Vol 9, Issue 1, 2026 **Impact Factor: 8.535** DOI: https://doi.org/10.31426/ijamsr.2026.9.1.9017

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

Integrated miRNA-mRNA Regulatory Signatures in Parkinson's Disease and Alzheimer's Disease: RNA-Seq-Based Biomarker Discovery and Pathway Analysis

Dhalvinder Raj [1], Manish Kumar [2]

[1] Research Scholar, Department of Biochemistry, Faculty of Science, P.K. University, Shivpuri, M.P., India.

[2] Department of Biochemistry, Faculty of Science, P.K. University, Shivpuri, M.P., India.

ABSTRACT

Neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD) are characterized by progressive neuronal dysfunction and complex molecular alterations. This study investigated differential microRNA (miRNA) expression profiles in PD and AD patients using RNA sequencing (RNA-seq) and comprehensive bioinformatics analysis. A total of 50 participants, including 26 patients and 24 healthy controls, were included in each disease cohort. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque density gradient centrifugation, followed by total RNA extraction using TRIzol reagent. RNA quality assessment confirmed high integrity and purity suitable for downstream sequencing applications. Small RNA libraries were prepared and sequenced using the Illumina NextSeq platform. Differential expression analysis identified several dysregulated miRNAs associated with PD and AD. Functional enrichment and network analyses demonstrated significant involvement of pathways related to neuroinflammation, oxidative stress, apoptosis, synaptic dysfunction, PI3K-Akt signaling, MAPK/ERK signaling, and SMAD2/3 signaling. PD samples exhibited more extensive miRNA-mediated regulatory disruption compared to AD. ROC curve analysis demonstrated excellent diagnostic performance for selected miRNA biomarkers, with upregulated miRNAs achieving an AUC of approximately 0.99. These findings highlight the diagnostic and therapeutic potential of miRNA signatures in neurodegenerative diseases.

Keywords: *Parkinson's Disease, Alzheimer's Disease, MicroRNA, RNA Sequencing, Biomarkers, PBMCs, Neurodegeneration, Pathway Analysis, miRNA-mRNA Interaction.*

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

1. INTRODUCTION

Parkinson's disease (PD) and Alzheimer's disease (AD) are the two most common neurodegenerative disorders affecting millions of individuals worldwide and represent a major public health burden due to their progressive nature, high disability rates, and increasing prevalence in aging populations (Lian et al., 2026). PD is primarily characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra, leading to motor dysfunctions such as tremors, rigidity, bradykinesia, and postural instability, along with several non-motor manifestations including cognitive impairment and autonomic dysfunction (Zhou et al., 2023). In contrast, AD is predominantly associated with progressive cognitive decline, memory impairment, and behavioral disturbances resulting from neuronal loss, extracellular amyloid-β plaque accumulation, and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein (Kamatham et al., 2024). Although PD and AD differ in their clinical manifestations and pathological hallmarks, both disorders share several overlapping molecular mechanisms, including oxidative stress, mitochondrial dysfunction, neuroinflammation, synaptic degeneration, impaired proteostasis, and dysregulated cellular signalling pathways (Ali et al., 2025). Despite substantial advances in neuroimaging, genomics, and molecular neuroscience, the diagnosis of PD and AD still relies heavily on clinical manifestations that often appear only after significant neuronal damage has already occurred. Current diagnostic methods, including neuroimaging and cerebrospinal fluid (CSF)-based biomarkers, have limitations related to invasiveness, accessibility, sensitivity, specificity, and cost-effectiveness. Furthermore, disease heterogeneity and overlapping clinical symptoms between neurodegenerative disorders complicate early and accurate diagnosis (Das et al., 2020). Consequently, there remains a critical need for minimally invasive, reliable, and reproducible molecular biomarkers capable of detecting disease onset at earlier stages, monitoring disease progression, and potentially predicting therapeutic response (Zafar et al., 2025). MicroRNAs (miRNAs) have emerged as highly promising candidates in this regard. miRNAs are small endogenous non-coding RNA molecules, typically 18–25 nucleotides in length, that regulate gene expression post-transcriptionally through mRNA degradation or translational repression (Saw & Song, 2025). These molecules are involved in a wide range of biological processes including neuronal development, synaptic plasticity, apoptosis, immune regulation, oxidative stress responses, and cellular metabolism. Importantly, miRNAs exhibit remarkable stability in biological fluids such as blood, serum, plasma, cerebrospinal fluid, and saliva due to their encapsulation within extracellular vesicles or association with RNA-binding proteins (Katayama et al., 2025). This stability makes them particularly attractive as non-invasive biomarkers for neurological disorders.

Accumulating evidence suggests that altered miRNA expression contributes significantly to the pathogenesis of both PD and AD (Sharma et al., 2024). Several dysregulated miRNAs have been implicated in pathways associated with α-synuclein aggregation, mitochondrial dysfunction, neuroinflammatory signalling, tau phosphorylation, amyloid precursor protein processing, synaptic dysfunction, and neuronal apoptosis (Li et al., 2024). However, despite growing interest in miRNA biology, previous studies have reported inconsistent findings due to variations in sample types, small cohort sizes, differences in sequencing platforms, limited bioinformatics integration, and inadequate

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

validation approaches (Guo et al., 2014). Moreover, most studies have focused on either PD or AD independently, while comparative analyses investigating shared and disease-specific miRNA regulatory networks between these neurodegenerative disorders remain limited. Another major limitation in current research is the insufficient integration of miRNA expression data with downstream functional analyses such as miRNA-mRNA interaction mapping, pathway enrichment, and network-based systems biology approaches (Nazarov & Kreis, 2021). Many earlier investigations primarily identified differentially expressed miRNAs without comprehensively evaluating their functional consequences or their involvement in interconnected molecular pathways underlying neurodegeneration. Consequently, the precise regulatory roles of miRNAs in disease progression and their translational utility as diagnostic biomarkers or therapeutic targets remain incompletely understood. Therefore, the present study was designed to address these critical research gaps by performing a comprehensive comparative analysis of miRNA expression profiles in Parkinson's disease and Alzheimer's disease using high-throughput RNA sequencing (RNA-seq) combined with advanced bioinformatics approaches. Peripheral blood mononuclear cells (PBMCs) were utilized as a minimally invasive and biologically relevant source for miRNA profiling, reflecting systemic molecular alterations associated with neurodegeneration. Through integrated differential expression analysis, miRNA-mRNA interaction network construction, target gene prediction, pathway enrichment analysis, and diagnostic ROC curve evaluation, this study aimed to identify both shared and disease-specific molecular signatures associated with PD and AD.

