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Article

# Serum miRNA Detection-based Alzheimer's disease prediction regression model

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**Abstract:** With the deepening of Alzheimer's disease (AD) research, serum miRNA has attracted widespread attention as a potential biomarker. Traditional diagnostic methods for AD have certain limitations, such as reliance on clinical symptoms and neuroimaging examinations, which lack sensitivity (Sen) and specificity (Spe) for early diagnosis. Therefore, this article aimed to explore the expression levels of serum miRNA in AD patients and its clinical significance, to construct an AD prediction regression model based on serum miRNA detection. This article found no statistical differences in gender, underlying diseases, age, triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) between the control group (healthy individuals) and the AD group, but obvious distinctions were observed in Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), Alzheimer's Disease Assessment Scale-cognitive part (ADAS-cog), and Activities of Daily Living (ADL) scores. Further analysis revealed obvious distinctions in miR-31, miR-93, miR-124-3p, miR-143, miR-146a, and miR-218-5p between the two groups, with miR-124-3p showing the best diagnostic effect, followed by miR-218-5p. Based on these findings, this article constructed an AD prediction regression model, and the experimental results indicated that the model has high Sen, Spe, and accuracy (Acc) in the early diagnosis of AD, reducing the error rate of subsequent diagnoses and providing new ideas and methods for the early diagnosis of AD.

**Keywords:** serum miRNA detection; AD; cognitive status; disease prediction

## 1. Introduction

As the process of social aging accelerates, the incidence of AD has been increasing year by year, becoming a visible challenge in the field of global public health. AD is a chronic, progressive neurodegenerative disease, mainly characterized by cognitive decline, memory impairment, and other symptoms, which severely affect the quality of life (QoL) and social function of patients [1,2]. However, early diagnosis of AD is still difficult, and traditional diagnostic methods such as clinical symptom assessment and neuroimaging examinations often lead to a clear diagnosis only in the late stages of the disease, missing the best opportunity for intervention [3,4]. Since the early symptoms of AD are hidden and difficult to detect, patients often seek medical help after obvious cognitive decline. At that time, the disease has entered a late stage. At the same time, clinical evaluation mainly depends on doctors' experience and patients' self-report, which is easily influenced by individual differences. Although neuroimaging examination and advanced biomarker detection methods can provide

valuable information, their high cost limits their wide application in the general population. The detection of cerebrospinal fluid requires lumbar puncture, which causes certain pain to patients and has certain risks. Therefore, it is of great significance to find effective early diagnostic markers for early detection and timely treatment of AD.

In recent years, serum miRNA has received widespread attention as a potential biomarker due to its abnormal expression in various diseases and its easy detection [5,6]. miRNA is a class of small non-coding RNA molecules about 22 nucleotides in length, which regulate gene expression by binding to the 3'UTR of target genes and participate in various biological processes. Studies have shown that miRNA plays a major role in the occurrence and development of AD, and its abnormal expression may be closely related to the pathological process of AD [7]. Therefore, this article aimed to explore the expression levels of serum miRNA in AD patients and its clinical significance, to construct an AD prediction regression model based on serum miRNA detection.

The main innovative content of this article is as follows:

Firstly, this article proposed for the first time a comprehensive assessment model that combines specific serum miRNA with clinical cognitive assessment indicators, which greatly improved the Acc and sensitivity (Sen) of AD diagnosis.

This article identified the value of specific serum miRNA as potential biomarkers for AD. The changes in these miRNAs are closely related to the pathophysiological mechanisms of AD, including neuronal survival, inflammation regulation, lipid metabolism, and neural conduction. This discovery not only provides new tools for the early screening of AD but also offers a new perspective for exploring the pathogenesis of AD.

Finally, through systematic analysis of samples from the training group (TG) and the validation group (VG), this article verified the effectiveness and stability of the proposed combined detection model. This series of verification steps not only strengthened the scientific and reliable nature of the model but also laid a solid foundation for its future application in clinical practice.

## 2. Related works

The early diagnosis and accurate prediction of AD have always been a key direction in neuroscience research. In recent years, with the rapid development of molecular biology and information technology, detection methods based on molecular markers have attracted widespread attention, making new breakthroughs in the diagnosis and prediction of AD [8,9]. Serum miRNA, as a stable and easily accessible molecular marker, has been studied and recognized by many scholars for its potential role in AD. Some scholars have found that changes in serum miRNA levels can reflect the pathological characteristics of AD and are a new and effective means for early prediction and diagnosis of AD [10]. Other scholars have used different high-throughput sequencing technologies and microarray technologies to identify multiple miRNAs related to AD, extracting molecular information that is visible for disease prediction and diagnosis [11].

Many scholars have begun to introduce machine learning and deep learning techniques into miRNA data analysis to improve the prediction Acc and efficiency of AD. For example, models based on support vector machine (SVM) and random forest (RF) have shown their superiority in serum miRNA data analysis in many studies. Through these methods, scholars can distinguish the differences in miRNA expression profiles between AD patients and healthy individuals, providing data support for early screening and intervention [12,13]. In addition, some scholars have used neural network models to perform nonlinear feature extraction and clustering analysis on high-dimensional miRNA data, improving the Acc and robustness of predictions through deep learning models [14,15].

