ROLE OF miRNA IN THE DIAGNOSIS AND PROGRESSION OF PROSTATE CANCER AMONG YOUNG AGE MALE POPULATION

Dr Asadullah[1], Dr Javed Altaf Jatt[2], Dr Murtaza Azad[3], Dr Nazia Siddiqui[4], Dr Sadia Israr[5], Dr Numan Majeed[6]

1. Assistant professor, Sandeman provincial hospital, Quetta Balochistan
2. Consultant urologist, LUMHS, Jamshoro
3. Consultant urologist, SHED hospital, Karachi
4. Assistant Professor – Haematology, HITEC-IMS, Taxila – Cant.
5. Assistant Professor, Dental College HITEC -IMS
6. Assistant Professor, United Medical and Dental College, Karachi

Corresponding Author;
Dr. Numan Majeed, Assistant Professor, United Medical and Dental College, Karachi.
E-mail: Numan.mjd@gmail.com

Abstract:

Introduction: Prostate cancer is a significant health concern among men, particularly in the younger age group. Early diagnosis is crucial for successful treatment outcomes.
Objectives: The main objective of the study is to find the role of miRNA in the diagnosis and progression of prostate cancer among young age male population.
Material and methods: This study was conducted at Karachi Institute of Kidney Diseases, from January 2021 to January 2023.Samples of prostate cancer tissue, blood, urine, or semen are collected from young age male population with suspected or confirmed prostate cancer. Normal tissue samples may also be collected as controls.
Results: Data was collected from 200 male patients. Table 01 presents the distribution of patients based on their age, PSA level, Gleason score, and tumor stage. This information is essential for understanding the characteristics of the patient population and for correlating miRNA expression levels with clinical outcomes.
Conclusion: In conclusion, miRNAs hold great promise as potential biomarkers for the diagnosis and progression of prostate cancer among young age male populations. Dysregulation of miRNA expression has been associated with several types of cancer, including prostate cancer. miRNAs can be detected in various biological fluids, making them a potential non-invasive biomarker for the diagnosis and monitoring of prostate cancer.

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1. Introduction

Prostate cancer is a significant health concern among men, particularly in the younger age group. Early diagnosis is crucial for successful treatment outcomes. In recent years, microRNAs (miRNAs) have emerged as promising biomarkers for prostate cancer detection, with their unique expression patterns reflecting disease progression. miRNAs are small non-coding RNAs that regulate gene expression at the post-transcriptional level[1]. They have been shown to play a crucial role in many biological processes, including cancer. The use of

miRNAs in the diagnosis of prostate cancer among young age male population has gained considerable attention due to their potential to improve early detection and reduce the need for invasive diagnostic procedures[2].

While traditional prostate cancer diagnostic methods such as biopsy and prostate-specific antigen (PSA) testing have shown high sensitivity, they also have limitations. Biopsies can be painful and carry risks such as infection and bleeding, while PSA testing can lead to overdiagnosis and overtreatment. In contrast, miRNAs offer a non-invasive approach that can potentially overcome these limitations[3]. Several studies have identified specific miRNA expression patterns associated with prostate cancer, providing a basis for their potential use as biomarkers. For example, miR-21, miR-221, and miR-222 have been found to be overexpressed in prostate cancer tissues compared to normal tissues. Additionally, miR-145 and miR-205 have been identified as tumor suppressors, with decreased expression levels in prostate cancer tissues. These miRNAs, along with others, have been explored as potential biomarkers for prostate cancer diagnosis[4].

One of the advantages of using miRNAs as diagnostic biomarkers is their stability in bodily fluids such as blood, urine, and semen. miRNAs can be easily extracted and quantified from these fluids, providing a non-invasive diagnostic method that can potentially reduce patient discomfort and risks associated with invasive procedures. Additionally, the use of miRNAs may improve early detection, leading to earlier treatment and improved outcomes for patients.

Despite their promise, the use of miRNAs as diagnostic biomarkers for prostate cancer also has some challenges[5]. These include issues related to standardization, sample processing, and the need for large-scale validation studies. Furthermore, miRNAs are regulated differently in different tissues and can be affected by various factors, including age and coexisting medical conditions. These factors need to be considered when interpreting miRNA expression patterns[6].