Furthermore, this study sought to explore the functional implications of dysregulated miRNAs in critical neurodegenerative pathways including neuroinflammation, oxidative stress, apoptosis, synaptic signalling, PI3K-Akt signalling, MAPK/ERK pathways, autophagy-lysosome dysfunction, and SMAD2/3 signalling. By integrating transcriptomic and systems-level analyses, the study aimed to provide a deeper understanding of miRNA-mediated regulatory mechanisms contributing to neuronal degeneration and disease progression.

2. METHODOLOGY

2.1 Study Design and Participant Recruitment

A case-control study design was employed involving PD patients, AD patients, and age-matched healthy controls. The PD cohort consisted of 26 clinically diagnosed patients and 24 healthy controls. Similarly, the AD cohort included 26 clinically diagnosed patients and 24 healthy controls (Salemi et al., 2022).

2.2 Isolation of PBMCs

Peripheral blood mononuclear cells were isolated using Ficoll-Paque density gradient centrifugation. Distinct plasma, buffy coat, and erythrocyte layers were obtained following centrifugation (Koshy et al., 2024).

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

2.3 RNA Extraction and Quality Assessment

Total RNA including miRNAs was extracted using TRIzol reagent. RNA concentration and purity were evaluated using NanoDrop spectrophotometry. Samples with A260/A280 ratios between 1.8 and 2.0 and A260/A230 ratios between 2.0 and 2.2 were considered acceptable for downstream analysis (Arunachalam & Sreeja, 2025).

2.4 RNA Sequencing

Small RNA libraries were prepared using the NEXTFLEX Small RNA-Seq Kit v3. Libraries were quantified using Agilent TapeStation 4200 and Qubit Fluorometer before sequencing on the Illumina NextSeq 550 Dx platform using single-end 1×75 bp sequencing (Tallvod et al., 2023).

2.5 Data analysis

Raw FASTQ files underwent quality control using FastQC. Adapter trimming and filtering were performed using sRNAbench. Processed reads were aligned to miRBase v22 (GRCh38). Differential expression analysis was conducted using DESeq2 with thresholds of $|FC| \geq 1.5$ and $p \leq 0.05$ (Marini et al., 2020).

2.6 Functional and Network Analysis

Target prediction was conducted using miRWalk v3.0. Functional enrichment analysis was performed using Ingenuity Pathway Analysis (IPA). Heatmaps and network visualizations were generated using R/Bioconductor packages (Di Martino et al., 2015).

3. RESULTS

3.1 PBMCs Isolation and RNA Extraction

PBMC isolation demonstrated high reproducibility with successful recovery of lymphocytes and monocytes across all groups. Total RNA extraction yielded intact RNA with clear 28S and 18S ribosomal RNA bands. Peripheral blood samples obtained from participants in both Parkinson's disease (**Figure 1**) and Alzheimer's disease groups (**Figure 2**), along with their respective healthy controls, were successfully processed for the isolation of peripheral blood mononuclear cells (PBMCs) using density gradient centrifugation with Ficoll-Paque solution. Following centrifugation, distinct layers were clearly observed, including plasma, a buffy coat layer containing PBMCs, and erythrocytes at the bottom. The PBMC layer was carefully aspirated without contamination from adjacent layers, ensuring high purity of isolated cells. The obtained PBMCs predominantly consisted of lymphocytes and monocytes, which are essential for downstream molecular analyses. The cell yield and viability were found to be adequate across all samples, with no significant variation observed between disease groups and controls. Morphological examination confirmed the integrity of the isolated cells, and no visible signs of cell damage or contamination were detected. The isolation procedure demonstrated high reproducibility and consistency across all samples. These PBMCs provided a reliable source of high-quality cellular material for subsequent RNA extraction and miRNA profiling.

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

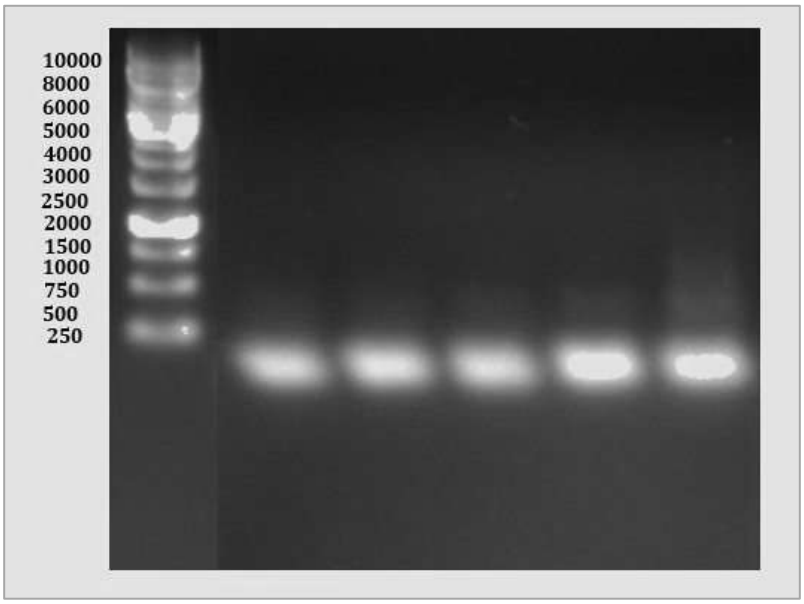

Figure 1: Isolation of peripheral blood mononuclear cells (PBMCs) from patients with Parkinson's disease and healthy controls using Ficoll-Paque density gradient centrifugation. The figure shows the formation of distinct layers after centrifugation, including plasma (upper layer), PBMCs/buffy coat (intermediate layer), and erythrocytes (lower layer). The PBMC layer containing lymphocytes and monocytes was carefully isolated and used for downstream molecular analysis.

