		diabetic + stroke	3	.8507	.67717	.39097	
		prostate	4	.8568	.64018	.32009	
		No	12	1.8395	1.00229	.28934	
Blood	Chemotherapy	No	16	1.5042	1.05600	.26400	0.2
		Yes	9	1.0634	.33125	.11042	
	Radiotherapy	No	20	1.3740	.97871	.21885	0.7
		Yes	5	1.2314	.33290	.14888	
	Family history	No	17	1.3481	.71097	.17774	0.9
		Yes	8	1.4409	1.18013	.39338	
	Other disease	diabetic	6	1.0962	.51160	.22879	0.6
		diabetic + stroke	3	1.1258	.24891	.14371	
		prostate	4	1.0528	.37065	.18533	
		No	12	1.6482	1.16520	.33636	

Urine	Chemotherapy	No	16	1.8939	.89128	.22282	0.4
		Yes	9	1.6737	.39507	.13169	
	Radiotherapy	No	20	1.8167	.82103	.18359	0.9
		Yes	5	1.8063	.39852	.17822	
	Family history	No	17	1.8019	.72216	.18054	0.8
		Yes	8	1.8372	.83768	.27923	
	Other disease	diabetic	6	1.4981	.80183	.35859	0.4
		diabetic + stroke	3	1.3368	.76612	.44232	
		prostate	4	1.8289	.57141	.28571	
		No	12	1.9869	.76927	.22207	

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