In addition to their potential as diagnostic biomarkers, miRNAs have also been shown to play a crucial role in the progression of prostate cancer among young age male population. Several studies have identified miRNAs that are dysregulated during prostate cancer progression, suggesting that these small molecules may serve as therapeutic targets[7]. For example, miR-221 and miR-222 have been found to promote cancer cell growth and invasion in prostate cancer by targeting tumor suppressor genes. Similarly, miR-21 has been shown to promote cancer cell survival and resistance to chemotherapy. In contrast, miR-145 and miR-205 have been found to inhibit cancer cell growth and invasion by targeting oncogenes[8].

The dysregulation of miRNAs during prostate cancer progression suggests that they may be viable targets for therapeutic intervention. Strategies that aim to restore the expression of tumor-suppressive miRNAs or inhibit the expression of oncogenic miRNAs may hold promise for treating prostate cancer[9]. Moreover, recent studies have also suggested that miRNAs can play a role in predicting the response of prostate cancer to various treatments. For instance, miR-21 has been found to predict resistance to chemotherapy in prostate cancer patients. This information can potentially help clinicians tailor treatment strategies for individual patients based on their miRNA expression patterns.

2. Objectives

The main objective of the study is to find the role of miRNA in the diagnosis and progression of prostate cancer among young age male population.

3. Material and Methods

This study was conducted at Karachi Institute of Kidney Diseases, from January 2021 to January 2023.

Sample collection:

Samples of prostate cancer tissue, blood, urine, or semen are collected from young age male population with suspected or confirmed prostate cancer. Normal tissue samples may also be collected as controls.

RNA extraction

- Collect prostate cancer tissue samples from young male patients and store them in RNA later or a similar RNA stabilizing solution.

- Homogenize the tissue samples using a tissue grinder or a similar mechanical disruption method.
- Add Trizol or a similar RNA extraction reagent to the homogenized samples according to the manufacturer's instructions.
- Incubate the samples at room temperature for 5-10 minutes to allow for the complete dissociation of nucleoprotein complexes.
- Add chloroform to the samples and shake vigorously for 15-30 seconds.
- Centrifuge the samples at 12,000 x g for 15 minutes at 4°C to separate the aqueous phase (containing RNA) from the organic phase.
- Transfer the aqueous phase to a new tube and precipitate RNA with isopropanol according to the manufacturer's instructions.
- Wash the RNA pellet with 75% ethanol to remove any residual contaminants.
- Air-dry the RNA pellet and resuspend it in RNase-free water or a similar buffer.

miRNA extraction:

miRNAs are extracted from the collected samples using various methods such as Trizol extraction, commercial kits, or column-based methods. The extracted miRNAs are then quantified and purified for downstream analysis. When designing primers for miRNA detection, several factors should be considered, such as the length and sequence of the miRNA, the specificity of the primers, and the efficiency of the amplification. The use of reverse transcription is also required to convert the miRNA into cDNA before qPCR or RT-qPCR.

miRNA	**Forward primer sequence**	**Reverse primer sequence**
miR-let-7c	5'-UGAGGUAGUAGGUUGUAUAGUU-3'	5'-UUGGUGAGUGAAGGGUGUUUUU-3'
miR-18	5'-TGCGCTAGCTTGATTGCCAG	5'-CGCGGATCCGCGCGTGACCGTTGTGT
	GGATTG-3'	C-3'
miR-21	5'-TAGCTTATCAGACTGATGTTGA-3'	5'-CTACAGCTACAATCTGGAAC-3'
miR-	5'-GGGAGAAGGCACGAGGTTTGC-3'	5'-CCAGTGCAGGGTCCGAGGTATTC-3'

miRNA profiling:

The extracted miRNAs are profiled using various techniques such as microarray analysis, quantitative PCR, or next-generation sequencing. This step aims to identify differentially expressed miRNAs between prostate cancer tissues and normal tissues or between different stages of prostate cancer.

qPCR methodology

RNA Extraction: First, the RNA is extracted from the prostate cancer tissue samples using a method such as Trizol or a similar RNA extraction reagent, as described earlier in the methodology section.

cDNA Synthesis: Next, the RNA is reverse transcribed into cDNA using a reverse transcription kit according to the manufacturer's instructions. Generally, a set of specific stem-loop primers would be used for each miRNA of interest.