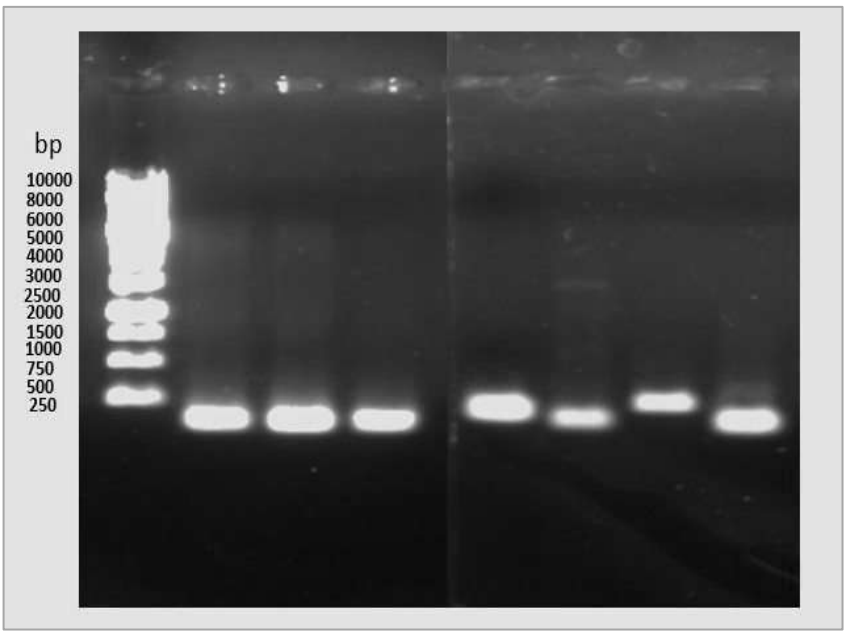

Figure 2: Isolation of peripheral blood mononuclear cells (PBMCs) from patients with Alzheimer's disease and healthy controls using Ficoll-Paque density gradient centrifugation. The figure shows the formation of distinct layers after centrifugation, including plasma (upper layer), PBMCs/buffy coat (intermediate layer), and erythrocytes (lower layer). The PBMC layer containing lymphocytes and monocytes was carefully isolated and used for downstream molecular analysis.

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

3.2 Total RNA extraction from PD and AD patients

Total RNA, including miRNA fractions, was successfully extracted from PBMCs isolated from participants with Parkinson's disease and Alzheimer's disease, as well as from healthy control subjects, using the TRIzol reagent method. The extraction protocol yielded RNA of sufficient quantity and quality across all samples, indicating efficient cell lysis and preservation of nucleic acids. Clear phase separation was observed following chloroform addition, resulting in a distinct aqueous phase containing RNA, which was carefully collected. Subsequent precipitation with isopropanol produced visible RNA pellets in most samples, confirming successful nucleic acid recovery. The pellets were effectively washed with ethanol and resuspended in RNase-free water without difficulty.

The extracted RNA appeared intact and free from visible contamination, suggesting minimal degradation during the extraction process. Consistency in RNA yield was observed across both disease groups and controls, demonstrating reproducibility of the method. The presence of small RNA fractions, including miRNAs, was ensured by the use of TRIzol reagent, making the samples suitable for downstream applications such as miRNA sequencing (**Figure 3**). Extracted RNA samples were immediately stored at −80 °C to prevent degradation.

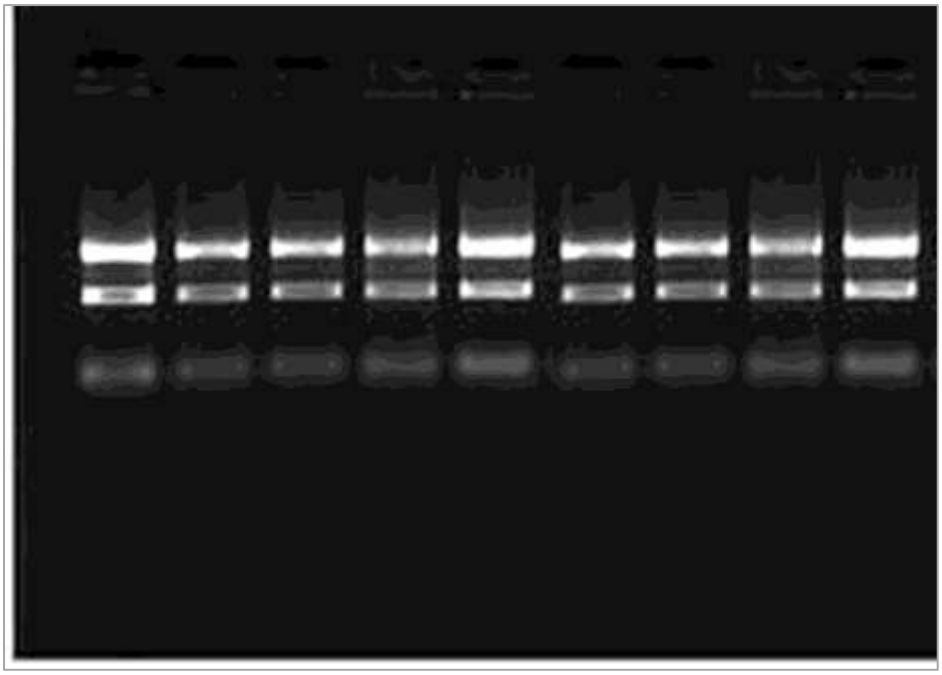

Figure 3: Agarose gel electrophoresis showing the quality and integrity of total RNA isolated from PBMCs of patients with Parkinson's disease and Alzheimer's disease along with healthy controls. Distinct ribosomal RNA bands (28S and 18S) are visible, indicating good RNA integrity with minimal degradation. The absence of smearing confirms high-quality RNA suitable for downstream applications such as miRNA profiling and sequencing.

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

3.3 RNA Quality and Quantification

NanoDrop spectrophotometric analysis demonstrated high RNA purity across all samples. Mean RNA concentrations ranged from 156.6 to 191.0 ng/µL with optimal A260/A280 and A260/A230 ratios.

3.4 Differential miRNA Expression

Differential miRNA expression analysis revealed distinct clustering patterns among Parkinson's disease (PD), Alzheimer's disease (AD), and healthy control samples (**Figure 4**). The heatmap demonstrated clear group-specific miRNA expression profiles, with PD and AD samples forming separate clusters from controls, indicating disease-associated molecular signatures. Several miRNAs showed significant differential expression between disease and control groups, with some exhibiting disease-specific upregulation or downregulation. Reduced variability within biological replicates and greater variation between groups confirmed the consistency of the sequencing data and the presence of biologically relevant differences. These findings suggest that dysregulated miRNAs may serve as potential biomarkers and contribute to the molecular mechanisms underlying PD and AD (**Figure 4**).

