Expression of miR-28-3p in patients with Alzheimer's disease before and after treatment and its clinical value

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Abstract. Expression of miR-28-3p in patients with Alzheimer's disease (AD) before and after treatment and clinical value of miR-28-3p were determined. There were three groups: 68 AD patients treated with donepezil combined with basic therapy in The People's Hospital of Shouguang collected as an AD group, 70 patients with mild cognitive impairment (MCI) as an MCI group, and 75 healthy people as a normal group. Serum miR-28-3p was detected by qRT-PCR. The Montreal cognitive assessment scale (MoCA), mini mental state examination scale (MMSE), activities of daily living scale (ADL) and homocysteine (Hcy) were adopted to assess patients before and after treatment. miR-28-3p in normal group was significantly lower than that in other two groups, and miR-28-3p in MCI group was significantly lower than that in AD group ($P<0.001$). miR-28-3p correlated with the course and severity of patients. miR-28-3p in AD group after treatment was significantly lower than that before treatment ($P<0.001$). ADL and Hcy of AD patients after treatment were significantly lower than before treatment ($P<0.05$), and MMSE and MoCA after treatment were significantly higher than before treatment ($P<0.05$). Before and after treatment, miR-28-3p was significantly positively correlated with ADL score and Hcy level, but negatively correlated with MMSE score and MoCA score. Analysis of the working characteristic curve of the patients indicated that miR-28-3p can be used for diagnosis of AD patients. Donepezil therapy may reduce miR-28-3p level to alleviate the symptoms of AD patients, and miR-28-3p level can be used as an early diagnosis and prognosis evaluation of AD patients.

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Introduction

Alzheimer's disease (AD) is a common neurodegenerative disease in the elderly, mainly manifested as cognitive dysfunction, memory decline, social disorders, and behavioral abnormalities (1,2). AD patients account for more than 80% of dementia cases among people aged over 65 years in the world (3). According to previous reports, it was estimated that 47.5 million people worldwide would suffer from dementia in 2015, and the incidence rate of AD shows an increasing trend with changes in lifestyle (4,5). Previous studies showed that the loss of cholinergic nerve cells in basal forebrain and the increase of acetylcholinesterase (ACh E) activity in AD patients lead to the decrease of neurotransmitter acetylcholine (ACh). Therefore, the therapeutic purpose was mainly achieved by inhibiting ACh E activity (6). Donepezil, one of acetylcholinesterase inhibitors, is a commonly used drug for the treatment of AD and has a good effect, which can significantly improve the cognitive function of AD patients (7).

At present, many researchers are looking for biomarkers that can detect the disease and monitor the course of the disease, especially before symptoms appear or in early stages (8,9). In recent years, the exploration of peripheral blood microRNA (miRNA) has become a research hot-spot. Some studies have reported that there are some changes in miRNA levels in postmortem brain studies and changes in miRNA in whole blood, plasma or serum. Therefore, the detection of the changes of miRNA in AD has high clinical value for the early diagnosis and curative effect evaluation of the disease (10-13). miRNA belongs to an endogenous non-coding protein RNA gene (with the length of about 18-24 nucleotides), which can play an important role in a variety of diseases. For example, it mediates the regulation of protein production by interacting with target messenger RNA (mRNA) (14). miR-28-3p is a miRNA that targets a variety of cancer-related genes and can participate in epithelial-mesenchymal transition (EMT), cell proliferation, invasion and migration (15,16). Moreover, studies have showed that miR-28-3p was significantly downregulated in nasopharyngeal carcinoma tissues and could accelerate the invasion and migration of nasopharyngeal carcinoma cells (16). Hong *et al* (17) showed that miR-28-3p was significantly upregulated in AD APP/PS1 transgenic mouse model. Paltsev *et al* (18) reported that miR-132 was involved

in the pathogenesis of senile dementia by detecting the serum miR-132 level of dementia patients, and it could be used as a detection index for early diagnosis and treatment of senile dementia. However, there is no research report on whether miR-28-3p plays the same role in AD patients.

In this study, miR-28-3p in serum of AD patients before and after treatment was determined, and its correlation with clinical indicators was analyzed, to provide certain reference for early diagnosis and treatment of AD.

Patients and methods

Clinical general data. Altogether 68 AD patients admitted to The People's Hospital of Shouguang (Weifang, China) were collected as the AD group, including 31 males and 37 females, with an average age of 70.12±2.09 years, with the MoCA score of 14.67±2.01 and MMSE score of 15.48±1.68. Further 70 patients with early cognitive impairment were treated as the MCI group, including 32 males and 38 females, with an average age of 69.68±2.11 years, with the MoCA score of 20.76±1.69 and MMSE score of 22.67±0.73. Additionally 75 healthy people who underwent physical examination in The People's Hospital of Shouguang during the same period were selected as the normal group, including 34 males and 41 females, with an average age of 69.47±1.98 years, with the MoCA score of 27.82±1.42 and MMSE score of 28.03±1.52. This study was approved by the Ethics Committee of The People's Hospital of Shouguang. The patients and their families were informed in advance and signed a complete informed consent form.

