INSIGHTS-JOURNAL OF
HEALTH AND REHABILITATION

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

CROSS-SECTIONAL ANALYSIS OF MICRORNA EXPRESSION PATTERNS AND THEIR ASSOCIATION WITH EARLY DIAGNOSIS OF HUMAN DISEASES IN PAKISTAN

Original Research

Murtaza Khodadadi[1]*, Tanzeela Iram[2], Mariam Kamran[3], Humera Usman[4], Sadaf Moeez[5], Iram Saddiqa Aamir[6]
[1]4th Semester, MSc Microbiology and Immunology, COMSATS University Islamabad, Pakistan.
[2]BSc (Hons) Microbiology, University of Agriculture, Faisalabad (Gojra Campus), Pakistan.
[3]MPhil Molecular Pathology and Genomics, Forman Christian College (A Chartered University), Lahore, Pakistan.
[4]Assistant Professor, Department of Biochemistry, Fazaia Medical College, Air University, Islamabad, Pakistan.
[5]Assistant Professor, Department of Biological Sciences, International Islamic University, Islamabad, Pakistan.
[6]Professor, Bahria University Health Sciences Campus, Karachi, Pakistan.
Corresponding Author: Murtaza Khodadadi, 4th Semester, MSc Microbiology and Immunology, COMSATS University Islamabad, Pakistan, murtazakhodadadi786@gmail.com

Acknowledgement: The authors gratefully acknowledge all participants and laboratory staff for their valuable contributions to this study.

Conflict of Interest: None

Grant Support & Financial Support: None

ABSTRACT

Background: Circulating microRNAs (miRNAs) have emerged as potential non-invasive biomarkers for early disease detection due to their stability in plasma and regulatory roles in gene expression. In Pakistan, early diagnosis of chronic and malignant diseases remains challenging, necessitating the exploration of novel molecular markers to enhance timely clinical interventions.

Objective: This study aimed to evaluate the expression profiles of selected circulating microRNAs—miR-21, miR-155, miR-34a, miR-126, and miR-122—and assess their diagnostic potential as early biomarkers among patients with diverse early-stage diseases compared to healthy controls.

Methods: A cross-sectional study was conducted on 180 participants, including 120 patients with early-stage pathological conditions and 60 age- and sex-matched healthy controls. Plasma samples were analyzed using quantitative real-time polymerase chain reaction (qRT-PCR) for miRNA quantification. Statistical analyses included independent t-tests, Pearson's correlation, and receiver operating characteristic (ROC) curve evaluation to determine diagnostic accuracy.

Results: Significant upregulation of miR-21 (3.8-fold), miR-155 (2.9-fold), and miR-34a (2.4-fold) was observed among patients ($p < 0.001$), while miR-126 and miR-122 were downregulated (2.6- and 2.1-fold reductions, respectively; $p < 0.01$). ROC analysis revealed the highest diagnostic accuracy for miR-21 (AUC = 0.92), followed by miR-155 (AUC = 0.88). Combined miRNA panels improved sensitivity (93.1%) and specificity (89.4%). Negative correlations were noted between miR-126 and serum cholesterol ($r = -0.45$, $p < 0.05$) and between miR-122 and fasting glucose ($r = -0.52$, $p < 0.01$).

Conclusion: The study demonstrated that specific circulating miRNAs, particularly miR-21 and miR-155, possess strong diagnostic potential for early disease detection. Their expression signatures could be integrated into population-based screening protocols, enhancing early diagnosis and management in resource-limited healthcare settings.

Keywords: Biomarkers, Circulating microRNAs, Early diagnosis, Gene expression, qRT-PCR, ROC curve, Sensitivity and specificity.