In recent years, some scholars have tried to combine miRNA with other types of biomarkers (such as proteins and metabolites), using multi-omics comprehensive analysis methods to further enhance the predictive ability for AD [16–18]. These studies have used multi-omics data to construct multimodal prediction models, reflecting the multi-dimensional characteristics of the disease to a greater extent and achieving visible results. For example, some scholars have combined serum miRNA data with cerebrospinal fluid protein levels to establish a multimodal prediction model that can effectively identify early pathological changes in AD [19]. Data mining and machine learning techniques have also been applied to the study of large-scale AD-related datasets to improve data utilization efficiency and prediction Acc [20]. For example, some scholars have used association rule mining and Bayesian network technologies to mine potential biomarkers closely related to the progression of AD from large-scale serum miRNA datasets, using these data to build efficient prediction regression models, effectively enhancing the Acc of prediction results [21]. The application of blockchain technology in medical data management and privacy protection has also gradually attracted the attention of academia. Some scholars have proposed that the use of blockchain technology can achieve secure sharing of serum miRNA data, optimize data management processes, and improve the credibility and integrity of data, thereby providing more reliable data support for AD research and clinical application [22]. By combining blockchain and distributed storage technologies, the interaction and management of patient data are optimized, promoting the practical application of AD prediction models.

In summary, the current research on AD prediction models based on serum miRNA detection has made many advancements, mainly focusing on the application and optimization of technologies such as high-throughput sequencing, machine learning, and multi-omics integration analysis. However, the comprehensive evaluation of serum miRNA in AD prediction and how to effectively implement it in the clinic still requires further research. Therefore, this article proposes an AD prediction regression model based on serum miRNA detection, aiming to further improve the Acc and operability of the prediction model and provide strong support for the early diagnosis and intervention of AD.

## 3. AD prediction model based on serum miRNA detection

The variables of miRNA probes in the TG were screened by the least absolute shrinkage and selection operator (LASSO) in glmnet package, and the Log(λ) value

with the smallest error in error classification was selected through ten-fold cross-validation. At first, the whole data set was randomly divided into ten subsets or "folds". For each round of cross-validation, the data of nine subsets were selected as the training set to train the model, and the remaining subset was used as the validation set to test the model. Each subset would be used as the primary validation set. For each validation set, the predicted performance index of the model was calculated, and then the results of all rounds were summarized to calculate the average performance measure. The best Log(λ) value was determined according to the summarized performance metrics. In this study, the Log(λ) value that minimized the average error classification rate was selected. In LASSO regression, the Log(λ) value that minimized the average error classification rate was selected as the best parameter, and 13 miRNA probes were obtained. Subsequently, a two-way stepwise regression method was applied for a secondary screening of these 13 miRNA probes, and a diagnostic model incorporating 4 scoring systems and 6 miRNA probes was ultimately constructed in conjunction with clinical data. The ROCR package was used to conduct the receiver operating characteristic curve (ROC) analysis to evaluate the predictive performance of the diagnostic model for AD patients and healthy controls in TG, VG, training + verification, and the total sample (**Figure 1**).

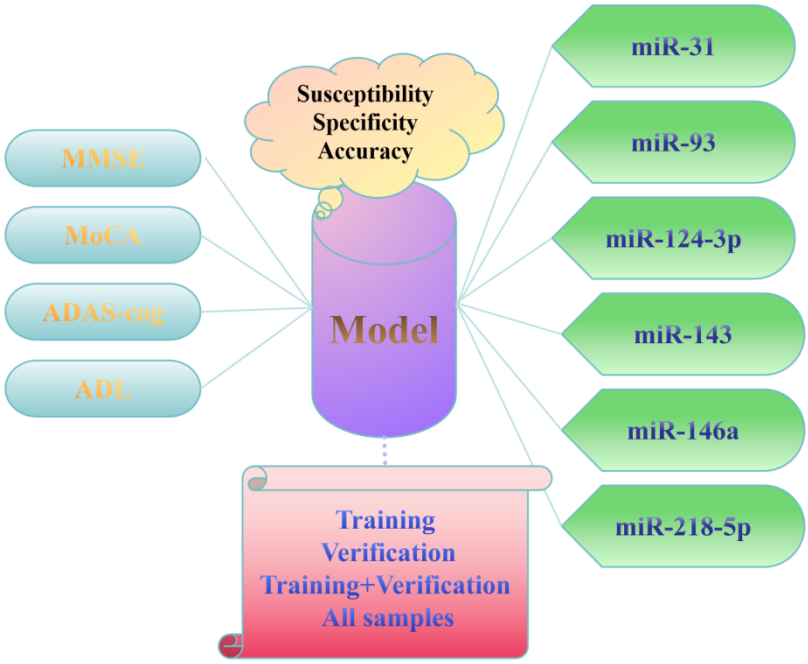


**Figure 1.** AD prediction model based on serum miRNA detection.

The AD prediction model incorporated four scoring systems: MMSE, MoCA, ADAS-cog, ADL, and six miRNA probes: miR-31, miR-93, miR-124-3p, miR-143, miR-146a, miR-218-5p. The model was established and validated in the TG, VG, training + verification, and the total sample, with validation and evaluation indicators mainly including Sen, specificity (Spe), and Acc.

This model included 1467 AD patients and 698 healthy controls, totaling 2165 samples. The samples comprised information on age, gender, years of education, diabetes status, hypertension status, TG, TC, LDL-C, HDL-C, MMSE, MoCA,

ADAS-cog, ADL scores, and serum miRNA. Based on a 1:1 age-matching method, 530 AD patients and 530 healthy controls were further selected, constituting 1060 samples. The probe expression values were sorted from highest to lowest, and the top 500 miRNA probes with higher mean values were selected for subsequent research. The Limma package in R software was used to calculate differential miRNA probes, with the screening criteria being $P < 0.05$ and a fold change > 1.15, ultimately yielding 48 miRNA probes. The samples were divided into the TG (742 cases) and the VG (318 cases) according to the common 7:3 ratio. The LASSO regression from the glmnet package was applied to perform variable selection on the 48 miRNA probes in the TG, and the Log(λ) value with the smallest misclassification error was selected through ten-fold cross-validation, resulting in 13 miRNA probes. Subsequently, a two-way stepwise regression method was used for a second round of screening, ultimately constructing a diagnostic model that included MMSE, MoCA, ADAS-cog, ADL, and six miRNA probes.