Inclusion criteria: Patients with good compliance and complete clinical data; the educational level of the patients was primary school or above; all patients received routine examinations on urine routine, blood routine, electrocardiogram, liver and kidney functions after admission; patients were accompanied by their family when they were admitted to hospital.

Exclusion criteria: Patients with previous history of mental illness, liver dysfunction, severe organ lesions, craniocerebral trauma, and autoimmune system defects; patients who had a history of drug dependence and had taken antidepressants; patients who had cognitive impairments.

Treatment methods. Patients with AD were treated with donepezil as basic therapy. After admission, patients were given drugs to improve cerebral blood circulation and promote brain cell metabolism. According to the specific conditions of the patients, symptomatic treatment was given, and the rest condition and diet of the patients were adjusted. Patients should take moderate rehabilitation exercise and get adequate sleep. At the same time, donepezil hydrochloride tablets (Chinese Eisai Pharmaceutical Co., Ltd., SFDA approval no. H20050978) were given on this basis, once a day and 5 mg for the first time. After one month of continuous treatment, the stable blood drug concentration and clinical response to the drug were evaluated. According to the specific situation, the dosage was increased to 10 mg once a day for one year.

Detection index. A total of 4 ml of fasting elbow venous blood were collected from the three groups of patients after admission and from AD patients after discharge. After coagulation for 60 min (20-25˚C), each sample was centrifuged at 1,006.2 x g for 10 min to collect the upper serum. The centrifugation radius was 10 cm and the centrifugation temperature was 4˚C. The collected serum was placed in a refrigerator at -80˚C for testing. The level of homocysteine (Hcy) was detected by cyclic enzyme method (19). The level of miR-28-3p was detected by real-time fluorescence quantitative PCR (qRT-PCR). Total RNA in serum was extracted according to the instructions of TRIzol kit (Shanghai Sangon Biotech). The template RNA was digested and treated with DNase I (Shanghai Sangon Biotech) to eliminate DNA contamination. The ultraviolet spectrophotometer (Beijing Up General Technology Co., Ltd.) was used to measure the purity and concentration, and OD 260/280 value more than 1.8 was considered usable. The RNA sample was then reverse transcribed into cDNA, and the operation was strictly carried out in accordance with the instructions of cDNA reverse transcription kit (Takara Bio). qRT-PCR was used for detection. The qRT-PCR reaction was carried out on ABI 7500 system (Applied Biosystems) using SYBR-Green PCR Master Mix (Thermo Fisher Scientific). PCR reaction conditions were as follows: pre-denaturation at 95˚C for 10 min, denaturation at 95˚C for 10 sec, and annealing and extension at 60˚C for 60 sec, for a total of 40 cycles. Primers for this experiment were designed by Premier 5.0 (Premier) and generated by Tianjin Saierbio Co., Ltd. U6 was used as internal reference. The specific primer sequences are shown in Table I. The above system configuration was strictly in accordance with the instructions. The level of miR-28-3p was calculated by $2^{-\Delta Ct}$.

Observation indicators. i) The miR-28-3p levels of patients in MCI group, normal group and AD group were compared. ii) The relationship between miR-28-3p level and clinical data in AD patients was compared. iii) Montreal cognitive assessment scale (MoCA) (20) and mini mental state examination scale (MMSE) (21) were used to evaluate the cognitive function and mental state of MCI group, normal group and AD group before treatment and 3 months after treatment, with a total score of 30 points. The high score was closely related to the good cognitive function. The levels of activity of daily life (ADL) and Hcy were observed before treatment and 3 months after treatment in AD group. ADL was used to evaluate the patients' activity of life. The high score was closely related to poor activity of life (22). iv) The correlation among serum miR-28-3p level, score, and Hcy level in AD patients was analyzed. v) The clinical value of miR-28-3p in diagnosing AD patients was explored.

Statistical analysis. SPSS 20.0 (IBM Corp.) was used for statistical analysis. GraphPad Prism 7 (GraphPad Software Co., Ltd.) was used to illustrate the collected data. The count data was expressed as n (%), and chi-square test was used for inter-group comparison. The measurement data was expressed as mean ± standard deviation (mean ± SD). The t-test was used for comparison between the two groups, one-way ANOVA was used for comparison among multiple groups, which was expressed as F. LSD-t-test was used for post-event analysis, Pearson's analysis was used for bivariate correlation analysis, and receiver operating characteristic curve (ROC) was used for diagnosis of AD patients. $P<0.05$, was considered a statistically significant difference.