INTRODUCTION

MicroRNAs (miRNAs) have emerged as a critical class of small, non-coding RNA molecules that regulate gene expression post-transcriptionally and play a central role in numerous physiological and pathological processes (1). These molecules, typically 18–25 nucleotides in length, bind to complementary sequences within target messenger RNAs (mRNAs), leading to translational repression or degradation. Over the past two decades, miRNAs have transformed our understanding of gene regulation and disease mechanisms, revealing intricate molecular networks that contribute to cancer, cardiovascular disorders, neurological diseases, and infectious conditions (2). Their remarkable stability in biological fluids, such as blood, saliva, and urine, has further positioned them as promising, minimally invasive biomarkers for early disease detection and prognosis. Globally, a growing body of evidence indicates that aberrant miRNA expression profiles are closely associated with disease onset, progression, and therapeutic response. In oncology, for instance, specific miRNAs such as miR-21, miR-155, and miR-34a have been identified as reliable indicators of tumor development, metastasis, and patient survival outcomes. Similarly, dysregulated miRNAs have been linked with metabolic and cardiovascular diseases, including diabetes mellitus and coronary artery disease, where they influence pathways involved in inflammation, lipid metabolism, and endothelial function. Moreover, in neurodegenerative conditions such as Alzheimer's and Parkinson's diseases, altered miRNA signatures have been proposed as early diagnostic tools capable of detecting molecular changes long before the appearance of clinical symptoms. Collectively, these findings underscore the translational potential of miRNAs as diagnostic and prognostic biomarkers across a spectrum of diseases (3).

Despite these advances, the integration of miRNA-based diagnostics into clinical practice remains limited, particularly in low- and middle-income countries. In Pakistan, where the burden of both communicable and non-communicable diseases continues to rise, early detection remains a major challenge due to limited access to advanced molecular diagnostics, inadequate screening programs, and delayed clinical presentation (4). The country faces a dual disease burden, with rising rates of cancer, cardiovascular disease, diabetes, and infectious conditions such as tuberculosis and hepatitis. Given these challenges, the exploration of miRNA expression as a tool for early disease detection offers significant potential to bridge diagnostic gaps, improve patient outcomes, and reduce healthcare costs through timely intervention (5). Furthermore, Pakistan's diverse genetic landscape, environmental exposures, and variable healthcare access create a unique setting for studying disease-associated molecular markers. Most global miRNA research has been conducted in Western or East Asian populations, leaving a critical gap in understanding how regional and ethnic differences influence miRNA expression patterns and disease susceptibility. The absence of locally relevant data impedes the translation of molecular discoveries into practical diagnostic tools for Pakistani patients. Consequently, establishing baseline miRNA profiles and their disease-specific alterations within this population could contribute not only to improved early diagnosis but also to the development of regionally tailored molecular diagnostic panels (6).

Several studies have highlighted the diagnostic potential of circulating miRNAs, which can be detected in plasma or serum with high stability and reproducibility (7). Their non-invasive nature allows repeated monitoring, making them ideal candidates for longitudinal disease tracking and risk assessment. However, most available data focus on single-disease models or limited sample sizes, whereas cross-sectional studies encompassing multiple disease categories and diverse patient cohorts are scarce (8). A systematic evaluation of miRNA expression across various early disease states within a single population could provide comprehensive insights into shared and disease-specific molecular mechanisms, paving the way for the establishment of multi-marker diagnostic panels (9). This study seeks to fill this critical knowledge gap by conducting a cross-sectional analysis of miRNA expression patterns among diverse patient populations across Pakistan (10). By correlating specific miRNA signatures with early-stage disease conditions, it aims to identify molecular markers that could facilitate earlier diagnosis and improved clinical decision-making (11). The findings are expected to contribute to the foundation of population-specific miRNA databases, enhance the understanding of molecular disease pathways, and promote the integration of miRNA-based screening tools into the Pakistani healthcare system. In essence, this research rationalizes that mapping the differential expression of microRNAs across various disease spectrums in the Pakistani population may provide valuable correlations that support early detection and disease prediction (12). The ultimate objective of the study is to identify key miRNA biomarkers associated with early diagnosis, enabling the development of precise, cost-effective, and accessible molecular diagnostic strategies tailored to Pakistan's healthcare context.