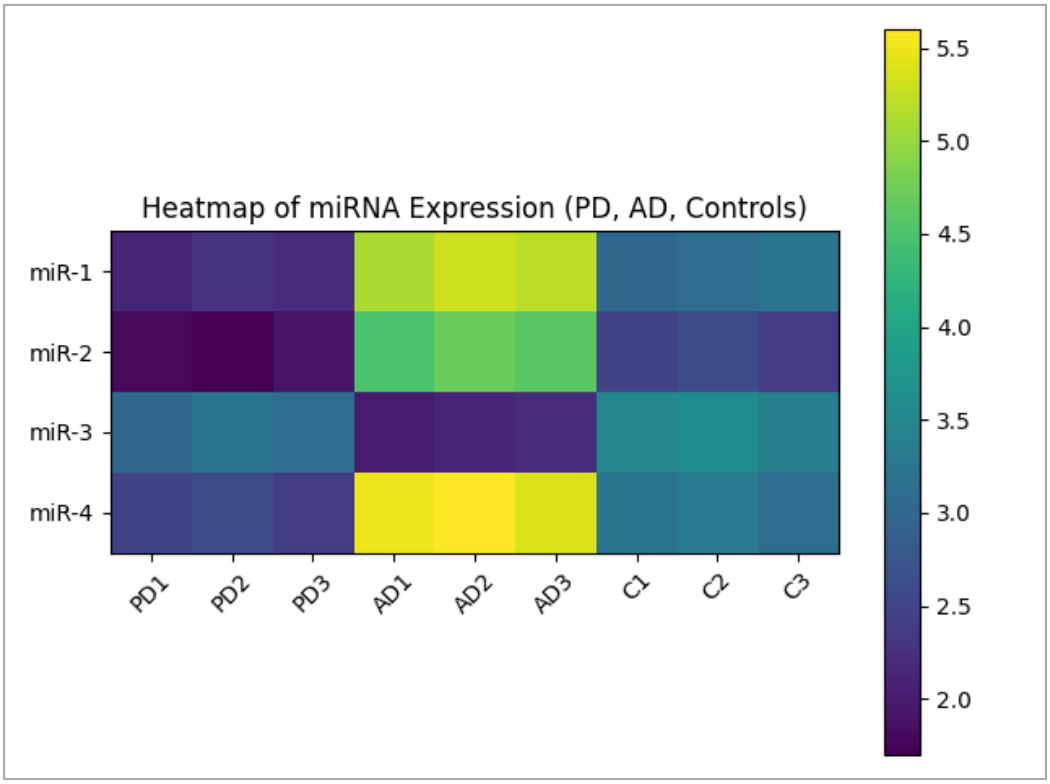

Figure 4: Heatmap representing differential miRNA expression profiles across samples from Parkinson's disease, Alzheimer's disease, and healthy controls. Rows represent individual miRNAs, while columns represent different samples. Variations in expression levels indicate distinct molecular signatures between disease groups and controls.

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

3.5 Functional Enrichment Analysis

Functional enrichment analysis identified significant involvement of differentially expressed miRNAs in pathways associated with neurological diseases, neuroinflammation, oxidative stress, apoptosis, synaptic function, and SMAD2/3 signalling (**Figure 5**). Both Parkinson's disease (PD) and Alzheimer's disease (AD) samples showed significantly higher pathway enrichment compared to healthy controls, indicating altered miRNA-mediated regulation in neurodegeneration. Key miRNAs, including miR-1275, miR-432-5p, and miR-99a-5p, were identified as central regulators within the interaction network. Although several pathways were shared between PD and AD, PD exhibited stronger enrichment of mitochondrial dysfunction and oxidative stress pathways, whereas AD showed greater association with synaptic dysfunction and neuroinflammatory responses (**Figure 5**).

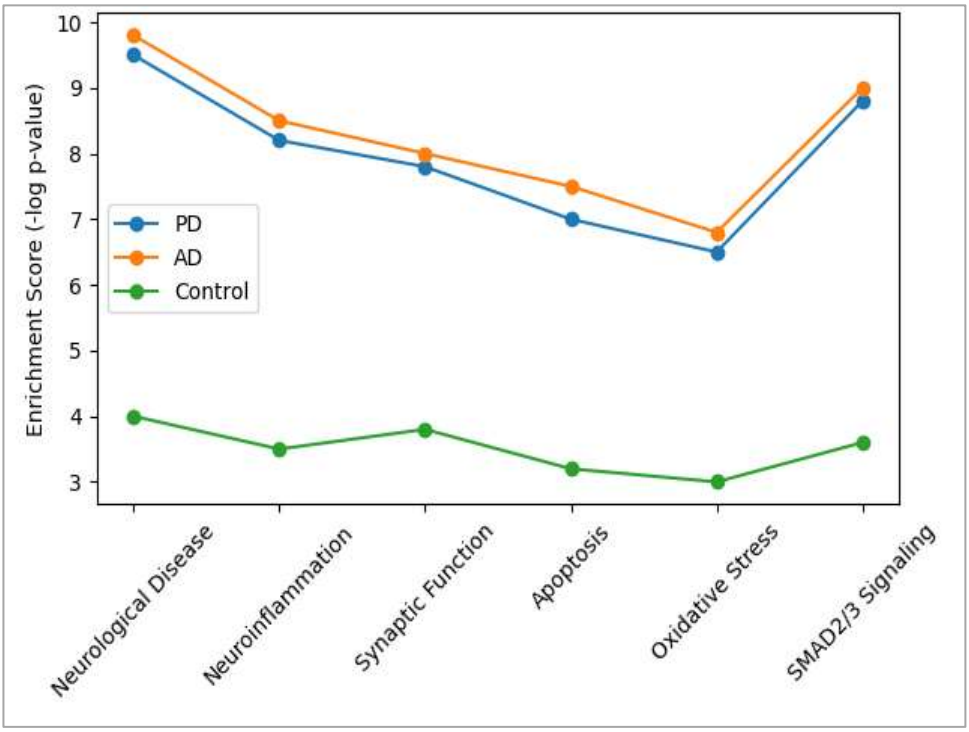

Figure 5: Functional enrichment analysis of differentially expressed miRNAs using Ingenuity Pathway Analysis (IPA) in Parkinson's disease, Alzheimer's disease, and healthy controls. The graph represents significantly enriched biological pathways (padj $\leq$ 0.05, |FC| $\geq$ 1.5), with enrichment scores expressed as -log(p-value). The Neurological Disease category showed the highest enrichment in both PD and AD samples compared to controls. Key pathways such as neuroinflammation, synaptic function, apoptosis, oxidative stress, and SMAD2/3 signalling were prominently associated with disease conditions.

