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ORIGINAL ARTICLE

Evaluation of miRNA- 155 Expression and Alzheimer's disease

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ABSTRACT

Background: *Our aging domestic population is disproportionately affected by senile dementia and neurological impairment caused by Alzheimer's disease (AD), a complex and progressive age-related disorder. In the field of human pathogenic neurobiology, as crucial regulators of gene expression, microRNAs (miRNAs). Brain cells are known to have a high transcriptome, which means they are actively involved in gene transcription and mRNA generation.* ***Objectives:*** *This research aimed to examine the correlation between the expression of miRNAs-155 and patients diagnosed with Alzheimer's disease.* ***Methodology:*** *The study enrolled 100 participants: 50 healthy controls and 50 AD patients diagnosed according to DSM-V criteria, the quantification of miRNA-155 expression was performed; through the use of real-time PCR.* ***Results:*** *The expression of miRNA-155 is significantly greater in the Alzheimer's disease group (9.94±3.134-fold change) than in the control group (1.07±0.52) (P= 0.000). In order to evaluate miR-155's expression efficiency, a receiver operating characteristic (ROC) curve was constructed. Both the sensitivity and specificity were found to be quite high in the study, measuring 1.000 for each. The area under the curve (AUC) was also 1.000, indicating excellent performance. The 95% confidence interval for the AUC was from 0.1.000 to 1.000. The cut-off value used in the analysis was 3.88.* ***Conclusion:*** *Ultimately, miRNA-155 might potentially be used as a diagnostic tool to differentiate individuals with Alzheimer's disease from those who are healthy. According to the findings, that miRNA-155 has the potential to function as a genetic indicator for Alzheimer's disease. This work offers significant insights for future investigations on miRNAs as molecular indicators in medical domains and as promising targets for therapeutic interventions.*

INTRODUCTION

Deterioration of cognitive abilities is a hallmark of Alzheimer's Disease (AD), a complex neurological disorder. leading to difficulties in doing everyday tasks. This includes problems with remembering past events and difficulties with spatial and temporal awareness. Dementia and cognitive decline are most often attributed to this condition in individuals aged 65 and above[1]. Alzheimer's disease (AD) is an increasingly significant worldwide public health problem with profound societal repercussions. It is a common occurrence among older adults, often increasing in frequency every 5 years beyond the age of 65[2]. The illness's development is thought to be significantly influenced by Down's syndrome, type 2 diabetes mellitus, cardiac disease, hypertension, obesity, and inflammatory processes[3], Oxidative stress has been connected to the progressing of many diseases, Increased concentrations of (ROS) can lead to oxidative stress and/or a reduction in antioxidants[4]. Epigenetics is the scientific field that focuses on investigating the modifications to the chromatin structure that may influence the observable characteristics of an organism without changing its genetic makeup. These modifications can occur regardless of whether the cells are dividing or not.[5], One subfield of epigenetics deals with microRNAs, which are short noncoding RNAs that typically have between twenty-one and twenty-three base pairs[6]. It affects cell development, differentiation, and death, contributing to the etiology of numerous diseases[7]. It is evident that miRNAs are involved in a range of physiological and pathological processes, and they have significant roles in controlling gene expression at the post-transcriptional level. They achieve this by slowing down or inhibiting the translation process through binding to a complementary sequence in the 3' untranslated region (UTR) of messenger RNAs (mRNA)[8]. Acute inflammatory responses induced by pathogens via Toll-like receptors (TLRs) are mostly regulated by miR-155, miR-146, and miR-223, according to recent studies [9]. There is a strong link between changes in their expression and many clinical diseases. Multiple studies have shown substantial evidence supporting the involvement of dysregulated miRNAs in the development of AD[10]. The function of inflammatory and immunological responses is vital in the development of AD. Therefore, effectively controlling various kinds of T-cells might potentially relieve the severe symptoms associated with AD. In an

inflammatory environment, miR155 controls the survival, differentiation, proliferation, and activation of Th1, Th2, Th17, Treg, and CD8+ T-cells. Undoubtedly, determining the precise positive or detrimental role of miR-155 in regulating inflammation in Alzheimer's disease (AD) via T cell control is challenging. This is due to its association with diverse T cell responses and complex T cell communication[11]. Assessing miR155 and other miRNAs can enhance diagnostic accuracy, differentiate AD from mild cognitive impairment, and provide insights into disease progression and severity. The diagnostic potential miR155 in distinguishing AD from healthy controls has not been previously explored. Therefore, this study aims to compare levels of in AD miR155 patients and healthy controls.