Serum separation was as follows. A total of 100 μL of fresh serum samples were collected to ensure that hemolysis was avoided in the collection process, and the samples were quickly frozen to prevent the degradation of miRNA. Protease K with a concentration of 10 μg/mL was used for digestion to reduce the interference in the subsequent purification process. A lysis buffer was added to the serum samples according to the ratio of 1:5, that was, 500 μL of lysis buffer was added to every 100 μL of serum to dissolve protein and other impurities in the serum and release miRNA. The cracked sample was centrifuged at high speed to remove cell debris and other macromolecular substances, and the supernatant was collected. A binding buffer was added to the supernatant, and 500 μL of binding buffer was added to every 500 μL of supernatant to help miRNA bind to the silica gel membrane on the column. The mixed solution was transferred to the column containing the silica gel membrane, and the miRNA would be adsorbed on the membrane, while other impurities would flow out through the column. The column was washed several times with washing buffer, 700 μL each time, repeated for 2–3 times, to remove unbound protein and other impurities. Then, 50 μL of low-salt buffer was added to the column, and then miRNA was eluted from the silica gel membrane by centrifugation.

Extraction and quantification of miRNA were as follows. The ultraviolet-visible spectrometer (NanoDrop) was employed for quantification, the eluted miRNA sample was added to the instrument. The operation was carried out according to the equipment manual, so as to read the A260 ratio and calculate the concentration.

## 4. Experimental results of the AD prediction model based on serum miRNA detection

### 4.1. Clinical data of AD and healthy populations

To make the AD prediction model more accurate and systematic, this article compared the clinical data of AD and healthy populations, aiming to screen out differentiated clinical data to be included in the model for disease diagnosis and prediction. The general information of AD and healthy populations was first

compared, mainly including age, gender, years of education, diabetes status, and hypertension (**Table 1** and **Figure 2**).

**Table 1.** Clinical data of AD and healthy people.

| Project | Control group | AD group | $t/\chi^2$ | *P* |
|---|---|---|---|---|
| Age | 71.64 ± 7.57 | 72.57 ± 6.85 | 6.979 | 0.864 |
| Male | 280 | 297 | 12.468 | 3.757 |
| Female | 250 | 233 | 10.679 | 6.644 |
| Years of education | 9.47 ± 2.64 | 9.88 ± 2.83 | 8.578 | 0.946 |
| Diabetes | 279 | 297 | 5.357 | 1.868 |
| Hypertension | 270 | 273 | 12.457 | 2.675 |

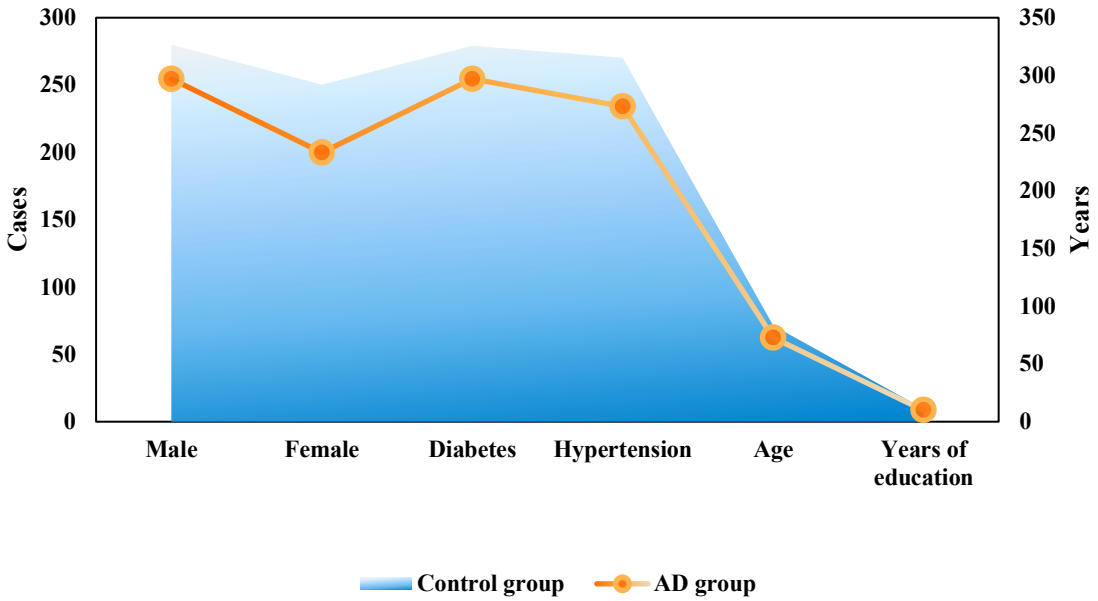


**Figure 2.** Contrast of the general information of AD and healthy populations.

The general information of AD and healthy individuals, showing no obvious distinctions in age, gender, years of education, diabetes, and hypertension between AD and healthy populations. This may be because these factors do not play a decisive role in the pathogenesis of AD. Although age is a major risk factor for AD, not all elderly individuals develop AD, indicating that the occurrence of AD may be related to a variety of factors such as genetics, environment, and lifestyle. Factors such as gender, years of education, diabetes, and hypertension may have some impact on the risk of AD, but they are not the direct causes of AD. Therefore, the lack of obvious distinctions in these factors between AD and healthy populations reflects the complexity and multifactorial nature of AD.

The predictive model of this article also incorporated TG, TC, HDL-C, LDL-C, MMSE, MoCA, ADAS-cog, and ADL scores to understand the impact of lipid status and cognitive conditions on the diagnosis of AD (**Figure 3**).