Table I. Primer sequences.

Gene	Upstream	Downstream
miR-28-3p	CGCGCACTAGATTGTGAGCT	AGTGCAGGGTCCGAGGTATT
U6	CGACAAGACGATCCGGGTAAA	GGTTGAGGAGTGGGTCGAAG

Table II. Comparison of general clinical data of three groups [mean ± SD, n (%)].

Clinical data	AD group (n=68)	MCI group (n=70)	Normal group (n=75)	F/χ^2 value	P-value
Sex				0.002	0.999
Male	31 (45.59)	32 (45.71)	34 (45.33)		
Female	37 (54.41)	38 (54.29)	41 (54.67)		
Average age (years)	70.12±2.09	69.68±2.11	69.47±1.98	1.832	0.163
Smoking				0.273	0.873
Yes	24 (35.29)	22 (31.43)	24 (32.00)		
No	44 (64.71)	48 (68.57)	51 (68.00)		
Drinking				3.496	0.174
Yes	47 (69.12)	48 (68.57)	42 (56.00)		
No	21 (30.88)	22 (31.43)	33 (44.00)		
Body mass index (kg/m^2)	23.49±3.27	24.07±3.31	23.95±4.06	0.505	0.604
Complication					
Diabetes	27 (39.71)	25 (35.71)	22 (29.33)	1.736	0.420
Hypertension	36 (52.94)	33 (47.14)	37 (49.33)	0.473	0.790
TG (mmol/l)	1.68±0.72	1.61±0.67	1.57±0.77	0.420	0.657
TC (mmol/l)	5.29±1.12	5.15±1.04	5.10±0.99	0.620	0.5391
HDL-C (mmol/l)	1.30±0.56	1.53±0.62[a]	1.91±0.87[a,b]	13.460	<0.001
LDL-C (mmol/l)	2.98±1.13	2.82±1.07	2.54±1.10[a]	2.951	0.055
MoCA score	14.67±2.01	20.76±1.69[a]	27.82±1.42[a,b]	1.055	<0.001
MMSE score	15.48±1.68	22.67±0.73[a]	28.03±1.52[a,b]	1.491	<0.001

[a]P<0.05, compared with AD group; [b]P<0.05, compared with MCI group. AD, Alzheimer's disease; MCI, mild cognitive impairment; TG, triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; MoCA, Montreal cognitive assessment scale; MMSE, mini mental state examination scale.

Results

Comparison of general clinical data of three groups. There was no significant difference in sex, average age, smoking and drinking, body mass index, complications, triglyceride (TG) or total cholesterol (TC) among AD, MCI and normal groups (P>0.05). The high density lipoprotein cholesterol (HDL-C), MoCA score and MMSE score of AD group were significantly lower than those of the other two groups, while the low density lipoprotein cholesterol (LDL-C) of AD group was higher than that of the normal group (P<0.05) (Table II).

Comparison of miR-28-3p levels in three groups. Comparing the serum miR-28-3p levels of AD group, MCI group and normal group, as shown in Fig. 1, it was clear that the miR-28-3p content of normal group was significantly lower than that of AD group and MCI group (P<0.05), while the serum miR-28-3p level of MCI group was significantly lower than that of AD group patients (P<0.001).

Relationship between serum miR-28-3p level and clinical data in AD patients. The clinical data of AD patients were collected as shown in Table III. The miR-28-3p level had no significant correlation with sex, age, smoking or drinking (P>0.05), however, miR-28-3p level had a significant correlation with disease course and severity (P<0.05).

Comparison of serum miR-28-3p levels of AD patients before and after treatment. Comparison of the serum miR-28-3p level of AD patients before and after treatment in Fig. 2, indicated that the serum miR-28-3p level of AD patients before treatment was 3.07±0.71, the serum miR-28-3p level after

Table III. Relationship between serum miR-28-3p level and clinical characteristics in AD patients (mean ± SD).

Clinical characteristics	n	miR-28-3p	F/t value	P-value
Sex			0.487	0.628
Male	37	3.11±0.65		
Female	31	3.04±0.51		
Age			0.785	0.435
<65 years	20	3.02±0.45		
≥65 years	48	3.12±0.49		
Course of disease			3.058	0.003
<2 years	42	2.89±0.44		
≥2 years	26	3.25±0.49		
Smoking			0.405	0.687
Yes	24	3.10±0.46		
No	44	3.05±0.50		
Drinking			1.101	0.275
Yes	47	3.15±0.51		
No	21	3.01±0.42		
Severity			10.280	<0.001
Mild	22	2.75±0.37		
Medium	31	3.09±0.42		
Severe	15	3.37±0.47		

AD, Alzheimer's disease.