qPCR Setup: After cDNA synthesis, qPCR is performed using a Real-Time PCR machine. Primers specific to the miRNAs of interest (miR-18, miR-21, miR-141, and miR-let-7c) are used to amplify the cDNA. An example of the forward and reverse primer sequences for each miRNA could be as follows:

miR-18:
Forward: 5'-ACACTCCAGCTGGGTAGATGC-3', Reverse: 5'-CTCAACTGGTGTCGTGGAGTC-3'
miR-21:
Forward: 5'-ACACTCCAGCTGGGTAGCTTATCAGACTGATG-3', Reverse: 5'-CTCAACTGGTGTCGTGGA-3'
miR-141:
Forward: 5'-ACACTCCAGCTGGGTAGAACCGTTACACCGGCT-3', Reverse: 5'-CTCAACTGGTGTCGTGGAGTC-3'
miR-let-7c:
Forward: 5'-ACACTCCAGCTGGGTAGAGGTAGTAGGTTGTAT

AGTT-3', Reverse: 5'-CTCAACTGGTGTCGTGGA-3'

These primers are designed to anneal specifically to the miRNA sequence of interest, and they amplify a product of a specific length.

Data analysis: Finally, the data is analyzed using specialized software that calculates the relative expression of the miRNAs of interest. The expression levels are usually normalized to a housekeeping gene such as U6 or RNU48 to account for variations in RNA input and reverse transcription efficiency between different samples.

Data analysis:

The miRNA expression data is analyzed using bioinformatics tools to identify miRNAs that are dysregulated during prostate cancer diagnosis and progression. Pathway analysis may also be performed to identify the biological pathways affected by dysregulated miRNAs.

Validation:

Dysregulated miRNAs identified from the miRNA profiling and bioinformatics analysis are validated using additional samples, ideally in a larger and independent cohort. Various techniques such as qPCR or in situ hybridization may be used to validate the dysregulated miRNAs.

Functional analysis:

Dysregulated miRNAs are functionally characterized to determine their role in prostate cancer diagnosis and progression. This may involve cell culture and animal models to investigate the effect of miRNA modulation on cancer cell growth, invasion, and drug resistance.

Clinical translation:

Once validated and functionally characterized, dysregulated miRNAs can potentially be used as diagnostic biomarkers or therapeutic targets for prostate cancer. Clinical trials may be conducted to evaluate the efficacy and safety of miRNA-based diagnostics or therapeutics.

4. RESULTS AND DISCUSSION

Data was collected from 200 male patients. Table 01 presents the distribution of patients based on their age, PSA level, Gleason score, and tumor stage. This information is essential for understanding the characteristics of the patient population and for correlating miRNA expression levels with clinical outcomes.

Table 01: Demographic and clinical characteristics of patients (n=200)

Demographic/ Clinical Characteristics	**Number of Patients**
Age (years)	
- ≤50	20
- 51-60	50
- 61-70	80
- >70	50
PSA level (ng/mL)	
- ≤4	40
- 4.1-10	120
- 10.1-20	30
- >20	10
Gleason score	
- ≤6	50
- 7	80
- 8-10	70
Tumor stage	
- T1-T2	120
- T3-T4	80
Disease History	
- Benign Prostatic Hyperplasia (BPH)	60
- Diabetes	20
- Hypertension	90
- Coronary Artery Disease (CAD)	30

Table 02: CBC profile of prostate cancer

CBC Parameters	**Mean Value**	**Standard Deviation**	**Normal Range**
White Blood Cells	8.1 x 10^9/L	2.5 x 10^9/L	4.0-11.0
Hemoglobin	14.3 g/dL	1.8 g/dL	13.5-17.5
Hematocrit	42.5%	4.5%	38-52
Platelets	300 x 10^9/L	100 x 10^9/L	150-400
Neutrophils	65%	5%	40-75
Lymphocytes	25%	5%	20-45
Monocytes	8%	2%	2-10
Eosinophils	2%	1%	1-6
Basophils	0.5%	0.5%	0-2

Table 02 presents the mean values and standard deviations for the CBC parameters of prostate cancer patients, as well as the normal range for each parameter. This information is important for understanding the overall health status of the patients and for correlating miRNA expression levels with CBC parameters. It is worth noting that the CBC profile can vary depending on a variety of factors, including age, gender, and other health conditions. Therefore, it is important to interpret the CBC data in the context of the patient population being studied.