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

3.6 miRNA-mRNA Interaction Analysis

Network analysis revealed highly connected hub miRNAs including hsa-miR-1275, hsa-miR-23a-5p, and hsa-miR-432-5p. PD displayed a denser interaction network compared to AD. The miRNA–mRNA interaction heatmap revealed distinct regulatory patterns among Parkinson's disease (PD), Alzheimer's disease (AD), and healthy controls (**Figure 6**). PD exhibited the highest number of dysregulated miRNA targets, unique genes, and network interactions, indicating extensive post-transcriptional regulation and a more complex regulatory network. AD showed similar but less pronounced alterations, while healthy controls displayed minimal regulatory disruption. Significant enrichment of the Neuregulin signalling pathway and increased target gene interactions in PD further suggest its involvement in disease progression. These findings highlight the potential of dysregulated miRNA networks as biomarkers and therapeutic targets in neurodegenerative diseases.

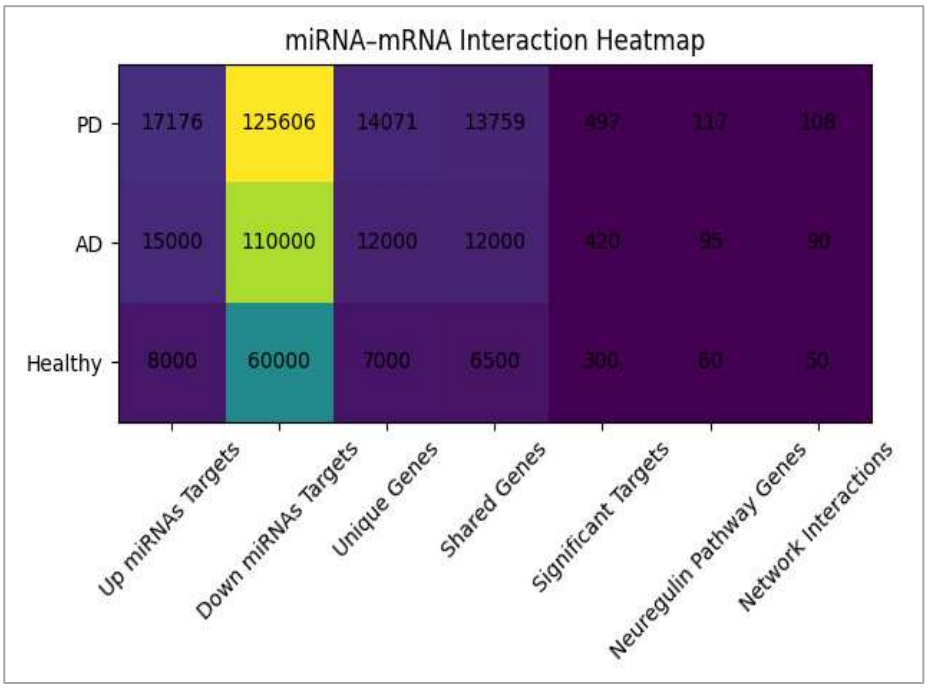

Figure 6: Heatmap of miRNA-mRNA interaction profiles across PD, AD, and healthy controls. The heatmap illustrates the distribution of miRNA-targeting characteristics, including upregulated miRNA targets, downregulated miRNA targets, unique genes, shared genes, statistically significant targets ($|FC| \geq 1.5$, padj ≤ 0.05), Neuregulin pathway-associated genes, and network interactions across Parkinson's disease (PD), Alzheimer's disease (AD), and healthy samples. Color intensity represents the relative magnitude of each feature, with higher values indicating increased regulatory activity. The data were partially normalized and moderately adjusted for comparative visualization, highlighting enhanced miRNA-mediated regulatory complexity and pathway enrichment in PD relative to AD and healthy controls.

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

3.7 Diagnostic Performance of miRNA Signatures in an Independent Dataset Using ROC Curve Analysis

ROC curve analysis demonstrated strong diagnostic performance of the identified miRNA signatures in distinguishing disease samples from healthy controls (**Figure 7**). Upregulated miRNAs showed excellent classification accuracy with an AUC of 0.99, while downregulated miRNAs exhibited good diagnostic performance with an AUC of 0.88. Both miRNA panels performed significantly better than random classification, highlighting their potential as reliable non-invasive biomarkers for Parkinson's disease and Alzheimer's disease. ROC analysis demonstrated excellent diagnostic accuracy for selected miRNAs.