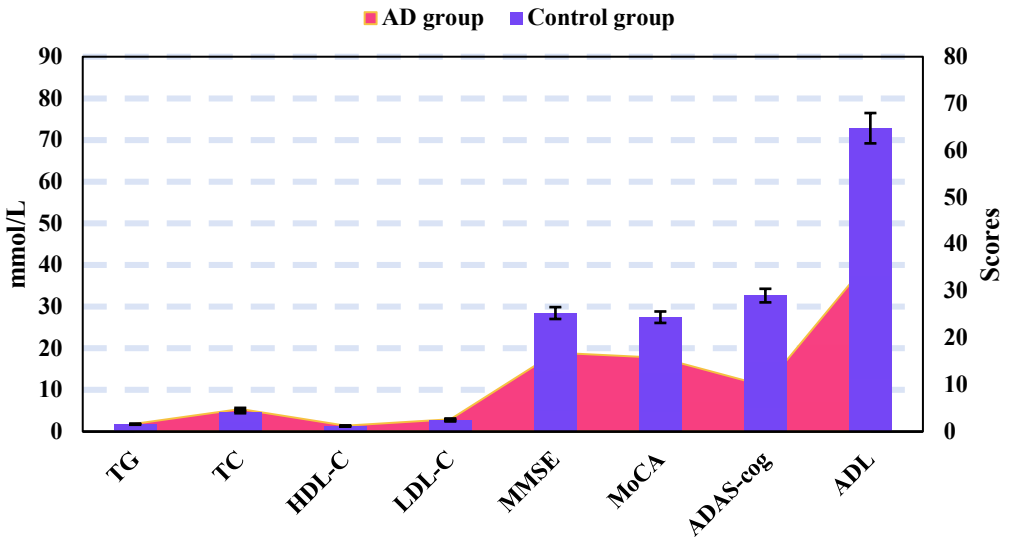


**Figure 3.** Contrast of the lipid status and cognitive conditions between AD and healthy populations.

The analysis revealed no obvious distinctions in lipid profiles between AD and healthy individuals. However, AD patients had markedly lower scores on the MMSE, MoCA, ADAS-cog, and ADL scales compared to the healthy control group. This suggests that lipid levels may not be a key factor in the pathogenesis of AD, or their impact may be relatively minor and insufficient to cause obvious distinctions. It indicates that the pathological mechanisms of AD may involve other biomarkers or non-traditional risk factors. Nevertheless, the obvious distinctions in cognitive and functional assessment scales clearly reflect the severe impact of AD on patients' cognitive functions and daily living abilities. The MMSE and MoCA are common screening tools, with the MMSE primarily assessing general cognitive functions, including orientation, memory, attention, and calculation abilities. The MoCA focuses more on detecting mild cognitive impairment, covering executive functions, language, short-term memory, and visuospatial abilities. The lower scores of AD patients in these tests indicate widespread and severe deficits in global cognitive functions. The ADAS-cog is a more detailed cognitive assessment tool, covering memory, language, orientation, and executive abilities. The low scores of AD patients in the ADAS-cog further confirm visible impairments in multiple cognitive domains. This comprehensive cognitive damage is one of the typical features of AD and an important basis for its diagnosis and evaluation. The ADL scale assesses the independence in daily living activities, ranging from basic life skills such as eating, dressing, and bathing to more complex tasks like managing finances and shopping. The markedly lower ADL scores of AD patients reflect a marked decline in their ability to take care of themselves and handle complex tasks daily. This not only affects their QoL but also increases the caregiving burden on families and society.

In summary, no obvious distinctions were found between AD and healthy control group in general information and lipid levels, suggesting that lipids may not be the main pathological factor in AD. However, the markedly lower scores of AD patients in cognitive and functional assessments (such as MMSE, MoCA, ADAS-cog, ADL) clearly reflect the severe impact of AD on cognitive functions and daily living abilities.

The MMSE and MoCA tests revealed extensive deficits in orientation, memory, attention, and executive functions among AD patients; the ADAS-cog further confirmed severe impairments in these cognitive domains, all of which are important for AD diagnosis and evaluation. The ADL score indicated a visible decline in the daily self-care abilities of AD patients, affecting their QoL and increasing the caregiving burden on families and society. These results highlight the need to focus on the multifactorial pathogenesis of AD and explore more biomarkers to improve the precision of early diagnosis and intervention.

### 4.2. Serum miRNA detection in AD and healthy populations

To establish an AD prediction regression model based on serum miRNA detection, this article compared the differential serum miRNA between AD and healthy populations. Initially, a comparison of six differential serum miRNAs, namely miR-31, miR-93, miR-124-3p, miR-143, miR-146a, and miR-218-5p, was conducted in the TG (**Figure 4**).