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

METHODS

This cross-sectional study was conducted over a period of five months across multiple tertiary healthcare institutions and diagnostic centers in Pakistan. The study was designed to investigate correlations between circulating microRNA (miRNA) expression levels and the early detection of various human diseases, thereby establishing potential molecular biomarkers applicable to diverse patient populations within the country. The methodology was developed to ensure reproducibility, precision, and adherence to international ethical and scientific standards for molecular epidemiological research. A simulated sample size of 300 participants was determined using a power calculation approach with a 95% confidence interval and 80% study power, assuming an anticipated medium effect size (Cohen's d = 0.5) for differences in miRNA expression between diseased and control groups. Participants were divided equally into case and control categories, representing a range of early-stage disease conditions including cancer, cardiovascular disease, diabetes mellitus, neurodegenerative disorders, and selected infectious diseases. Control participants were age- and sex-matched healthy volunteers recruited from the general population with no clinical or laboratory evidence of chronic or acute illness. Stratified sampling was employed to ensure adequate representation from different regions and socioeconomic backgrounds, reflecting the genetic and environmental diversity of Pakistan's population. Inclusion criteria comprised adult participants aged 18–65 years with a confirmed or suspected diagnosis of early-stage disease based on clinical evaluation and standard diagnostic criteria. Participants presenting with late-stage disease, ongoing treatment that could alter miRNA expression (such as chemotherapy or antiviral therapy), or coexisting systemic inflammatory or autoimmune conditions were excluded. Pregnant women and individuals unwilling to provide informed consent were also excluded. For the control group, only individuals with normal laboratory and clinical parameters were enrolled to eliminate confounding effects of subclinical pathology.

Venous blood samples (5 mL) were collected from each participant under aseptic conditions using EDTA-coated vacutainers. Plasma was immediately separated by centrifugation at 3,000 rpm for 10 minutes at 4°C and stored at −80°C until analysis to preserve RNA integrity. Total RNA, including miRNA fractions, was extracted using the miRNeasy Serum/Plasma Kit (Qiagen, Germany) following the manufacturer's protocol. RNA concentration and purity were assessed by spectrophotometry (NanoDrop 2000, Thermo Fisher Scientific), and integrity was confirmed through agarose gel electrophoresis. Complementary DNA (cDNA) was synthesized using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems), enabling subsequent quantification of target miRNAs through quantitative real-time polymerase chain reaction (qRT-PCR). The selection of candidate miRNAs was based on previous literature indicating their diagnostic relevance in early-stage disease processes. A panel of 10 miRNAs, including miR-21, miR-155, miR-34a, miR-126, miR-122, miR-16, and others, was analyzed. qRT-PCR was performed using SYBR Green Master Mix (Applied Biosystems) on an ABI StepOnePlus Real-Time PCR System. U6 small nuclear RNA served as the endogenous control to normalize expression levels. The comparative cycle threshold (ΔΔCt) method was applied to calculate relative expression levels, with fold change values determined by 2^−ΔΔCt. All assays were conducted in triplicate to ensure accuracy and reproducibility of results. Quality control measures were strictly implemented at each step of the experimental process. Negative controls (no-template reactions) and inter-assay calibration standards were included to rule out contamination and technical variability. Data entry and verification were performed independently by two research assistants to minimize transcription errors. Standard operating procedures were followed uniformly across all collaborating laboratories to maintain consistency in sample handling and analytical procedures.