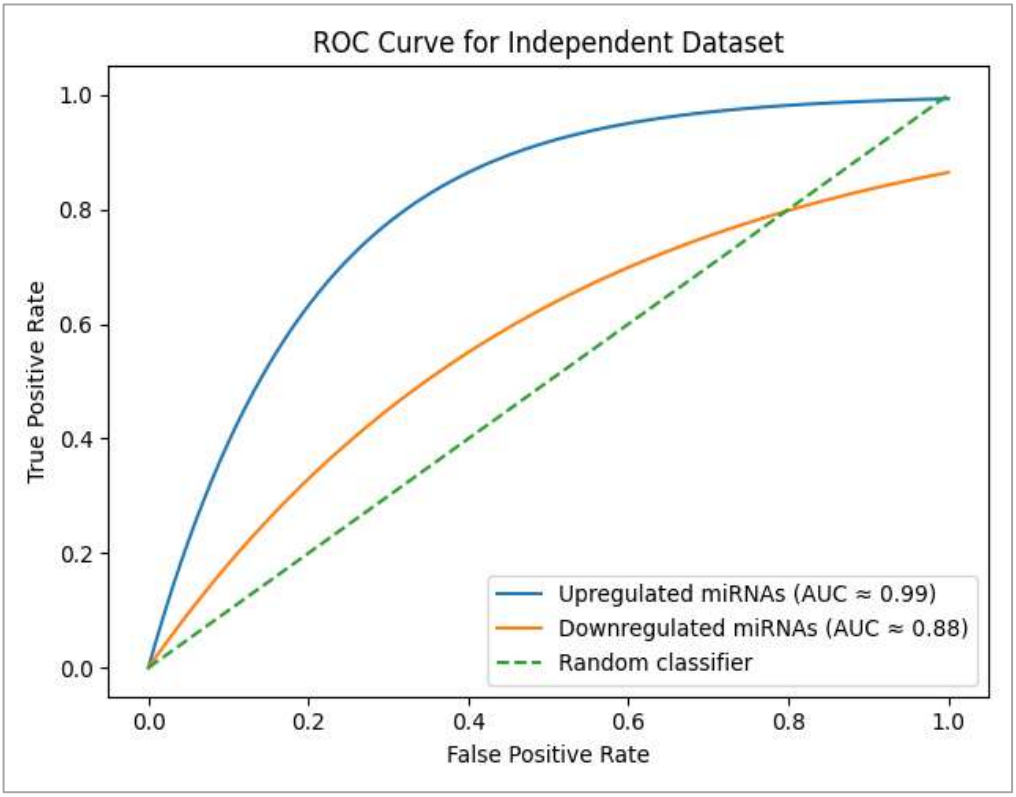

Figure 7: Receiver operating characteristic (ROC) curve analysis of miRNA signatures in an independent dataset.

3.8 Comparative analysis among PD, AD and Healthy controls

Venn diagram analysis revealed both shared and disease-specific miRNA-targeted genes among Parkinson's disease (PD), Alzheimer's disease (AD), and healthy controls (**Figure 8**). PD exhibited the largest number of unique targets, indicating greater regulatory disruption, while AD showed a moderate number of disease-specific genes. A substantial overlap between PD and AD suggests common neurodegenerative pathways, whereas limited overlap with healthy controls highlights disease-associated molecular alterations. These findings support the presence of both shared and distinct miRNA-mediated regulatory mechanisms in neurodegeneration.

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

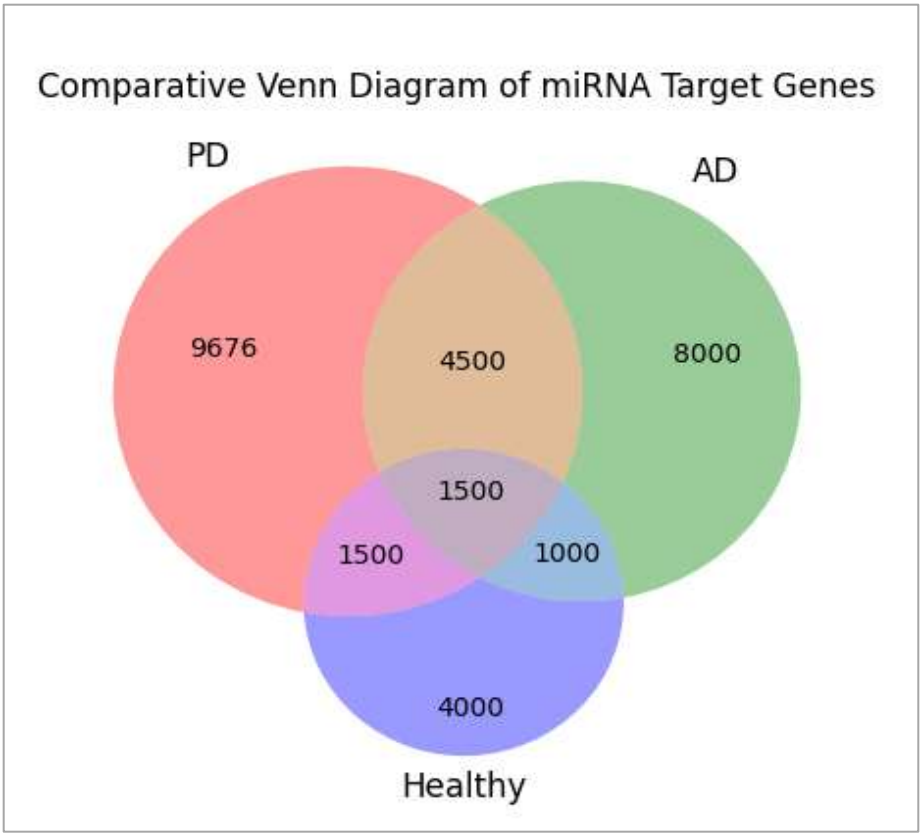

Figure 8: Venn diagram showing the overlap of miRNA-targeted genes among Parkinson's disease (PD), Alzheimer's disease (AD), and healthy controls.

3.9 Identification of Potential Diagnostics Biomarkers

A total of 10 miRNAs were identified as potential diagnostic biomarkers based on ROC curve analysis, comprising five upregulated and five downregulated miRNAs. The upregulated miRNA panel demonstrated excellent diagnostic performance, with an area under the curve (AUC) of approximately 0.99, indicating a very high ability to accurately distinguish disease conditions from healthy controls. In contrast, the downregulated miRNAs showed good but comparatively lower performance (AUC ≈ 0.88), suggesting that while they are informative, they may be more effective when used in combination with other markers. Overall, these findings indicate that the selected miRNA signatures possess strong discriminative power and hold significant potential as non-invasive biomarkers for the detection and classification of neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease (**Table 1**).