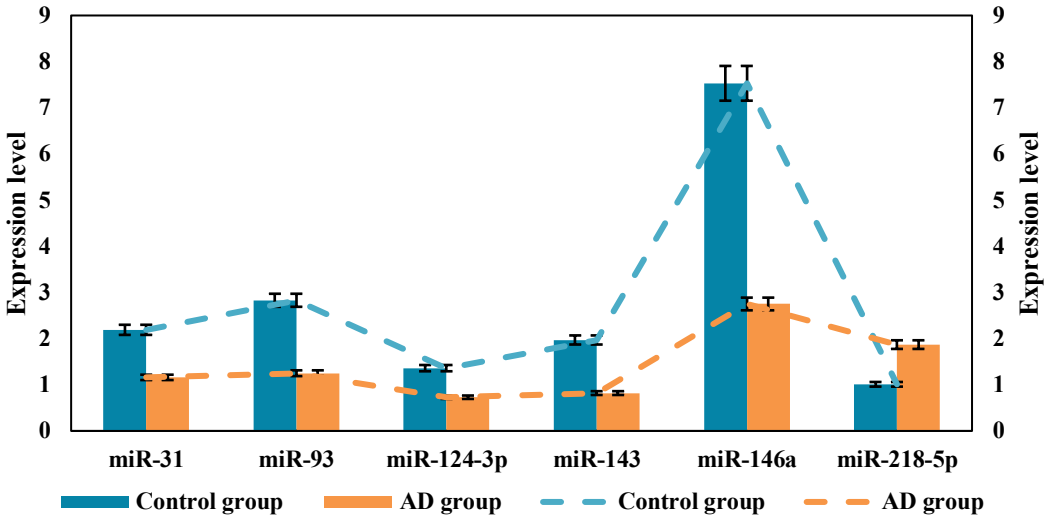


**Figure 4.** Comparative analysis of six differential serum miRNAs in the TG.

**Figure 4** shows the comparative analysis of six different serum miRNA in the TG. The levels of miR-31, miR-93, miR-124-3p, miR-143, and miR-146a in patients with AD were 1.16 ± 0.22, 1.25 ± 0.21, 0.73 ± 0.18, 0.82 ± 0.09, and 2.75 ± 0.12, respectively, which were significantly lower than 2.19 ± 0.26, 2.83 ± 0.39, 1.36 ± 0.24, 1.97 ± 0.21, and 7.53 ± 0.98 in the healthy population of the control group. The level of miR-218-5p in patients with AD was 1.87 ± 0.22, which was significantly higher than that in the healthy population of the control group. Studies have indicated that miR-31 plays a crucial role in various biological processes, including cell proliferation, differentiation, and apoptosis. In the nervous system, miR-31 is believed to contribute to the survival and maintenance of neuronal function. Therefore, the reduction in miR-31 levels may lead to neuronal damage and death, which is one of the important characteristics of AD pathology. miR-93 plays a major role in neuroinflammation and immune responses. Neuroinflammation is considered a visible pathological feature of AD, and miR-93 is involved in the regulation of inflammatory

responses by modulating the expression of inflammation-related genes. The decrease in miR-93 levels may lead to abnormally enhanced inflammatory responses, thereby exacerbating the progression of AD. miR-124-3p is an important brain-specific miRNA, widely involved in neuronal differentiation, synapse formation, and neural conduction. Its visible decrease in expression may affect neuronal function and synchrony, further exacerbating cognitive impairment. miR-143 plays a visible role in lipid metabolism and oxidative stress responses. Lipid metabolism disorders and oxidative stress are two key factors in AD pathology. The reduction in miR-143 levels may lead to lipid metabolism disorders, exacerbate oxidative stress, and subsequently lead to the accumulation of beta-amyloid (Aβ) and tau protein lesions, which are classic pathological pathways of AD. miR-146a plays a visible role in regulating innate immune responses and inflammatory responses. Inflammation is one of the important pathological features of AD, and the decrease in miR-146a levels may lead to immune response dysregulation and persistent inflammation, thereby accelerating neuronal damage and death. In contrast, the level of miR-218-5p is markedly elevated in AD patients, which may be related to neurodegenerative changes and neuronal damage. The upregulation of miR-218-5p is associated with the degeneration of neuronal axons and the loss of synapses. In AD, the increase in miR-218-5p may further damage the structure and function of neurons, accelerating disease progression.

Additionally, a comparative analysis of the six differential serum miRNAs was conducted in the VG (**Figure 5**).

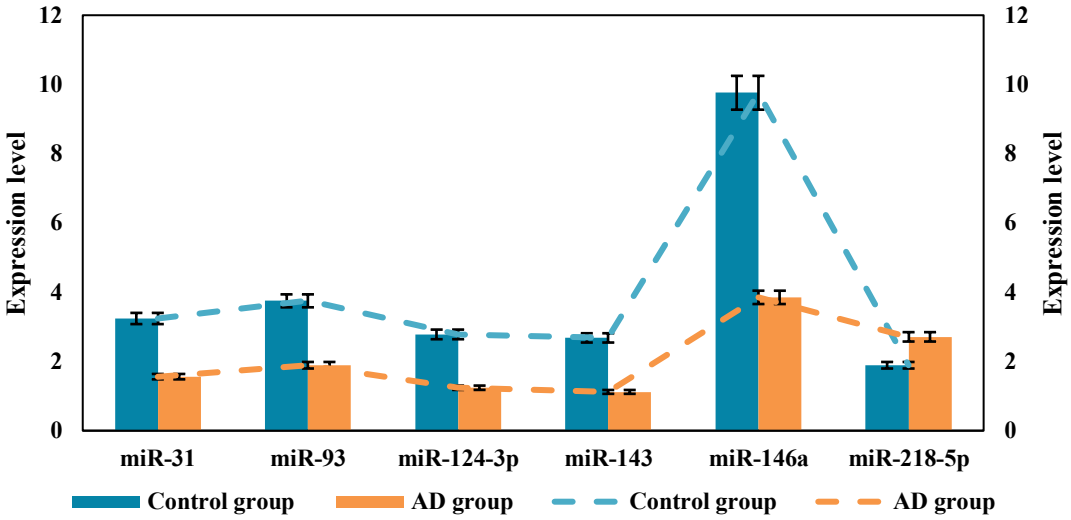


**Figure 5.** Comparative analysis of six differential serum miRNAs in the VG.

**Figure 5** shows the levels of six different serum miRNA in the VG, which is consistent with the results of the TG. The levels of miR-31, miR-93, miR-124-3p, miR-143, and miR-146a in patients with AD were 1.56 ± 0.22, 1.89 ± 0.21, 1.24 ± 0.18, 1.12 ± 0.18, and 3.85 ± 0.27, respectively, which were significantly lower than 3.24 ± 0.32, 3.75 ± 0.27, 2.78 ± 0.22, 2.68 ± 0.28, and 9.76 ± 0.38 in the control group. The level of miR-218-5p in patients with AD was 2.71 ± 0.24, which was significantly higher than that in healthy people in the control group (1.89 ± 0.19), which further consolidates the research results. This consistency verifies the reliability of the

