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Expression Analysis of miR-132, miR-182, miR-124, miR-let7b and miR-let7c in Patients with Depression

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Abstract:
Objective: The aim of this research is to analyze the impact of miRNA-mediated regulation on individuals with depression
Methodology: This analysis is a segment of a randomized controlled trial organized at Mercy Teaching Hospital in Peshawar, involving 102 patients diagnosed with depression. The trial began in February 2019 and concluded in November 2019, spanning a period of ten months. Peripheral blood samples were collected from all participants, with an additional sample taken after 12 weeks of antidepressant treatment. Laboratory investigations included quantifying miRNA levels. Total blood RNA was drawn out, utilizing the Trizol method, and the levels of miRNA-132, miR-182, miR-124, miR-let7b, and miR-let7c were measured using real-time Polymerase Chain Reaction (PCR). The expression analysis of miRNAs before and after treatment were evaluated using an independent sample t-test. A p-value, of below 0.05 were reflected as statistically significant.
Results: Patients with depression exhibited significantly elevated levels of plasma miRNA-132, miRNA-182, and miRNA-124. During the pre-tests, miRNA-132 showed a 2.96-fold increase, miRNA-182 exhibited a 2.90-fold increase, and plasma miR-124 displayed a 1.90-fold increase. However, following treatment, a decrease to 1.38, 1.32, and 1.22-fold respectively. When comparing the next two, miR-let7b exhibited a reduction of 0.72-fold, while miR-let7c decreased by 0.78-fold during the initial tests. However, after three months of antidepressant treatment, miR-let7b changed to 0.68, and miR-let7c increased to 0.93.
Conclusion: MicroRNAs have a crucial function in the pathophysiology of depression and could potentially serve as a valuable indicator for diagnosing and predicting depression by analyzing microRNA levels in relevant tissues.

Key Words: MicroRNAs, depression, real time PCR and expression analysis

Introduction:

Depression is a widespread medical disorder that manifests as prolonged periods of low mood and lack of interest in daily activities, leading to an incapacity to carry out regular tasks for at least two weeks.[1] Depression affects over 300 million individuals of all age group globally, and it constitutes a remarkable contributor to the global burden of disease.[2] Epidemiological research indicates that ecological elements, particularly subjection to traumatic life incidents, play a prominent part in activating major depression.[3] A proposal, come across suggesting that the interplay between specific ecological constituents and hereditary tendency can lead to an enduring malfunctioning of cerebral gene layouts through epigenetic mechanisms. This malfunctioning is considered to come up to the occurrence of psychiatric disorders, including depression, and their phenotypic manifestations.[4] The detection of depression relies essentially on the patient's communicated symptoms, mental state assessment, and clinical evaluation. However, there is a growing demand in the medical community for the identification of specific biological markers to assist in the diagnosis and treatment of depression, explicitly in speculating the feedback to particular medicinal approaches. [5].
MicroRNAs (miRNAs) are short RNA molecules without coding capability, consisting of approximately 22 nucleotides. They participate a significant task in adjusting gene expression after transcription by mainly suppressing translation processes. [6] The majority of miRNAs undergo a transcription process out of DNA sequences, initially forming primary miRNAs. These primary miRNAs are eventually refined into precursor miRNAs before ultimately maturing into functional miRNAs.[7] miRNAs play a settling role in supervising a widely distributed, developmental and physiological activities. [6] Furthermore, they have the ability to be released into the extracellular fluid, where they act as signaling molecules, enabling

and facilitating cell-to-cell communication. [8] The abnormal expressions of miRNAs are associated to the progress of cancer[9], aging[10], and neuro-psychiatric disorders. [11]. Consequently, the expression levels of miRNAs could serve as biomarkers for these diseases. Specifically, miRNAs are proposed as promising pharmacological targets and diagnostic biomarkers for addressing and identifying depression and anxiety. [12,13]

The presence of miRNAs in biological secretions like blood [14] and saliva [15] has sparked the interest in exploring their potential as biomarkers for diseases [16]. When it comes to depression, various miRNAs, including miRNA-132, miRNA-182[17], miRNA let-7b, let-7c[5], and miRNA-124.[18], have exhibited potential as biomarkers.[18] The target goal of the present-time investigation is to assess the effects of antidepressant treatment on the expression levels of miRNA-132, miRNA-182, miRNA let-7b, let-7c, and miRNA-124 in individuals diagnosed with depression.