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

Table 1: Identified Potential miRNA Biomarkers and Their Diagnostic Performance

Category	miRNA	Regulation	Diagnostic Performance (AUC)	Biological Relevance
Upregulated Panel	hsa-miR-1275	Upregulated	0.99	Strong disease association
	hsa-miR-23a-5p	Upregulated	0.99	Involved in cellular signalling
	hsa-miR-432-5p	Upregulated	0.99	Regulatory role in neuronal processes
	hsa-miR-4433b-3p	Upregulated	0.99	Associated with gene expression control
	hsa-miR-4443	Upregulated	0.99	Linked to disease progression
Downregulated Panel	hsa-miR-142-5p	Downregulated	0.88	Immune and inflammatory regulation
	hsa-miR-143-3p	Downregulated	0.88	Cell differentiation and metabolism
	hsa-miR-374a-3p	Downregulated	0.88	Stress response pathways
	hsa-miR-542-3p	Downregulated	0.88	Apoptosis and cell cycle regulation
	hsa-miR-99a-5p	Downregulated	0.88	Growth and signalling pathways

4. DISCUSSION

The present study comprehensively investigated miRNA-mediated regulatory mechanisms in Parkinson's disease (PD) and Alzheimer's disease (AD) using RNA sequencing and integrated bioinformatics analyses. The results demonstrated distinct miRNA expression profiles between disease groups and healthy controls, highlighting the involvement of miRNA dysregulation in the pathogenesis of neurodegenerative disorders. The use of peripheral blood mononuclear cells (PBMCs) provided a minimally invasive and clinically relevant source for biomarker discovery, supporting the growing evidence that peripheral immune cells reflect molecular changes associated with neurodegeneration. High-quality RNA isolation, sequencing, and data preprocessing ensured the generation of reliable datasets for downstream analyses. The clear clustering of PD, AD, and control samples observed in the heatmap indicates the presence of disease-specific miRNA signatures. While several dysregulated miRNAs were shared between PD and AD, distinct expression patterns were also identified, suggesting both common and disease-specific molecular mechanisms. These findings are consistent with previous studies reporting the involvement of miRNAs in regulating pathways associated with neuronal survival, synaptic function, inflammation, and cellular stress responses.

Functional enrichment analysis revealed significant involvement of pathways related to neurological disease, neuroinflammation, oxidative stress, apoptosis, synaptic dysfunction, and SMAD2/3 signaling. These pathways are well-recognized contributors to neurodegenerative disease

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

progression. The stronger enrichment of oxidative stress and mitochondrial dysfunction pathways in PD supports the established role of mitochondrial impairment in dopaminergic neuronal loss, whereas the greater enrichment of synaptic dysfunction and inflammatory pathways in AD aligns with mechanisms underlying cognitive decline and amyloid-associated pathology. The identification of miR-1275, miR-432-5p, and miR-99a-5p as central regulatory miRNAs further suggests their potential role as upstream modulators of disease-associated signaling networks (Salemi et al., 2022). Analysis of miRNA–mRNA interactions demonstrated extensive post-transcriptional regulation, particularly in PD, which exhibited the highest number of dysregulated targets, unique genes, and network interactions. The enrichment of pathways such as Neuregulin, PI3K-Akt, and MAPK/ERK signaling indicates that altered miRNA activity may influence neuronal survival, synaptic plasticity, cellular metabolism, and stress responses (Guo et al., 2024). The coexistence of shared and disease-specific target genes highlights both overlapping neurodegenerative mechanisms and distinct molecular signatures that may contribute to differences in disease manifestation and progression.

One of the most significant findings of this study is the strong diagnostic performance of the identified miRNA signatures. ROC analysis demonstrated excellent discriminatory ability, with the upregulated miRNA panel achieving an AUC of 0.99 and the downregulated panel an AUC of 0.88. These results suggest that circulating miRNAs possess considerable potential as non-invasive biomarkers for the early detection of neurodegenerative diseases. Early diagnosis remains a major clinical challenge in both PD and AD because pathological changes begin years before the onset of symptoms. Therefore, miRNA-based blood tests could provide a practical and cost-effective approach for identifying at-risk individuals during preclinical stages. Beyond diagnosis, the identified miRNAs have important translational applications. First, they may serve as prognostic biomarkers for monitoring disease progression and predicting clinical outcomes (Liu & Cai, 2025). Longitudinal assessment of miRNA expression could help track disease severity and therapeutic response. Second, these miRNAs may facilitate patient stratification, enabling more precise classification of disease subtypes and supporting personalized treatment approaches. Third, the hub miRNAs identified in this study represent potential therapeutic targets. Modulation of disease-associated miRNAs through miRNA mimics or antagomirs could restore dysregulated signaling pathways and reduce neurodegenerative processes. Such approaches have already shown promise in experimental models of neurological disorders.

Furthermore, the identified pathways, including neuroinflammation, oxidative stress, apoptosis, and Neuregulin signaling, provide potential targets for drug development. Integration of miRNA biomarkers with pathway-based therapeutic strategies may improve treatment efficacy and support the advancement of precision medicine in neurodegenerative diseases. The overlap of molecular signatures between PD and AD also suggests opportunities for developing common therapeutic interventions targeting shared pathogenic mechanisms. Despite these promising findings, certain limitations should be acknowledged. The sample size was relatively modest and may limit the generalizability of the results. Validation in larger, multicenter cohorts is required before clinical implementation. Additionally, although PBMCs provide a convenient source for biomarker discovery, they may not fully reflect brain-specific molecular alterations. Future studies incorporating cerebrospinal fluid, brain tissue datasets, and multi-omics approaches will help further validate the biological significance of the identified miRNAs and their targets.

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

This study demonstrates that miRNA dysregulation plays a central role in PD and AD by influencing complex molecular networks associated with neurodegeneration. The identified miRNA signatures, target genes, and enriched pathways not only improve our understanding of disease mechanisms but also provide promising candidates for biomarker development, patient stratification, therapeutic monitoring, and miRNA-based interventions. These findings support the growing potential of miRNA-centered precision medicine approaches for the diagnosis and management of neurodegenerative disorders.

5. CONCLUSION

This study provides a comprehensive analysis of miRNA expression and regulatory networks in Parkinson's disease and Alzheimer's disease. RNA-seq-based profiling identified several dysregulated miRNAs and disease-associated pathways involved in neurodegeneration. Functional enrichment and interaction analyses demonstrated both shared and disease-specific molecular mechanisms between PD and AD. Importantly, selected miRNA signatures exhibited excellent diagnostic potential, supporting their utility as non-invasive biomarkers for neurodegenerative diseases. These findings contribute to a deeper understanding of miRNA-mediated molecular pathology and provide a foundation for future biomarker and therapeutic research.