METHODOLOGY

Subjects:

This is a case-control study to examine the following groups during the period from the first of November 2022 to the end of December 2023. This study included (50) patients mixed of males and females with age range 48-78 years old. Samples had been collected from the Nervous system center of AL-Sader Hospital in AL-Najaf Governorate. The patients were diagnosed clinically by physicians.

Total RNA extraction:

Using a Triazole Reagent kit, following the manufacturer's methodology, 100 blood samples were used to isolate and purify total RNA. (Invitrogen, USA), and then total RNA concentration and purity were Estimated by using a UV/Visible spectrophotometer instrument. RNA should be stored at –80Cº.

miRNA-155 and U6 reference gene quantification using RT-qPCR

Following RNA purification, miRNA profiling can be carried out by Primer sequences used for RT-qPCR analysis as shown in table (1). Reaction volume was set according to recommendations by the manufacturers (GoTaq® 1-Step RT-qPCR kit, Promega) as in table (2), and the Reaction conditions table (3).

Table 1: The sequence of primers used in the recent study

Primers of miRNA	Sequence 5ˈ to 3ˈ
microRNA-155Forward primer	AGGTGGCACAAACCAGGAA
microRNA-155Reverse primer	GTTGAACATCCCAGTGACCAG
U6 Forward primer	GTTTTGTAGTTTTTGGAGTTAGTGTTGTGT
U6 Reverse primer	CTCAACCTACAATCAAAAACAACACAAACA

Table 2: GoTaq® 1-Step RT-qPCR Reaction Mix

Component	Volume Final	Concentration
GoTaq® qPCR Master Mix, 2X	10 µl	1X
GoScript™ RT Mix for 1-Step RTqPCR (50X)	0.4 µl	1X
Forward Primer	2 µl	300 nM
Reverse Primer	2µl	300 nM
MgCl2	1.6 µl	25 mM
RNA template	3.7µl	100 ng
CXR	0.3µl	-

Table 3: One-step RT-qPCR programs

Step	Temperature	Duration	Cycles
Reverse transcription	37°C	15 min	1
Reverse transcriptase inactivation /and start activation of GoTaq DNA Polymerase.	95°C	10 min	1
Denaturation	95°C	10 sec	45
Annealing and data collection	58°C	30 sec	
Extension	72°C	30 sec	
Melt Curve	60-95°C	15 sec	1

Calculating Gene Expression (Gene Fold)

The expression of miRNA-١55for patients' blood samples was normalized to (RNU6-2) reference genes and compared with those in relatively normal healthy control. Finally, the fold-change between the healthy control and patient was calculated by using the 2-ΔΔct method described by Schmittgen and Livak [12].

Ethical Approval:

A study proposal to make human studies has been authorized by the Institutional Ethics Committees of AL-Kufa General Hospital and Kufa University's College of Science. In addition, all participants provided a written informed consent before participation in the study. Institutional Review Board (IRB) approval was granted by the College of Science at the University of Kufa in Iraq (3912/2022), in accordance with the Declaration of Helsinki's International Guideline for Human Research Protection.

Statistical Analysis:

The statistical measures used in the present research are the mean ± standard deviation (SD) and the independent t-test, conducted using the IBM SPSS program version 23. An analysis of the genotypes data was also conducted. A significance threshold of less than 0.05 was applied for all statistical analyses.

RESULTS

The average age of Alzheimer's patients and healthy group in the current study was between the (2.74±1.046) and (1.54±0.676) year respectively. The more common age group was (56-65) followed by (76+), (66-75) then (45-55), The (56-65) age group is more susceptible to AD than other age groups.