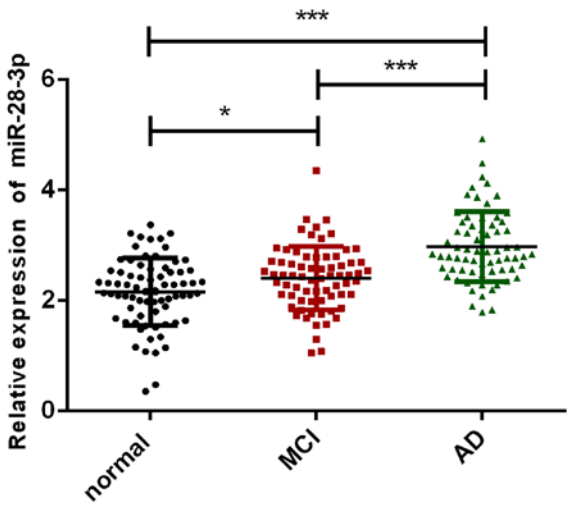

Figure 1. Comparison of serum miR-28-3p levels among the three groups. The miR-28-3p level in normal group was significantly lower than that in AD group and MCI group (P<0.05), while the serum miR-28-3p level in MCI group was significantly lower than that in AD group (P<0.001). *P<0.05 indicates the comparison between the two groups; ***P<0.001 indicates the comparison between the two groups. AD, Alzheimer's disease; MCI, mild cognitive impairment.

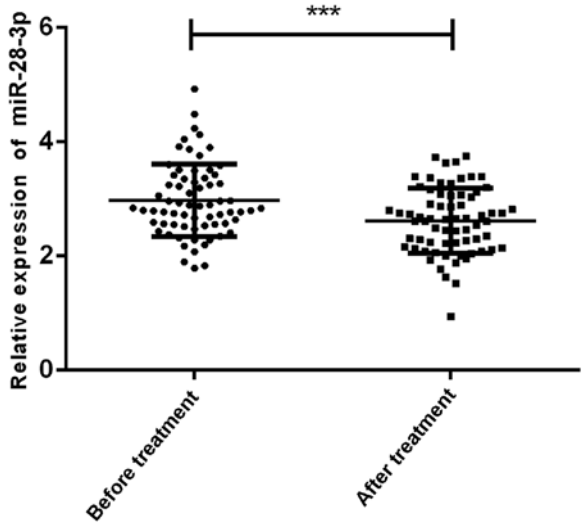

Figure 2. Serum miR-28-3p level of AD patients before and after treatment. The level of serum miR-28-3p in AD patients after treatment was significantly lower than that before treatment (P<0.001). ***P<0.001 indicates the comparison between the two groups. AD, Alzheimer's disease.

treatment was 2.55±0.61, and the serum miR-28-3p level after treatment was significantly lower than that before treatment (P<0.001).

Changes of score and Hcy level of AD patients before and after treatment. ADL score, MMSE score and Hcy level of AD after treatment were compared. As shown in Table IV, ADL score and Hcy level of AD patients after treatment were significantly lower than those before treatment (P<0.05). MMSE score and MoCA score after treatment were significantly higher than those before treatment (P<0.05).

Correlation of serum miR-28-3p level with score and Hcy level and clinical indicators of AD patients before treatment.

Table IV. Comparison of clinical indexes of AD patients before and after treatment (mean ± SD).

Treatment stage	ADL score	MMSE score	MoCA score	Hcy (μmol/l)
Before treatment	47.76±5.13	15.48±1.68	14.67±2.01	19.21±7.68
After treatment	43.23±4.58	20.76±1.87	19.89±1.88	11.52±8.02
t value	5.432	17.320	15.640	5.711
P-value	<0.001	<0.001	<0.001	<0.001

AD, Alzheimer's disease; MoCA, Montreal cognitive assessment scale; MMSE, mini mental state examination scale; ADL, daily living scale; Hcy, homocysteine.