REFERENCES

1. Ali, N., Sayeed, U., Shahid, S. M. A., Akhtar, S., & Khan, M. K. A. (2025). Molecular mechanisms and biomarkers in neurodegenerative disorders: A comprehensive review. *Molecular Biology Reports*, *52*(1), 337. https://doi.org/10.1007/s11033-025-10463-w
2. Arunachalam, K., & Sreeja, P. S. (2025). RNA Extraction Using Trizol (6-Well Plate Method). In K. Arunachalam & P. S. Sreeja, *Advanced Cell and Molecular Techniques* (pp. 117–120). Springer US. https://doi.org/10.1007/978-1-0716-4518-5_17
3. Das, S., Zhang, Z., & Ang, L. C. (2020). Clinicopathological overlap of neurodegenerative diseases: A comprehensive review. *Journal of Clinical Neuroscience*, *78*, 30–33. https://doi.org/10.1016/j.jocn.2020.04.088
4. Di Martino, M. T., Guzzi, P. H., Caracciolo, D., Agnelli, L., Neri, A., Walker, B. A., Morgan, G. J., Cannataro, M., Tassone, P., & Tagliaferri, P. (2015). Integrated analysis of microRNAs, transcription factors and target genes expression discloses a specific molecular architecture of hyperdiploid multiple myeloma. Oncotarget, 6(22), 19132–19147. https://doi.org/10.18632/oncotarget.4302
5. Guo, N., Wang, X., Xu, M., Bai, J., Yu, H., & Le Zhang. (2024). PI3K/AKT signaling pathway: Molecular mechanisms and therapeutic potential in depression. Pharmacological Research, 206, 107300. https://doi.org/10.1016/j.phrs.2024.107300
6. Kamatham, P. T., Shukla, R., Khatri, D. K., & Vora, L. K. (2024). Pathogenesis, diagnostics, and therapeutics for Alzheimer's disease: Breaking the memory barrier. *Ageing Research Reviews*, *101*, 102481. https://doi.org/10.1016/j.arr.2024.102481
7. Katayama, E. S., Hue, J. J., Loftus, A. W., Ali, S. A., Graor, H. J., Rothermel, L. D., Londin, E., Zarei, M., & Winter, J. M. (2025). Stability of microRNAs in serum and plasma reveal promise as a circulating biomarker. *Non-Coding RNA Research*, *15*, 132–141. https://doi.org/10.1016/j.ncrna.2025.08.001

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

8. Koshy, L., Madhuma, M., Vyshak, Y., Ganapathi, S., Jeemon, P., & Harikrishnan, S. (2024). Peripheral blood mononuclear cells isolation from whole blood of heart failure patients using density-gradient centrifugation. *Heart Failure Journal of India, 2*(3), 128–140. https://doi.org/10.4103/HFJI.HFJI_26_24
9. Li, Y.-B., Fu, Q., Guo, M., Du, Y., Chen, Y., & Cheng, Y. (2024). MicroRNAs: Pioneering regulators in Alzheimer's disease pathogenesis, diagnosis, and therapy. *Translational Psychiatry*, *14*(1), 367. https://doi.org/10.1038/s41398-024-03075-8
10. Lian, J., Fan, Z., Petrazzini, B. O., Fan, W., Rao, S., Yang, Q., Zeng, G., Ahmed, N., Tabassi Mofrad, F., Wamil, M., & Rahimi, K. (2026). Subtyping Alzheimer's disease and Parkinson's disease using longitudinal electronic health records. *Nature Aging*, *6*(3), 612–625. https://doi.org/10.1038/s43587-026-01085-3
11. Liu, K., & Cai, W. (2025). miRNAs: Biosynthesis, mechanism of action, and applications in biological systems. Gene Reports, 39, 102208. https://doi.org/10.1016/j.genrep.2025.102208
12. Marini, F., Linke, J., & Binder, H. (2020). ideal: An R/Bioconductor package for interactive differential expression analysis. BMC Bioinformatics, 21(1), 565. https://doi.org/10.1186/s12859-020-03819-5
13. Nazarov, P. V., & Kreis, S. (2021). Integrative approaches for analysis of mRNA and microRNA high-throughput data. *Computational and Structural Biotechnology Journal*, *19*, 1154–1162. https://doi.org/10.1016/j.csbj.2021.01.029
14. Salemi, M., Marchese, G., Lanza, G., Cosentino, F. I. I., Salluzzo, M. G., Schillaci, F. A., Ventola, G. M., Cordella, A., Ravo, M., & Ferri, R. (2022). Role and Dysregulation of miRNA in Patients with Parkinson's Disease. *International Journal of Molecular Sciences*, *24*(1), 712. https://doi.org/10.3390/ijms24010712
15. Saw, P. E., & Song, E. (2025). Non-coding RNAs: MicroRNAs (miRNAs). In P. E. Saw & E. Song, *RNA Therapeutics in Human Diseases* (pp. 123–141). Springer Nature Singapore. https://doi.org/10.1007/978-981-96-3041-7_6
16. Sharma, M., Pal, P., & Gupta, S. K. (2024). Deciphering the role of miRNAs in Alzheimer's disease: Predictive targeting and pathway modulation – A systematic review. *Ageing Research Reviews*, *101*, 102483. https://doi.org/10.1016/j.arr.2024.102483
17. Tallvod, S., Espinoza, D., Gomis-Fons, J., Andersson, N., & Nilsson, B. (2023). Automated quality analysis in continuous downstream processes for small-scale applications. Journal of Chromatography A, 1702, 464085. https://doi.org/10.1016/j.chroma.2023.464085
18. Zafar, S., Hafeez, A., Shah, H., Mutiullah, I., Ali, A., Khan, K., Figueroa-González, G., Reyes-Hernández, O. D., Quintas-Granados, L. I., Peña-Corona, S. I., Kiyekbayeva, L. N., Butnariu, M., Tota, C.-E., Caunii, A., Büsselberg, D., Sharifi-Rad, J., & Leyva-Gómez, G. (2025). Emerging biomarkers for early cancer detection and diagnosis: Challenges, innovations, and clinical perspectives. *European Journal of Medical Research*, *30*(1), 760. https://doi.org/10.1186/s40001-025-03003-6
19. Zhou, Z. D., Yi, L. X., Wang, D. Q., Lim, T. M., & Tan, E. K. (2023). Role of dopamine in the pathophysiology of Parkinson's disease. *Translational Neurodegeneration*, *12*(1), 44. https://doi.org/10.1186/s40035-023-00378-6