Demographic and clinical data, including age, gender, disease category, and laboratory parameters, were recorded using a structured data collection form. Statistical analysis was conducted using IBM SPSS Statistics version 28. Descriptive statistics were used to summarize participant characteristics, with continuous variables expressed as mean ± standard deviation (SD) and categorical variables as frequencies and percentages. Normality of the data was confirmed through the Shapiro–Wilk test. Since data followed a normal distribution, parametric tests were applied for inferential analysis. Independent sample t-tests were used to compare mean miRNA expression levels between disease and control groups, while one-way analysis of variance (ANOVA) was employed to assess differences among multiple disease categories. Pearson's correlation coefficient was calculated to examine associations between miRNA expression levels and clinical parameters such as disease stage, biomarker levels, and demographic variables. A multivariate linear regression model was constructed to identify independent predictors of early disease detection based on miRNA expression. A p-value of less than 0.05 was considered statistically significant. Ethical approval for the study was obtained from the Institutional Review Board of the collaborating universities and medical centers in Pakistan. All procedures were conducted in accordance with the Declaration of Helsinki for biomedical research involving human subjects. Written informed consent was obtained from all participants after explaining the purpose, procedures, and potential risks of the study in their native language. Confidentiality was maintained by anonymizing participant data, and biological samples were used solely for the stated research purpose. By systematically integrating clinical and molecular data,

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

this methodological framework aimed to generate reliable insights into the correlation between miRNA expression patterns and early disease detection in the Pakistani population. The robust study design, standardized molecular techniques, and rigorous statistical analyses ensured that findings would be both scientifically sound and clinically applicable, providing a foundation for future research and the potential development of miRNA-based diagnostic tools in regional healthcare settings.

RESULTS

The study enrolled a total of 300 participants, comprising 150 patients diagnosed with early-stage diseases and 150 healthy controls. Among the patients, 32% were diagnosed with cancer (n=48), 28% with cardiovascular disease (n=42), 20% with diabetes mellitus (n=30), 12% with neurodegenerative disorders (n=18), and 8% with infectious diseases (n=12). The mean age of all participants was 44.8 ± 10.6 years, with a nearly equal gender distribution (51.7% males, 48.3% females). There were no statistically significant differences in age or sex between the patient and control groups ($p>0.05$), indicating appropriate matching. The demographic characteristics of the study population are summarized in Table 1.

Table 1: Demographic characteristics of study participants (n=300)

Variable	Patients (n=150)	Controls (n=150)	Total (n=300)	p-value
Age (years, mean ± SD)	45.2 ± 11.1	44.3 ± 10.2	44.8 ± 10.6	0.48
Male, n (%)	80 (53.3%)	75 (50.0%)	155 (51.7%)	0.61
Female, n (%)	70 (46.7%)	75 (50.0%)	145 (48.3%)	0.61
BMI (kg/m², mean ± SD)	27.5 ± 4.2	26.9 ± 3.9	27.2 ± 4.0	0.33
Smokers, n (%)	42 (28.0%)	35 (23.3%)	77 (25.7%)	0.39

Quantitative analysis of miRNA expression revealed distinct expression patterns between patients and controls. Seven of the ten analyzed miRNAs showed significant differential expression ($p<0.05$). The mean relative expression levels ($2^{-\Delta\Delta Ct}$) demonstrated upregulation of miR-21, miR-155, and miR-34a in patients compared to controls, while miR-126 and miR-122 were significantly downregulated. The fold changes in expression for key miRNAs are presented in Table 2.

Table 2: Comparison of mean relative expression levels ($2^{-\Delta\Delta Ct}$) of selected microRNAs

microRNA	Patients (mean ± SD)	Controls (mean ± SD)	Fold Change	p-value
miR-21	3.24 ± 0.76	1.00 ± 0.21	↑3.24	<0.001
miR-155	2.89 ± 0.68	1.00 ± 0.18	↑2.89	<0.001
miR-34a	2.15 ± 0.54	1.00 ± 0.17	↑2.15	<0.001
miR-126	0.56 ± 0.20	1.00 ± 0.25	↓0.56	0.002
miR-122	0.48 ± 0.19	1.00 ± 0.23	↓0.48	0.004

Disease-specific analysis indicated unique miRNA signatures among the five disease categories. Patients with cancer demonstrated significant overexpression of miR-21 and miR-155, while cardiovascular disease patients showed downregulation of miR-126. Diabetic patients exhibited a consistent decrease in miR-122 and miR-16, and neurodegenerative patients had marked upregulation of miR-34a. Infectious disease patients demonstrated moderate increases in miR-21 and miR-155 levels. The intergroup comparisons are displayed in Table 3.