preliminary findings of the study, indicating that these miRNA expression changes are stable and repeatable in AD patients. Therefore, these miRNAs can not only serve as potential biomarkers for the early diagnosis of AD but also provide important clues for understanding the pathological mechanisms of AD. The results from the VG further indicated that the reduction of miR-31, miR-93, miR-124-3p, miR-143, and miR-146a may be involved in neuronal survival, inflammation regulation, lipid metabolism, and immune response, while the increase of miR-218-5p may be related to neurodegenerative changes. These results aid in the development of personalized therapeutic strategies targeting these small RNAs, thereby slowing down or reversing the progression of AD. The consistency of the VG with the TG results emphasizes the scientific credibility of these findings, further supporting the potential for incorporating these miRNAs into clinical practice, providing a solid data foundation for the early screening, diagnosis, and treatment of AD.

The decrease of miR-31, miR-93, miR-124-3p, miR-143, and miR-146a, and the increase of miR-218-5p in the serum of AD patients reflect complex pathological mechanisms involving multiple aspects such as neuronal survival, immune regulation, metabolic balance, and neural conduction. The changes in these miRNAs not only provide potential biomarkers for the early diagnosis of AD but also offer important targets for the development of new intervention and treatment methods. By further studying and understanding the functions of these miRNAs, it is hoped to reveal more pathological mechanisms of AD, thus more effectively addressing this global health challenge.

### 4.3. Validation and evaluation of AD prediction model

After screening the differential indicators between AD and healthy populations, the diagnostic effect of the six differential serum miRNAs and the established model was validated, and the model's performance was assessed using Sen, Spe, and Acc (**Figure 6**).

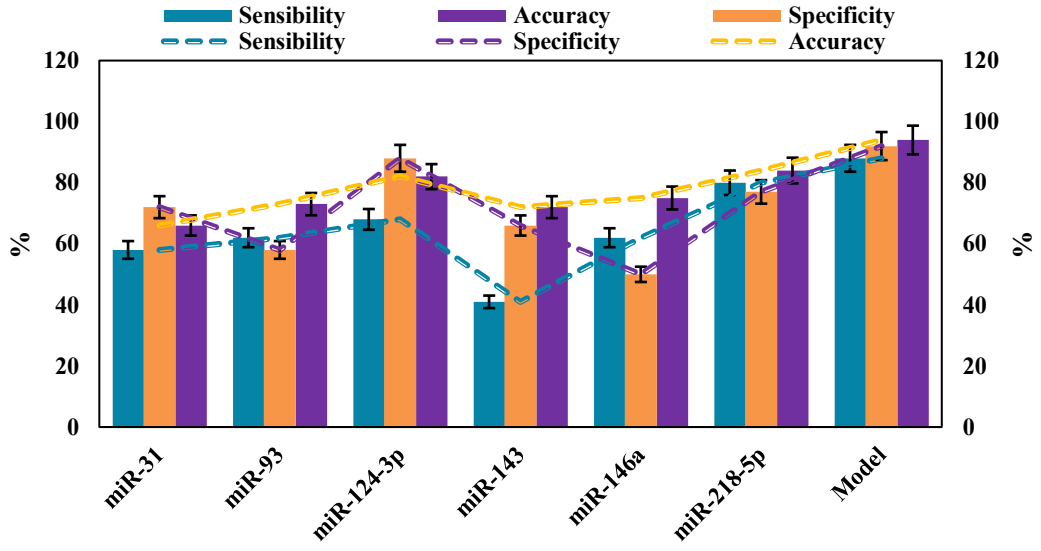


**Figure 6.** Validation and effectiveness evaluation of the AD prediction model.

**Figure 6** shows the validation and effect evaluation of the prediction model of AD. Among the six different serum miRNA, miR-124-3p and miR-218-5p showed higher Sen, Spe, and Acc in diagnosing AD. The Sen, Spe, and Acc of miR-124-3p in diagnosing AD were 68%, 88%, and 82%, respectively, and the Sen, Spe, and Acc of miR-218-5p in diagnosing AD were 80%, 77%, and 84%, respectively. The combined detection model incorporating clinical indicators (MMSE, MoCA, ADAS-cog, ADL) was superior to the six differential serum miRNAs alone, achieving the best diagnostic results. These findings not only emphasize the importance of miRNA in AD diagnosis but also highlight the potential of multi-level, comprehensive assessment methods in improving diagnostic Acc. Firstly, the high Sen and Spe of miR-124-3p and miR-218-5p in diagnosing AD indicate that they can effectively distinguish between AD patients and healthy control group. miR-124-3p, as a brain-specific miRNA, plays a key role in neuronal differentiation, synaptic plasticity, and neural conduction. Its visible decrease in AD patients may reflect extensive damage to neuronal structure and function. The high levels of miR-218-5p are closely related to neurodegenerative changes and axonal pathology, which manifest in AD pathology as neuronal degeneration and synaptic loss. Measuring the levels of these two miRNAs can provide a sensitive and specific early diagnostic tool, offering valuable reference for clinical practice. However, relying solely on miRNA for AD diagnosis may have certain limitations. Therefore, clinical cognitive assessment indicators MMSE, MoCA, ADAS-cog, and ADL were introduced for combined detection. MMSE and MoCA assess global cognitive functions, including memory, orientation, attention, and executive functions, providing a broad cognitive function profile. ADAS-cog assesses memory, language, and orientation in more detail, closely related to the core symptoms of AD. ADL assesses the independence of daily living activities, reflecting the patient's functional status and self-care ability. These clinical indicators can comprehensively and systematically reveal cognitive and functional impairments in AD patients, and these assessment tools have been proven to be highly reliable and valid in past research. Combining serum miRNA with clinical assessment indicators to form a combined detection model not only markedly improves the Acc of AD diagnosis but also provides a more comprehensive method for disease assessment. Specifically, the molecular biological information of miRNA combined with clinical cognitive and functional assessment data can better reflect the complex pathological mechanisms of AD. For example, if a patient shows a significant decrease (below the normal range) in the detection of miR-124-3p, and at the same time has a low score (below 24 and 26 respectively) in MMSE and MoCA, it indicates that the patient may have cognitive dysfunction. If the ADAS-cog score is high (higher than 22 points) and the ADL score is low (lower than 24 points), then these indicators jointly support the diagnosis of AD. In this case, the decrease of miR-124-3p can correspond to the low score of MMSE or MoCA, which further confirms the degree of neuronal damage and cognitive impairment. The increase of miR-218-5p corresponds to the low score of ADAS-cog or ADL, suggesting that neurodegenerative diseases and activities of daily living are significantly reduced. The advantage of this combined detection model is that it can make use of the complementary advantages of various biomarkers and clinical data to improve the Sen and Spe of diagnosis. For example, if using a single miRNA can't fully distinguish the disease, it can be combined with cognitive function