OBJECTIVES:

To assess the effectiveness of antidepressant treatment and its impact on the modulation of miRNA-132, miRNA-182, miRNA-124, miRNA let-7b, and miR-let-7c expression levels.

Material and Methods

After receiving approval from the ASRB, printed informed consent was attained from every candidate before they joined the survey. A total of 102 patients were recruited from the psychiatry outpatient department of Mercy Hospital, with recruitment beginning on February 23, 2021, and ending on November 19, 2021. The seriousness of depression was rated using the Hamilton Rating Scale for Depression (HAM-D), which was the primary outcome measure. Initial assessments were conducted for all patients, followed by a second assessment after twelve weeks.

Systemic blood specimens were gathered out of each and every patient for laboratory investigations, specifically for measuring miRNA levels using quantitative polymerase chain reaction (qPCR). RNA extraction was conducted using the TRIzol procedure, and the RNA was then converted to cDNA using the viva cDNA synthesis kit from Vivantis. Real-time polymerase chain reactions were carried out using the Mic PCR system from Bio Molecular System, following the instructions provided by the manufacturer. The qRT-PCR amplification included one-step reverse transcription and real-time PCR using specificied forward and reverse primers for microRNA-132, miRNA-182, miRNA-124, miRNA let-7b, and miR-let-7c expression levels.

Statistical Analysis:

The levels of miRNA-132, miRNA-182, miRNA-124, miRNA let-7b, and miR-let-7c expression in pre-tests and post-tests of patients with depression were subjected to analysis using an independent sample t-test. The data analysis was conducted using SPSS 26 software. A significance level of $p < 0.05$ was observed statistically remarkable

Results:

Table 1 displays the primary findings of the research, offering information such as the number of participants in the study and gender distribution among patients diagnosed with depression.

Gender	Number of patients	Percentage %
Females	67	65.69%
Males	35	34.31%
Total	102	100%

Based on the provided table, it is clear that among the individuals studied, 67 (65.69%) of them were female patients out of a total of 102, while 35 (34.31%) were male patients out of the same total.

Table 2: Comparison of miRNA-132, miRNA-182, miRNA-124, miRNA-let7b and miRNA-let7c expression levels in Pre-tests and Post-tests of patients with depression.

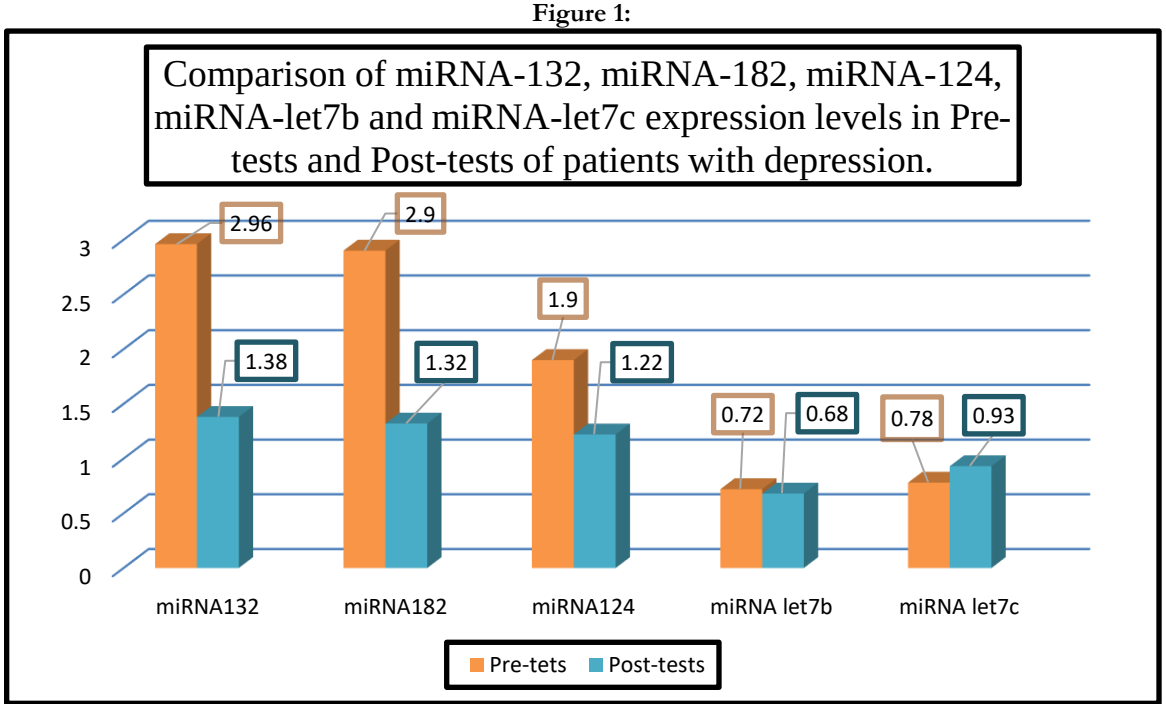
miRNAs	Pre-tests Expression levels	Post-tests Expression levels	p-value
miRNA-132	2.96	1.38	< 0.001
miRNA-182	2.90	1.32	< 0.001
miRNA-124	1.90	1.22	< 0.001
miRNA-let7b	0.72	0.68	0.383
miRNA-let7c	0.78	0.93	< 0.001