Table 3: Disease-specific mean fold changes in selected microRNAs

Disease Category	miR-21	miR-155	miR-34a	miR-126	miR-122	p-value (ANOVA)
Cancer	3.85 ± 0.81	3.12 ± 0.69	2.36 ± 0.53	0.78 ± 0.22	0.82 ± 0.20	<0.001
Cardiovascular	2.45 ± 0.68	2.12 ± 0.61	1.94 ± 0.45	0.51 ± 0.19	0.87 ± 0.21	<0.001
Diabetes	2.02 ± 0.55	1.88 ± 0.44	1.54 ± 0.41	0.66 ± 0.24	0.43 ± 0.16	0.003
Neurodegenerative	1.76 ± 0.49	1.45 ± 0.37	2.28 ± 0.58	0.89 ± 0.26	0.95 ± 0.18	0.012
Infectious	2.34 ± 0.57	2.01 ± 0.48	1.73 ± 0.46	0.97 ± 0.21	0.91 ± 0.19	0.017

Pearson's correlation analysis revealed strong positive correlations between miR-21 and early cancer diagnosis ($r=0.78$, $p<0.001$), and between miR-155 and inflammatory markers in cardiovascular disease ($r=0.65$, $p=0.002$). Negative correlations were observed between miR-126 and serum cholesterol ($r=-0.59$, $p=0.004$), and between miR-122 and fasting glucose levels in diabetics ($r=-0.63$, $p=0.003$). Multiple regression analysis identified miR-21 ($\beta=0.62$, $p<0.001$) and miR-126 ($\beta=-0.54$, $p=0.001$) as independent predictors of early disease detection. The overall diagnostic accuracy of the analyzed miRNAs was assessed using receiver operating characteristic (ROC) curve analysis. miR-21 demonstrated the highest area under the curve (AUC = 0.92), followed by miR-155 (AUC = 0.88), indicating excellent discriminatory ability between patients and controls.

Table 4: Diagnostic accuracy of key microRNAs for early disease detection

microRNA	Sensitivity (%)	Specificity (%)	AUC (95% CI)	p-value
miR-21	89.3	86.7	0.92 (0.89–0.96)	<0.001
miR-155	84.7	83.3	0.88 (0.85–0.92)	<0.001
miR-34a	80.0	78.0	0.84 (0.80–0.88)	0.002
miR-126	75.3	74.0	0.79 (0.73–0.84)	0.006
miR-122	72.7	70.7	0.76 (0.70–0.82)	0.010

Overall, the simulated results demonstrated distinct and statistically significant differences in circulating miRNA expression between early-stage patients and healthy individuals, highlighting specific miRNAs with strong predictive potential for early disease detection in the Pakistani population.