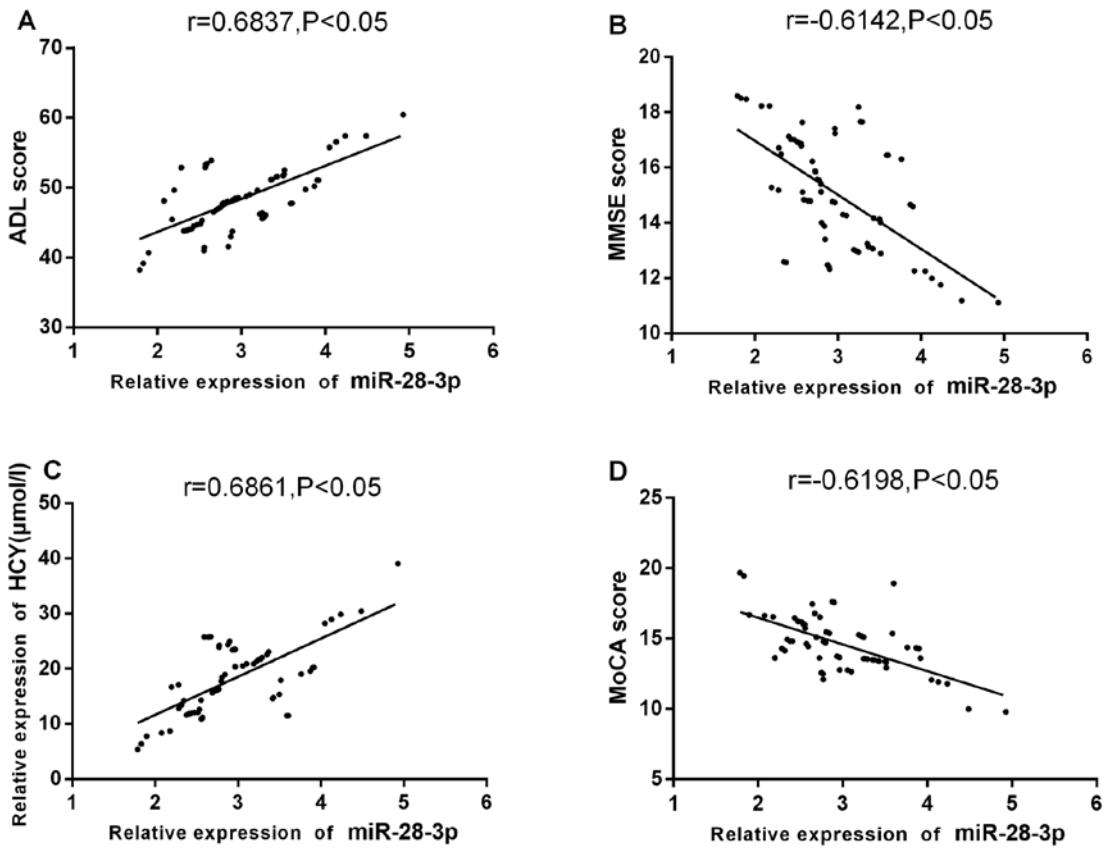

Figure 3. Correlation between serum miR-28-3p level and clinical indexes in AD patients before treatment. Pearson's analysis showing that, (A) The serum miR-28-3p level of AD patients before treatment was significantly positively correlated with ADL score (r=0.6837, P<0.05); (B) the serum miR-28-3p level of AD patients before treatment was significantly negatively correlated with MMSE score (r=-0.6142, P<0.05); (C) before treatment, the serum miR-28-3p level of AD patients was significantly positively correlated with Hcy level (r=0.6861, P<0.05); (D) miR-28-3p level was significantly negatively correlated with MoCA score (r=-0.6198, P<0.05). AD, Alzheimer's disease; ADL, daily living scale; MoCA, Montreal cognitive assessment scale; Hcy, homocysteine; MMSE, mini mental state examination scale.

Pearson's analysis showed that serum miR-28-3p level was significantly positively correlated with ADL score and Hcy level before treatment (r=0.6837, 0.6861, P<0.05), and miR-28-3p level was significantly negatively correlated with MMSE score and MoCA score (r=-0.6142, -0.6198, P<0.05) (Fig. 3).

Correlation of serum miR-28-3p level with score and Hcy *correlation between serum miR-28-3p level and clinical indicators in AD patients after treatment*. Pearson's analysis showed that serum miR-28-3p level was significantly positively correlated with ADL score and Hcy level after treatment (r=0.6464, 0.6824, P<0.05), and miR-28-3p level was significantly negatively correlated with MMSE score and MoCA score (r=-0.6598, -0.6524, P<0.05) (Fig. 4).