This table presents the average expression levels of miRNA-132, miRNA-182, miRNA-124, miRNA-let7b, and miRNA-let7c in the pre-tests and post-tests of individuals diagnosed with depression. The results indicate that the expression levels of miRNA-132, miRNA-182, and miRNA-124 were elevated in the pre-tests of all patients during their initial visit, measuring 2.96, 2.90, and 1.90 respectively. However, after three months of treatment, the post-test results showed improvement, with values of 1.38, 1.32, and 1.22 respectively. These improvements were statistically significant, with a p-value of less than 0.001.

Additionally, the miRNA-let7b and miRNA-let7c demonstrated low average expression levels of 0.72 and 0.78 respectively in the pre-tests conducted when patients first arrived at the hospital outpatient department (OPD). However, after three months of treatment, miRNA-let7b showed little improvement, maintaining a mean expression level of 0.68. On the other

hand, miRNA-let7c exhibited improvement, with its post-test level increasing to 0.93 from the initial pre-test level of 0.78. This improvement was statistically significant, as indicated by a p-value of less than 0.001.

Figure 1:



The figure no 1 illustrates a comparison of the average expression levels of miRNA-132, miRNA-182, miRNA-124, miRNA-let7b, and miRNA-let7c in depression patients prior to medication and subsequently undergoing medication. The pretest expression levels of miRNA-132, miRNA-182, miRNA-124, miRNA-let7b, and miRNA-let7c in depression patients, who were recruited from the psychiatry outpatient department (OPD), are represented by the orange columns. Conversely, the post-test expression levels of the same miRNAs after three months of treatment in the same group of patients are represented by the blue column. The initial three miRNAs, namely miRNA-132, miRNA-182, and miRNA-124, exhibited elevated expression levels, which significantly decreased after three months of treatment. In the case of miRNA-let7b, although there was a decrease in levels, no significant change was observed between the pre and post-tests. On the other hand, miRNA-let7c showed a decrease in expression levels during the pre-tests, with a mean level of 0.78, followed by an increase to a mean level of 0.93 after treatment.

Discussion:

This study investigated the expression levels of five miRNAs in patients with depression before and after treatment. The primary findings indicate an increase in the expression levels of miRNA-132, miRNA-182, and miRNA-124.The expression levels of miRNA-let 7b and miRNA-let 7c were found to be reduced. These findings indicate that miRNA levels have the prospective to promote as circulating predictive biomarkers for assessing the risk of developing depression.

MiRNAs have been associated as leading controllers of significant cellular functions, including development, differentiation, growth, and metabolism. Their crucial role in developmental processes underscores their potential impact on disease development. Malfunctioning of miRNAs has been associated to various ill health's, inclusive of malignancy, heart diseases, and neuro-developmental disorders. Notably, considering the discovery of circulating miRNAs, investigators have extensively explored their potential as valuable circulating biomarkers for diagnosing and predicting diseases.[19]

MiRNAs exhibit dynamic functions in the process of neurogenesis. [20] Hence, it comes as no surprise that miRNAs have been identified as significant contributors to neurodegenerative diseases. In individuals suffering from Alzheimer's disease, miRNAs showed distinct expression patterns in their brains.[21] Moreover, the dysregulation of miRNAs in mice brains led to a phenotype resembling Parkinson's disease. [22]

Under physiological conditions, miRNA and additional flowing nucleic acids can pass through the blood-brain barrier (BBB), allowing the miRNA levels in peripheral blood to reflect those in the central nervous system (CNS)[23]. The disruption of the blood-brain barrier (BBB) in pathological conditions can enhance its permeability, facilitating the unrestricted movement of particles into the flowing blood circulation. This suggests that the miRNA levels in peripheral blood are indicative of the miRNA levels in the neural system[23].