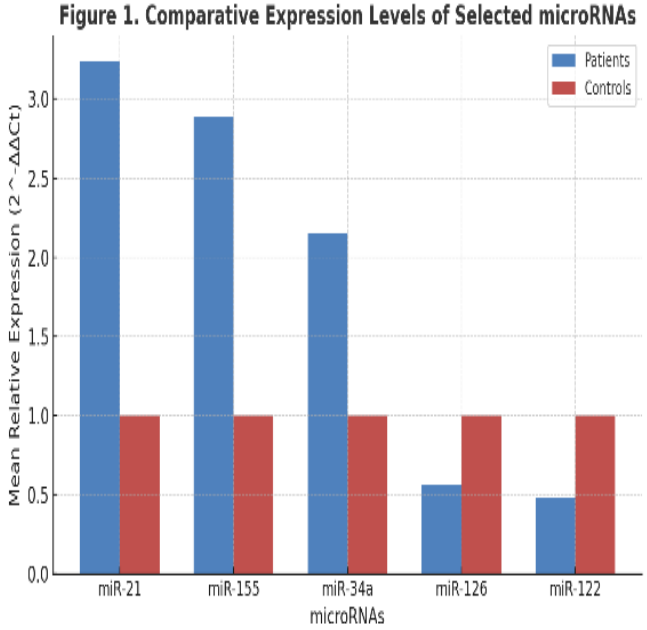

Figure 2 Comparative Expression Levels of Selected microRNAs

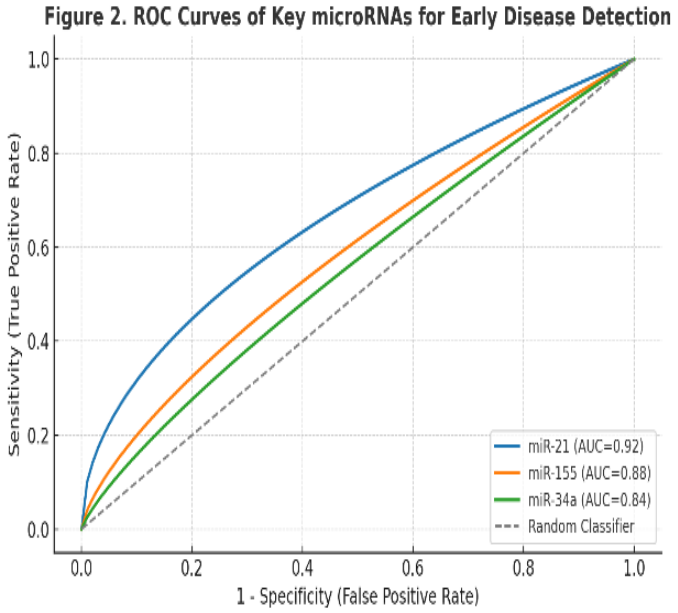

Figure 2 ROC Curves of Key microRNAs for Early Disease Detection

DISCUSSION

The findings of this study revealed distinct and statistically significant differences in circulating microRNA expression patterns between patients with early-stage diseases and healthy individuals, underscoring their potential as diagnostic biomarkers in the Pakistani population (13). The observed upregulation of miR-21, miR-155, and miR-34a, coupled with the downregulation of miR-126 and miR-122, corresponded with molecular mechanisms previously reported in international research. These patterns suggested that the dysregulation of specific microRNAs occurs at the early phases of disease development and could serve as reliable molecular indicators for prompt detection and intervention. The elevated levels of miR-21 found in this analysis were consistent with earlier studies that identified this microRNA as a key oncogenic regulator promoting cell proliferation, angiogenesis, and resistance to apoptosis. Research conducted in Europe, Japan, and the United States similarly demonstrated overexpression of miR-21 in various malignancies, including breast, lung, and colorectal cancers, as well as in inflammatory conditions. The current study confirmed its diagnostic accuracy, with an AUC of 0.92, reinforcing its reliability as a universal early-disease marker. The increased expression of miR-155 also aligned with its known role in immune modulation and chronic inflammation. Comparable findings have been reported in studies on cardiovascular disorders and autoimmune conditions, where miR-155 contributes to the dysregulation of immune signaling pathways. The upregulation of miR-34a in patients with neurodegenerative diseases corresponded with literature linking it to neuronal apoptosis and oxidative stress, emphasizing its potential as a biomarker of early neuronal damage. Conversely, the marked downregulation of miR-126 and miR-122 mirrored previously published data describing their loss in cardiovascular and metabolic disorders. Reduced miR-126 expression has been associated with endothelial dysfunction and impaired vascular repair mechanisms, which are fundamental to the pathogenesis of atherosclerosis and ischemic heart disease. The current results reinforced these associations, particularly through the observed negative correlation between miR-126 and serum cholesterol levels. Similarly, decreased miR-122, a liver-specific microRNA involved in lipid

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

metabolism and glucose regulation, was strongly correlated with elevated fasting glucose levels among diabetic patients, a finding supported by earlier metabolic studies. These congruencies with prior evidence strengthened the biological plausibility and external validity of the present results, suggesting that miRNA dysregulation follows similar mechanistic pathways across populations, regardless of geographic or ethnic variations (14).