Table4: Distribution of AD according to age groups.

Age groups	AD	
	Number	Percentage
45-55	6	12
56-65	17	34
66-75	11	22
76+	16	32
Total	50	100

Identification of the expression levels of miRNA-155 in the studied group

The results indicate a significant upregulation of miRNA-155 expression in Alzheimer's disease (AD) patients compared with the control group as illustrated in figures (1). The fold change of 9.94±3.134 in AD patients compared to 1.07±0.52 in controls indicates that miRNA-155 is markedly increased in AD patients. The large standard deviation (3.134) in the fold change among AD patients suggests variability in miRNA-155 expression levels within this group.

We used a receiver operating characteristic (ROC) curve to evaluate miRNA-155's sensitivity and specificity. The cutoff value was 3.88. Both sensitivity and specificity were 1.000, with an AUC of 1.000 and a 95% confidence interval of 1.000-1.000. These results are shown in table 5 and figure 2.

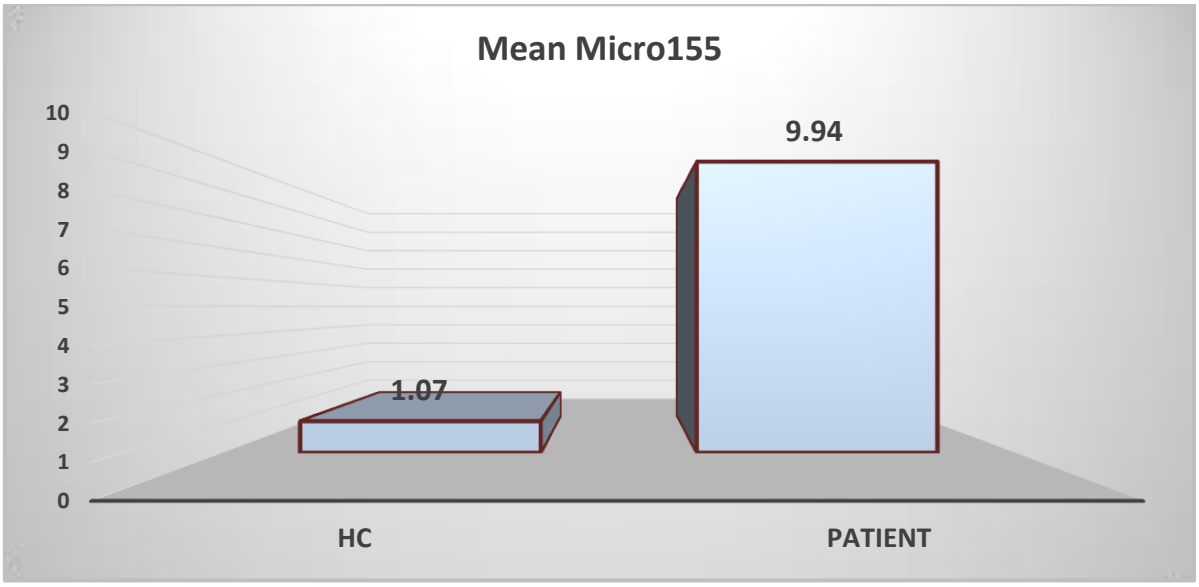

Fig. 1: Expression levels difference of miRNA-155 between AD patients and control groups.