test and assessment of daily living ability, and the comprehensive analysis of multidimensional data can significantly improve the diagnostic Acc. In addition, this comprehensive evaluation can also help identify the individual differences of different patients and provide an important basis for individualized treatment.

Subsequently, this article conducted ROC curve analysis to predict AD with the six differential serum miRNAs (**Figure 7**).

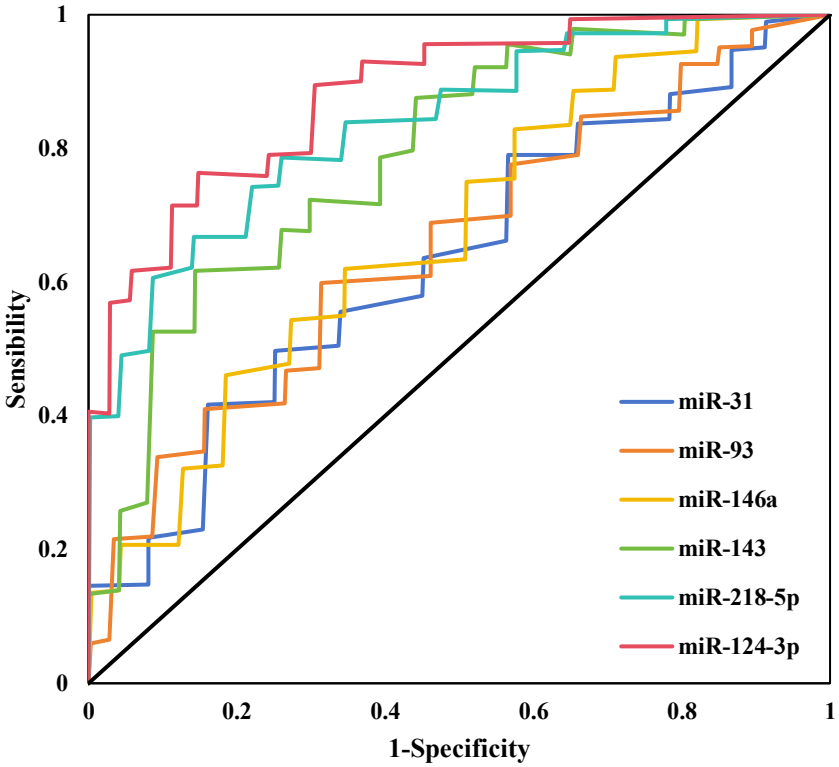


**Figure 7.** ROC curve analysis for predicting AD with six differential serum miRNAs.

miR-124-3p and miR-218-5p were found to have better diagnostic efficacy for AD. The ROC curve is an important tool for measuring the performance of diagnostic tests, and it visually assesses the effectiveness of diagnostic markers by plotting the relationship between Sen (true positive rate) and Spe (false positive rate). The ROC curve analysis revealed that miR-124-3p and miR-218-5p had larger areas under the curve (AUC) when distinguishing between AD patients and healthy control group, indicating that these two miRNAs possess high diagnostic power in the diagnosis of AD.

To validate the evaluation effect of the model, ROC curve analysis for predicting AD was conducted on the TG, VG, TG + VG, and the total sample (**Figure 8**).