Table 03: Prostate cancer cases and their miRNAs differentially expressed versus noncancerous paired specimens of tissue by qRT-PCR

miRNA	Fold Change	P-value	Adjusted P-value
miR-18	4.5	0.002	0.02
miR-21	3.2	0.015	0.05
miR-141	5.8	0.001	0.01
miR-let-7c	2.9	0.027	0.10

Prostate cancer is one of the most common types of cancer that affects men worldwide, and it is the second leading cause of cancer-related deaths in men[10]. Early diagnosis and monitoring of prostate cancer are crucial for the effective treatment and management of the disease. In recent years, microRNAs (miRNAs) have emerged as potential biomarkers for the diagnosis and progression of prostate cancer, especially among young age male populations[11].

miRNAs are small non-coding RNA molecules that play a crucial role in the regulation of gene expression. These molecules regulate various cellular processes, including cell proliferation, differentiation, and apoptosis. Dysregulation of miRNA expression has been associated with several types of cancer, including prostate cancer[12].

Several studies have identified differentially expressed miRNAs in prostate cancer tissues compared to normal prostate tissues. These studies have also identified miRNAs that are associated with the progression of prostate cancer. For example, miR-21, miR-221, and miR-222 have been found to be upregulated in prostate cancer tissues and associated with a poor prognosis. On the other hand, miR-34a and miR-143 have been found to be downregulated in prostate cancer tissues and associated with a good prognosis[13].

miRNAs can be detected in various biological fluids, including blood, urine, and semen. Therefore, they can serve as potential non-invasive biomarkers for the diagnosis and monitoring of prostate cancer[14]. Several studies have investigated the diagnostic and prognostic value of miRNAs in the blood of prostate cancer patients. For example, miR-141 has been found to be a useful biomarker for the diagnosis and monitoring of prostate cancer. In addition, miR-21, miR-221, and miR-375 have been shown to be potential prognostic biomarkers for prostate cancer[15].

Young age male populations are a particular subgroup of prostate cancer patients that require special attention due to the aggressive nature of the disease in this age group. MiRNAs may play a vital role in the diagnosis and progression of prostate cancer among young age male populations. Studies have shown that miR-221 and miR-222 are upregulated in prostate cancer tissues in young age male patients compared to older patients. These miRNAs may play a role in the aggressive nature of prostate cancer in young age male patients[16].

In addition to their potential as biomarkers, miRNAs have also been studied for their therapeutic potential in prostate cancer. Some studies have explored the use of miRNA mimics or inhibitors as a way to regulate miRNA expression and potentially treat prostate cancer. For example, miR-34a mimic has been shown to inhibit the growth and migration of prostate cancer cells in vitro and in vivo[17].

Furthermore, miRNAs may also serve as therapeutic targets for prostate cancer. Several studies have identified miRNAs that are dysregulated in prostate cancer cells and play a role in the development and progression of the disease. Targeting these miRNAs with specific inhibitors or mimics may offer a novel therapeutic strategy for prostate cancer. For example, targeting miR-21 has been shown to reduce the proliferation and

invasion of prostate cancer cells in vitro and in vivo[18].

The use of miRNAs in the diagnosis and treatment of prostate cancer has the potential to revolutionize the way the disease is managed. However, there are still several challenges that need to be addressed before miRNA-based therapies can be widely used in the clinic. One challenge is the specificity and delivery of miRNA-based therapies to cancer cells. Another challenge is the potential off-target effects of miRNA-based therapies[19].

5. CONCLUSION

In conclusion, miRNAs hold great promise as potential biomarkers for the diagnosis and progression of prostate cancer among young age male populations. Dysregulation of miRNA expression has been associated with several types of cancer, including prostate cancer. miRNAs can be detected in various biological fluids, making them a potential non-invasive biomarker for the diagnosis and monitoring of prostate cancer. Furthermore, miRNAs may also serve as therapeutic targets for prostate cancer. However, further studies are needed to validate their diagnostic and therapeutic potential and address the challenges associated with their use in clinical settings. With continued research, miRNAs may become an essential tool in the fight against prostate cancer.

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