Table 5: MiRNA-155 sensitivity and specificity in AD patients and control

Parameter	Cut off	AUC	Sensitivity	Specificity	95% CI	P-value
miRNA-155	3.88	1.000	1.000	1.000	1.000-1.000	0.000

AUC area under the curve, CI: Confidence interval

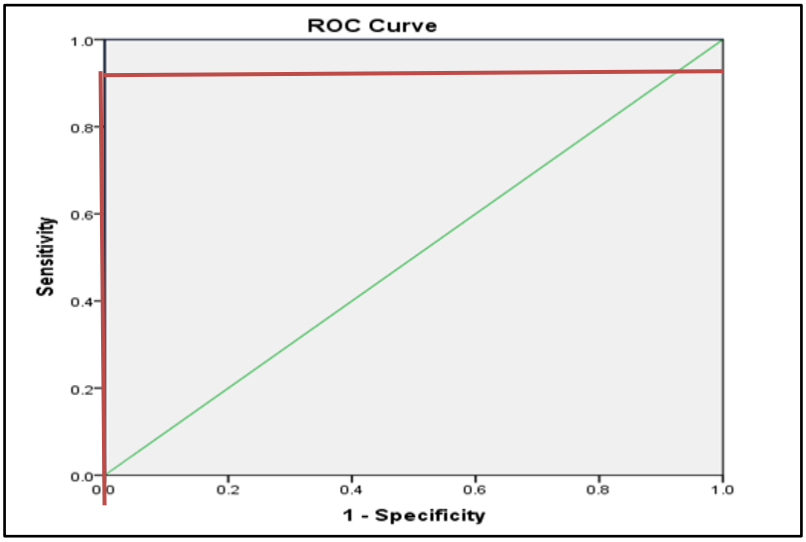

Fig. 2: Receiver operating characteristic (ROC) curve of miRNA -155

DISCUSSION

The change and increase in expression levels stem from when many factors. Growing evidence shows that exposure to environmental stimuli can cause a variety of epigenetic modifications, including miRNA expression, global or gene specific DNA methylation modifications, and histone changes[13]. The present results were accepted with Aloi et al.which reported that the expression level of miRNA-155 was significantly upregulated in patients with AD. Also, agreed with another study that has shown inecreased expression level of miR-155 in AD diseases[15,16].

miRNAs have a role in both activating and inhibiting inflammatory signals, along with contributing to progressing towards unregulated neuroinflammation, which may have negative pathogenic effects. MiR-155 is regarded as a pro-inflammatory agent in the CNS and is shown to be elevated in the brains of people suffering from different forms of neurodegeneration. After Toll-like receptors (TLR) are stimulated and the pro-inflammatory cytokine IFN-γ is released, the activation of microglia and macrophages is set off by the nuclear factor κB (NF-κB) [17,18,19].

MiR-155 triggers neuroinflammation by blocking factors that play a role in the inflammatory process. The presence of miR-155 leads to a decrease in the body's natural anti-inflammatory response, which in turn causes an increase in inflammation[20,21,22]. miR-155 specifically targets suppressors of cytokine signaling (SOCS1), SHIP1, IL-13 receptor alpha 1 (IL13Rα1), and SHIP2 are inhibitors of inflammation., which are negative regulators of cytokines and TNF-α, respectively[23.24.] A glycoprotein involved in suppressing the immune response, complement factor H (CFH) has its expression downregulated in Alzheimer's dementia (AD) due to an upregulation of miR-155. In neuroinflammation, CFH downregulation activates the complement system, which is essential for regulating the immune response and starting and advancing neurodegeneration[25-26].

CONCLUSION

Ultimately, miRNA-155 might potentially be used as a diagnostic tool to differentiate individuals with Alzheimer's disease from those who are healthy. According to the findings, that miRNA-155 has the potential to function as a genetic indicator for Alzheimer's disease. This work offers significant insights for future investigations on miRNAs as molecular indicators in medical domains and as promising targets for therapeutic interventions.

Recommendations:

Investigating the relationship between cytokines implicated in the pathophysiology of AD, miRNA155 gene expression. Researching for novel miRNAs that

could be used as AD susceptibility biomarkers. Study the polymorphism of miRNA-155 genes and their relationship with the activity of AD disease.

Ethical approval

The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki). Confidentiality of data, the authors declare that they have followed the protocols of their work center on the publication of patient data. Right to privacy and informed consent, the authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document. Use of artificial intelligence for generating text, the authors declare that they have not used any type of generative artificial intelligence for the writing of this manuscript, nor for the creation of images, graphics, tables, or their corresponding captions.

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