In the present study, patients diagnosed with depression exhibited significantly heightened plasma levels of miRNA-132, miRNA-182, and miRNA-124. In the pre-tests, there was a 2.96-fold increase in miRNA-132, a 2.90-fold increase in miRNA-182, and a 1.9-fold increase in plasma miR-124. These findings align with an investigation organized by Fang et al.,[24] which manifested that individuals identified with major depressive disorder (MDD) and not receiving any medication displayed plasma concentrations of miR-132 that were 2.4 times greater than those in the control group. Furthermore, they noted a positive correlation amongst the levels of miR-132 and the severity of depressive symptoms, suggesting that miR-132-5p levels in the bloodstream could potentially be used as a biomarker to evaluate the condition of depression.

In 2013, Li et al. also made a finding regarding the role of miR-182 in the progress of depression. [17,25] Through cellular modeling, it was found that miR-182 acts as a controller of the BDNF gene, much like miR-132. By employing a neuronal cell model, it was observed that both miR-182 and miR-132 downregulate the impression of BDNF. While, comparing individuals without depression (healthy controls) to patients identified with depression, it was found that depressed patients had lesser quantity of serum BDNF and elevated quntity of miR-132 and miR-182 and these findings resemble our study findings.

Furthermore, an inverse correlation was observed between the quantity of serum BDNF and miR-132/miR-182 in individuals with depression. Taken together, the particular discoveries indicate that miR-182 might act as a regulatory microRNA for BDNF and might serve as a valuable bio-marker for the diagnosis and treatment of depression.

Roy et al. in 2017a [26] has demonstrated in their research the potential application of brain-augmented miRNAs in anticipating clinical depression using serum samples obtained from 18 individuals diagnosed with Major Depressive Disorder (MDD). Following the adjustment for age, gender, and race within the Major Depressive Disorder (MDD) cohort, a serum-based finding unveiled a 3.5-fold upregulation in the expression of miR-124-3p. Our current study findings align with their results.

In our present study, we observed a significant 1.9-fold increase in plasma miR-124. These findings differ from the results reported by Wang et al. in 2018, where they identified a considerable decline in the expression of miR-124-3p specifically in Brodmann area 44 (BA44) among individuals diagnosed with Major Depressive Disorder (MDD).[27]

Additionally, our study findings align with the results reported by He et al. in 2016, who also observed a considerable decline in the expression of miR-124 among 32 patients diagnosed with Major Depressive Disorder (MDD) after undergoing an eight-week course of antidepressant treatment. [18].

The findings of the present study regarding miRNA-let7b indicate a decrease in the mean expression levels, but no considerable decline was observed amongst the pre and post-tests. Additionally, miRNA-let7c demonstrated a decrease in expression levels during the pre-tests, with a mean level of 0.78. Following treatment, there was a subsequent increase to a mean level of 0.93, accompanied by a significant p-value.

Roumans and colleagues discovered in their study that let-7b-5p showed a negative association with the risk of developing Major Depressive Disorder (MDD) .[20] Reinhart et al. identified the let-7 family of miRNAs as regulators of growth timing in C. elegans, with Let-7b-5p being one of its members. [29]. While Let-7 is remarkably preserved across animal species, higher species exhibit numerous isotypes of this microRNA. Among humans, nine adult isotypes have been identified, and one of them is known as let-7b-5p .[30]

The results of our study, showing a decrease in both miRNA-let 7b and miRNA-let7c, align with the findings of Gururajan et al. [5] They reported a considerable minimization in the expression of let-7b-5p in individuals with treatment-resistant depression (TRD) who underwent electroconvulsive stimulation therapy (ECT) when contrasted to control subjects. Additionally, they discovered a tendency approaching to higher post-treatment expression of let-7b-5p in patients whom sustained ketamine treatment (KET) approached to those who meet with ECT. Belzeaux et al. [31] reported inconsistent data, indicating an upregulation of let-7b-5p expression during major depressive episodes. However, it is important to note that their analysis took place on peripheral blood mononuclear cells (PBMC), which may not totally align with the miRNA adaptations in plasma utilized in the present study.

Conclusion:

Moreover, these miRNAs possess the capacity to function as biomarkers of diagnostic, prognostic, and predictive importance. They have also been identified as promising targets for therapy and may influence existing antidepressant treatments. As adaptable epigenetic regulators, we emphasize the importance of lifestyle interventions such as physical activity and diet, which offer new possibilities for clinical management approaches. Further research is needed to develop potential miRNA-based treatments for depression.

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