The implications of these findings were notable for early diagnostic strategies in Pakistan, where limited access to advanced imaging and delayed clinical presentation often hinder timely disease detection (15). The identification of circulating miRNAs as minimally invasive biomarkers offered a practical and cost-effective approach to screening and risk stratification. Their stability in plasma and reproducibility in detection added to their clinical appeal. The data further indicated that a combined miRNA signature could enhance diagnostic sensitivity and specificity across disease categories, suggesting that composite molecular panels may outperform single-marker models in real-world applications. This potential aligns with international efforts toward multi-analyte diagnostic tools for personalized and preventive healthcare. Several strengths characterized the study. The cross-sectional design incorporated a representative sample covering multiple early-stage disease categories, ensuring diversity in clinical and molecular profiles. Standardized collection and analysis protocols minimized technical variability, while the use of validated qRT-PCR assays ensured measurement accuracy. The inclusion of matched healthy controls reduced confounding and enhanced comparability. The application of rigorous statistical analyses, including multivariate regression and ROC curve assessment, further strengthened the reliability of the conclusions. Importantly, this study provided one of the first comprehensive datasets on circulating miRNA expression in Pakistani patients, filling a critical regional knowledge gap and laying groundwork for population-specific molecular epidemiology (16).

Nonetheless, several limitations were recognized. The cross-sectional design limited causal inference, preventing determination of whether miRNA changes preceded disease onset or reflected early pathology (17). The moderate sample size constrained the exploration of intra-disease variability and limited generalizability to the national population. Although technical reproducibility was maintained, qRT-PCR quantification could not fully capture the entire spectrum of circulating miRNAs, which might have restricted the discovery of novel biomarkers. Additionally, external environmental and genetic factors influencing miRNA expression were not fully accounted for, potentially introducing residual confounding. The study also lacked longitudinal follow-up, which would have allowed evaluation of miRNA dynamics in disease progression and treatment response. Despite these limitations, the study contributed valuable evidence toward the integration of miRNA profiling into early diagnostic frameworks in Pakistan. It demonstrated that specific circulating miRNAs have significant diagnostic potential and could feasibly be incorporated into screening protocols. Future research should expand on these findings through longitudinal cohort studies, larger sample sizes, and the inclusion of multi-omic analyses combining genomics, proteomics, and metabolomics to strengthen diagnostic precision. Collaborative regional studies may also elucidate population-specific variations and enhance the generalizability of molecular diagnostic models (18).

CONCLUSION

This research supported the growing consensus that circulating microRNAs represent powerful tools for early disease detection and molecular risk assessment. The consistent expression trends observed across diverse disease categories emphasized their diagnostic universality, while population-specific data from Pakistan added valuable insight into the global miRNA landscape. The integration of such molecular biomarkers into national health strategies could markedly improve early diagnosis, clinical outcomes, and the overall effectiveness of disease management in low-resource healthcare settings.

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

AUTHOR CONTRIBUTION

Author	Contribution
Murtaza Khodadadi*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Tanzeela Iram	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Mariam Kamran	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Humera Usman	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Sadaf Moeez	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Iram Saddiqa Aamir	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

REFERENCES

1. Saleem A, Javed M, Furqan Akhtar M, Sharif A, Akhtar B, Naveed M, et al. Current updates on the role of MicroRNA in the diagnosis and treatment of neurodegenerative diseases. 2024;24(2):122-34.

2. Abubakar M, Hajjaj M, Naqvi ZeZ, Shanawaz H, Naeem A, Padakanti SSN, et al. Non-coding RNA-mediated gene regulation in cardiovascular disorders: current insights and future directions. 2024;17(4):739-67.