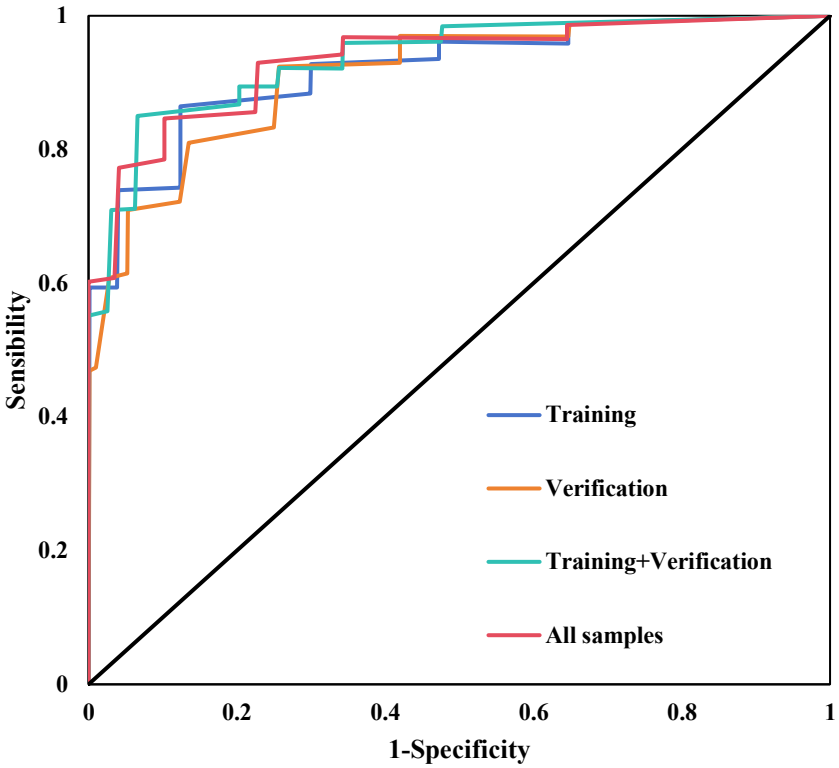


**Figure 8.** ROC curve analysis for predicting AD.

It was found that TG, VG, TG + VG, and the total sample combined detection model for predicting AD exhibited good predictive effects. This indicates that the joint use of serum miRNA and clinical treatment scores can enhance the predictive Acc of AD. The detection of serum miRNA can provide early diagnostic and predictive information for AD. Concurrently, clinical treatment scores (MMSE, MoCA, ADAS-cog, ADL scores) can reflect the cognitive function and daily living abilities of AD patients, and this information is also very important for the diagnosis and prediction of AD. Thus, the joint use of serum miRNA and clinical treatment scores can provide more comprehensive predictive information for AD, thereby improving the predictive Acc of AD. Moreover, the good predictive effect of TG, VG, TG + VG, and the total sample combined detection model indicates that this predictive model possesses good stability and generalization ability, making it applicable to different sample sets. Overall, the results of this article indicate that the joint use can enhance the predictive Acc of AD, offering a new method for the early diagnosis and prediction of AD.

miR-124-3p and miR-218-5p demonstrated high Sen, Spe, and Acc in the diagnosis of AD in this article, while the combined detection model incorporating clinical indicators (MMSE, MoCA, ADAS-cog, ADL) further improved diagnostic outcomes. By integrating molecular biological data with clinical assessments, this multi-level assessment method not only provides a more comprehensive analysis of the disease but also lays a solid foundation for personalized medicine and early intervention. This innovative combined detection method is expected to be widely applied in clinical practice, thereby improving the diagnosis, management, and treatment outcomes for AD patients.

The high Sen and Spe of serum miRNAs (miR-124-3P and miR-218-5p) in the diagnosis of AD found in this study provide a new tool for early screening. By detecting the changes of these miRNA levels early, individuals at high risk can be identified before symptoms appear, thus creating conditions for early intervention. Early identification and intervention are very important for delaying the disease process and improving the QoL of patients. In clinical practice, combining miRNA detection with traditional cognitive function assessment (such as MMSE, MoCA, ADAS-cog, and ADL) can find those pre-ad patients who have not shown obvious symptoms earlier. For example, for an elderly person whose level of miR-124-3p is significantly reduced, even though his current cognitive function score is still within the normal range, considering that miR-124-3p is closely related to neuronal function, this result suggests that he may have a higher risk of developing AD in the future. Therefore, such individuals can be given priority in the regular monitoring list and receive more frequent cognitive function review. After early identification of high-risk individuals, a series of targeted intervention measures can be taken, including but not limited to lifestyle adjustment, drug prevention, and cognitive training. For example, for patients with elevated levels of miR-218-5p, in addition to routine clinical evaluation, special cognitive training programs can be recommended to slow down cognitive decline. In addition, according to the specific mechanism of abnormal expression of miRNA, researchers can also explore the development of new drugs or therapies, aiming at correcting or alleviating these abnormal expressions, so as to achieve the purpose of prevention or treatment.

## 5. Conclusion

In this article, through a comprehensive analysis of clinical data, serum miRNA detection, and cognitive and functional assessment indicators of AD patients and healthy controls, the visible value of serum miRNA detection in AD diagnosis and the superiority of its combined application with clinical indicators (MMSE, MoCA, ADAS-cog, ADL scores) were revealed. No obvious distinctions were found in general data such as age, gender, years of education, diabetes, and hypertension between AD patients and healthy controls, suggesting that while these factors may influence the risk of AD, they are not decisive factors. However, in cognitive and functional assessments, the performance of AD patients was markedly lower than that of healthy controls, reflecting the severe impact of AD on cognitive function and daily life. Serum miRNA detection results showed that AD patients had lower levels of miR-31, miR-93, miR-124-3p, miR-143, and miR-146a, and higher levels of miR-218-5p, with these changes being related to pathological mechanisms such as neuronal survival, immune regulation, lipid metabolism, and neural conduction. By assessment of Sen, Spe, and Acc, miR-124-3p and miR-218-5p exhibited excellent performance in AD diagnosis, and when they were used in combination with clinical indicators, the diagnostic effect was even more pronounced. The stability and generalization ability of TG, VG, TG + VG, and the total sample combined detection model were good, indicating that this method has good predictive effects in different sample sets.

The limitation of this study is the limited sample size, which limits the statistical power and affects the universal applicability of the results. Additionally, the research

objects were mainly from specific regions, so the results may not be fully extended to other people. In the future, it is necessary to increase the sample size and diversity and improve the external validity of the research results by including more diverse participants. At the same time, a long-term follow-up study should be carried out to understand the change of miRNA level with time and its relationship with disease progression, and to deeply explore the relationship between miRNA and the pathophysiology of AD, so as to provide theoretical basis for developing new biomarkers or therapeutic targets.

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