Value of miR-28-3P in diagnosis of AD patients. AD group and normal group were selected to observe the diagnostic value of miR-28-3p, as shown in Fig. 5. The sensitivity of miR-28-3p for distinguishing AD patients from normal subjects was 79.41%, the specificity was 74.67%, the AUC was 0.8306 (0.7649-0.8963), and the cut-off value was 2.52. Patients in AD group and MCI group were selected, as shown in Fig. 6. As shown miR-28-3p had sensitivity of 64.71%, specificity of 75.71%, AUC of 0.7506 (0.6704-0.8309), and the

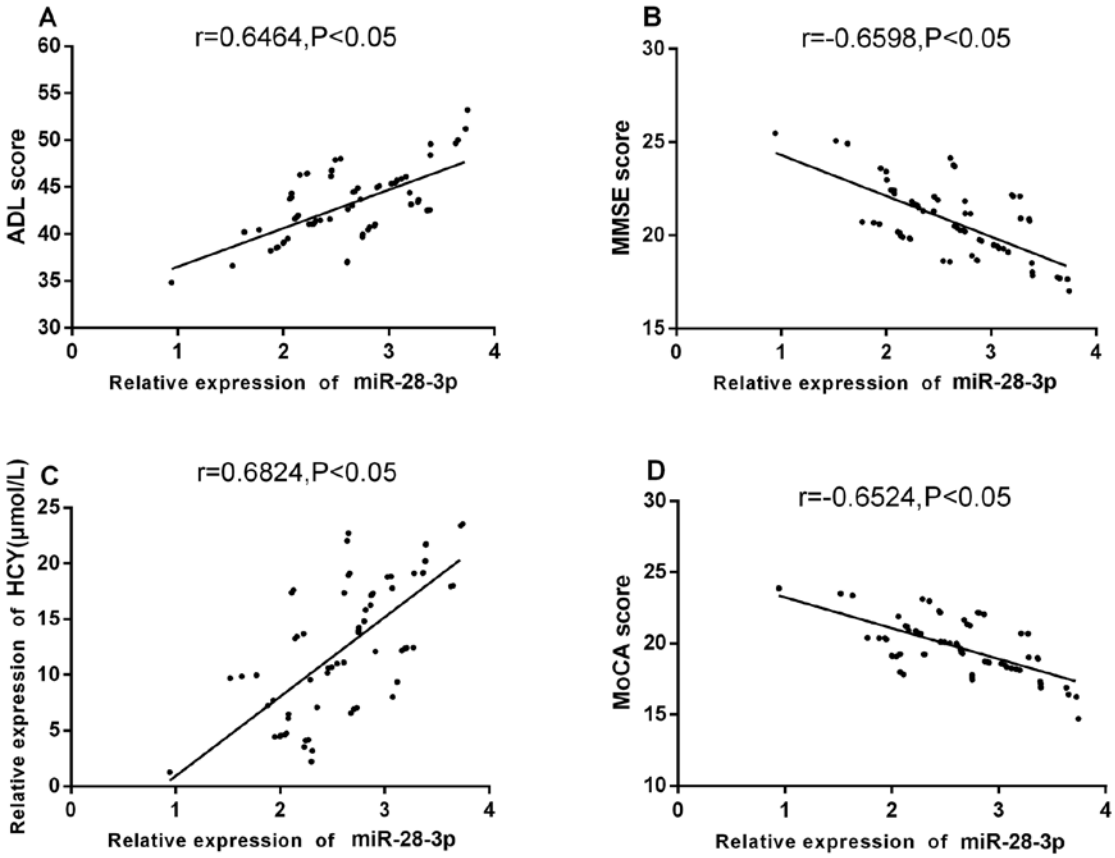

Figure 4. Correlation between serum miR-28-3p level and clinical indexes in AD patients after treatment. Pearson's analysis showing that, (A) after treatment, the serum miR-28-3p level of AD patients was significantly positively correlated with ADL score (r=0.6464, P<0.05); (B) miR-28-3p level was significantly negatively correlated with MMSE score (r=-0.6598, P<0.05); (C) after treatment, the serum miR-28-3p level of AD patients was significantly positively correlated with Hcy level (r=0.6824, P<0.05); (D) miR-28-3p level was significantly negatively correlated with MoCA score (r=-0.6524, P<0.05). AD, Alzheimer's disease; ADL, daily living scale; MoCA, Montreal cognitive assessment scale; Hcy, homocysteine; MMSE, mini mental state examination scale.

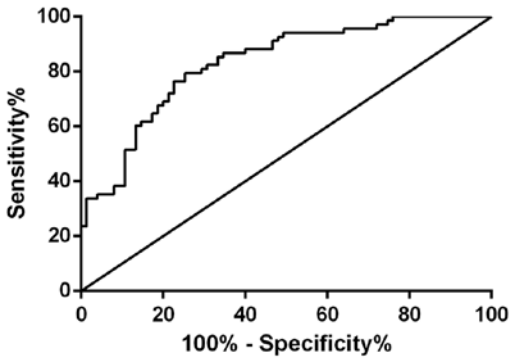

Figure 5. Value of miR-28-3p in diagnosing AD patients. The sensitivity, specificity, AUC and cut-off of miR-28-3p for distinguishing AD patients from normal subjects were 79.41%, 74.67%, 0.8306 (0.7649-0.8963), and 2.52 respectively. AD, Alzheimer's disease.