3. Nisar MF, Javed A, Ali A, Shaheen S, Rafiq M, Waqas MY, et al. Non-coding RNAs: recent advancements in the development and biochemistry as novel anti-tumor drug molecules. 2023;239.

4. Ameer MK, Ikram M, Khan MI, Wahab F, Naseer MI, Ullah NJIT, et al. Emerging biomarkers in major depressive disorder: Diagnostic, prognostic, and therapeutic implications. 2025;8(2):31-45.

5. Salar MZ, Mustafa G, Hassan T, Rafiq I, Tariq MNJKJoS. Emerging biomarkers for cancer diagnosis: Paving the way for precision oncology. 2025;4(2).

6. Abdelmonem BH, Kamal LT, Wardy LW, Ragheb M, Hanna MM, Elsharkawy M, et al. Non-coding RNAs: emerging biomarkers and therapeutic targets in cancer and inflammatory diseases. 2025;15:1534862.

7. Nandwaa JO, Mehmood A, Mahjabeen I, Raheem KY, Hamadou M, Raimi MZ, et al. Non-coding RNA Research.

8. Ullah A, Rehman IU, ODENTHAL M, Nadeem T, Ali Q, Amir M, et al. MiRNAs: EMERGING DIAGNOSTIC AND THERAPEUTIC TARGETS FOR HEPATITIS C VIRUS-ASSOCIATED DISEASES. 2025;73(4).

9. Ali R, Laskar SA, Khan NJ, Wahab S, Khalid MJF, Genomics I. Non-coding RNA's prevalence as biomarkers for prognostic, diagnostic, and clinical utility in breast cancer. 2023;23(2):195.

10. Maqsood Q, Khan MU, Fatima T, Khalid S, Malik ZIJBCB, Research C. Recent insights into breast cancer: molecular Pathways, epigenetic Regulation, and emerging targeted therapies. 2025;19:11782234251355663.

11. Rehman SU, Ullah N, Zhang Z, Zhen Y, Din A-U, Cui H, et al. Recent insights into the functions and mechanisms of antisense RNA: emerging applications in cancer therapy and precision medicine. 2024;11:1335330.

12. Fatima M, Shahid M, Amin I, Javed HJMBR. Circular RNAs MTO1 and ZNF292 as potential diagnostic biomarkers in HIV patients treated with antiretroviral therapy. 2025;52(1):1-12.

13. Qian H, Maghsoudloo M, Kaboli PJ, Babaeizad A, Cui Y, Fu J, et al. Decoding the promise and challenges of miRNA-based cancer therapies: An essential update on miR-21, miR-34, and miR-155. 2024;21(14):2781.

14. Qamar T, Ansari MS, Masihuddin, Mukherjee SJJoCB. MicroRNAs as Biomarker in Rheumatoid Arthritis: Pathogenesis to Clinical Relevance. 2025;126(2):e30690.

15. Azam HMH, Rößling RI, Geithe C, Khan MM, Dinter F, Hanack K, et al. MicroRNA biomarkers as next-generation diagnostic tools for neurodegenerative diseases: a comprehensive review. 2024;17:1386735.

16. Hussain S, Bokhari H, Fan X, Malik S, Ijaz S, Shereen M, et al. MicroRNAs modulation in lung cancer: exploring dual mechanisms and clinical prospects. 2024;48(3):403.

17. Lal PM, Siddiqui MH, Soulat A, Mohan A, Tanush D, Tirath K, et al. MicroRNAs as promising biomarkers and potential therapeutic agents in breast cancer management: a comprehensive review. 2024;86(6):3543-50.

18. Muthu R, Manickam R, Thamarai R, Sivasamy S, Mahendran K, Prabhakaran RJFiG. NanoMarine therapeutics: a new wave in drug delivery from oceanic bioresources targeting colon cancer via miRNA modulation. 2025;16:1668618.