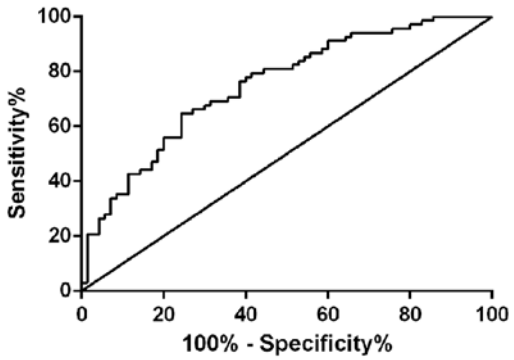

Figure 6. Value of miR-28-3p in diagnosing AD patients. The sensitivity, specificity, AUC and cut-off of miR-28-3p for distinguishing AD patients from MCI patients were 64.71%, 75.71%, 0.7506 (0.6704-0.8309), and 2.72 respectively. AD, Alzheimer's disease; MCI, mild cognitive impairment.

cut-off value of 2.72 for distinguishing AD patients from MCI patients.

Discussion

Pathogenesis of AD is relatively complex and correlated with inheritance, age, cytoskeleton changes, inflammatory response, nerve transmission obstruction and other factors. Most patients cannot receive timely treatment after suffering from the illness, which will lead to cognitive impairment of different degrees. The occurrence of the disease will bring economic burden and seriously affect the quality of life of patients (18). The pathological features of AD are mainly vascular amyloidosis, aggregation of abnormally phosphorylated tau protein in cells, formation of neurofibrillary tangles (NFTs), senile plaques formed by deposition of β-amyloid protein (Aβ), loss of neuronal cells in hippocampus and cerebral cortex (23,24). Donepezil can specifically hinder the degradation of ACh in the

brain, reduce the atrophy of brain tissue, and increase the level of choline in the cerebral cortex, thus improving the cognitive function of patients. Donepezil is widely used because of its low hepatotoxicity, long half-life and high safety (25,26).

Recent studies found that there are abundant miRNA in human brain, and about 20-25% of miRNA are significantly upregulated in AD brain, which is of great significance for identifying and/or diagnosis (27). Most miRNA are highly expressed or specifically expressed in the nervous system, participating in the processes of memory formation, synaptic plasticity and nerve differentiation, and regulating protein synthesis (28-30). Studies have found that miRNA plays an important role in the occurrence and development of AD, and miRNA interference may become a new target for the treatment of AD (31). miRNA has good stability and can be found in plasma and serum samples (32). Jia and Liu (33) detected the expression of miR-223 and miR-519 in serum of AD patients, and found that miR-223 was downregulated and miR-519 was upregulated. miRNA combined diagnosis can be used to predict the occurrence of AD disease. Therefore, detecting the number and types of miRNA in serum samples can effectively reflect the types and severity of the disease, and serum detection is convenient and non-invasive, thus, an ideal early screening method (34,35).

Relevant studies reported that miR-28-3p is a cell limiter, which can inhibit human T cell leukemia virus, type 1 (HTLV-1) replication and virus infection. miR-28-3p can target a sequence located in the mRNA of viral gag/pol genomic virus and reduce virus replication and gene expression in transient transgenic cells of HTLV-1 molecular clone (36). miR-28-3p can be detected in various diseases and participates in the occurrence and development of diseases (37-39). Related studies showed that miR-28-5p and miR-28-3p are downregulated in colorectal cancer samples compared with normal colon samples (15). In addition, the differential expression of 27 miRNA between AD patients and normal controls was identified, suggesting that miR-28-3p was significantly upregulated in AD patients (40). This indicated that miR-28-3p is expressed differently in different diseases. The results of this study showed that the miR-28-3p level in normal group was significantly lower than that in AD group and MCI group, while the serum miR-28-3p level in MCI group was significantly lower than that in AD group ($P<0.001$). It is suggested that the cognitive impairment of patients might be related to the upregulation of miR-28-3p expression and the occurrence of AD was related to the regulation of miRNA. Therefore, combined with previous literature, it was speculated that the upregulation of miR-28-3p in AD might be related to cell survival and cell communication. For example, PI3K-Akt signal transduction, ECM receptor and adhesion spot interaction may be induced and activated by compensatory response to extensive neurodegeneration of AD brain, but more basic experiments are needed to explore the specific action pathway of miR-28-3p (40). The expression of various miRNA in cerebrospinal fluid, the serum of patients with AD and Parkinson's disease, and its relationship with pathological features were detected. It was found that the expression of miRNA was significantly correlated with the severity of the disease, Braak stage, dementia status, plaque and entanglement density, and Louis body (41). However, there is scarce research on the relationship between miR-28-3p and pathological characteristics of AD patients. The present study found that the level of miR-28-3p had no significant correlation with sex, age, smoking or drinking, and the level of miR-28-3p was significant different in different disease courses and severity grades ($P<0.05$). It was suggested that the level of miR-28-3p increased gradually with the increase of course and severity, which was significantly correlated with the severity of the disease. Wang *et al* (42) suggested that miR-206-3p participated in the anti-dementia effect of donepezil and it might be a new pharmacological target for the treatment of AD. Rosignolo *et al* (43) showed that the level of miR-28-3p in patients with papillary thyroid cancer before operation was significantly higher than that in postoperative and healthy control groups. This study found that the serum miR-28-3p level of AD patients after treatment was significantly lower than that before treatment. This indicated that the combination of donepezil and basic therapy could significantly reduce the miR-28-3p level.

Hcy is produced by metabolism of methionine, which will produce oxidative stress, lead to cell damage and blood-brain barrier damage, and increase the level of brain Aβ. Many studies have shown that the increase of Hcy level in peripheral blood of AD patients was considered to be a risk factor for the occurrence of AD (44,45). Research by Gu *et al* (46) showed that Dihuang Yizhi recipe combined with donepezil hydrochloride could significantly improve ADL score, MMSE score and MoCA score of dementia patients with Parkinson's disease. Moreover, it was reported that donepezil could significantly reduce the serum Hcy level of AD patients and had good therapeutic effect (47). Our study found that ADL score and Hcy level of AD patients after treatment were significantly lower than those before treatment, and MMSE score and MoCA score after treatment were significantly higher than those before treatment ($P<0.05$). It was suggested that Hcy might be involved in the occurrence of AD, and ADL score, MMSE score and MoCA score could effectively reflect the therapeutic effect. Related studies found that serum miR-223 in AD patients was significantly positively correlated with MMSE score, while serum miR-519 was not significantly positively correlated with MMSE score (33). Correlation analysis showed that serum miR-132 level was negatively correlated with Hcy level, and serum miR-132 level was positively correlated with atrial natriuretic peptide (ANP) level and MMSE score (18). It was presumed that miR-28-3p had the same effect. After analysis, it was found that serum miR-28-3p level in AD patients was significantly positively correlated with ADL score and Hcy level, while miR-28-3p level was significantly negatively correlated with MMSE score and MoCA score. The results showed that with the increase of mental state and quality of life score and the decrease of serum Hcy level, the level of serum miR-28-3p decreased gradually, which effectively reflected the severity of cognitive impairment in AD patients and miR-28-3p level could be used as a therapeutic evaluation of AD disease. Reports showed that the AUC of miR-28-3p was 0.792 (95% confidence interval: 0.689-0.896), and the high expression of miR-28-3p in plasma could be used as a non-invasive and stable biomarker for detecting pulmonary embolism (48). Yu *et al* (49) used qRT-PCR and then analyzed

the operating characteristics of the subjects, and found that the expression of miR-28-3p, miR-143-3p, miR-151a-3p and miR-148a-3p were closely related to *Helicobacter pylori* infection. The above four plasma miRNA groups were expected to be used as non-invasive biomarkers for *Helicobacter pylori* infection. The present study found that the detection of serum miR-28-3p level could be used for early diagnosis of AD patients, but due to the small sample size and few related studies, a large number of studies are needed to further prove the research results.

This study provided references for the early diagnosis and treatment of AD patients by detecting the level of miR-28-3p in the serum of AD patients. However, no long-term follow-up of AD patients were carried out to observe their prognosis and whether the expression of miR-28-3p could be used as a prediction of long-term curative effect. Thus, the exact mechanism of miR-28-3p in the occurrence and development of AD needs further research.

In conclusion, donepezil therapy may reduce miR-28-3p level to improve the symptoms of AD patients, and miR-28-3p level can be used as early diagnosis and prognosis evaluation of AD patients.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

XZ wrote the manuscript, interpreted and analyzed the patient data. SW designed the study and performed the experiments. WS was responsible for the analysis and discussion of the data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The People's Hospital of Shouguang (Weifang, China). Patients who participated in this research had complete clinical data. Signed informed consents were obtained from the patients and/or guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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