Clinical Interventions in Aging

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ORIGINAL RESEARCH

Molecular Changes in Circulating microRNAs' Expression and Oxidative Stress in Adults with Mild Cognitive Impairment: A Biochemical and Molecular Study

This article was published in the following Dove Press journal:
Clinical Interventions in Aging

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Background: The release of miRNAs in tissue fluids significantly recommends its use as non-invasive diagnostic biomarkers for the progression and pathogenesis of mild cognitive impairment (MCI) in aged patients.

Objective: The potential role of circulated miRNAs in the pathogenesis of MCI and its association with cellular oxidative stress, apoptosis, and circulated BDNF, Sirtuin 1 (SIRT1), and dipeptidyl peptidase-4 (DPP4) were evaluated in older adults with MCI.

Methods: A total of 150 subjects aged 65.4±3.7 years were recruited in this study. The participants were classified into two groups: healthy normal (n=80) and MCI (n=70). Real-time PCR analysis was performed to estimate the relative expression of miRNAs; miR-124a, miR-483-5p, miR-142-3p, and miR-125b, and apoptotic-related genes *Bax*, *Bcl-2*, and *caspase-3* in the sera of MCI and control subjects. In addition, oxidative stress parameters; MDA, NO, SOD, and CAT; as well as plasma DPP4 activity, BDNF, SIRT1 levels were colorimetrically estimated.

Results: The levels of miR-124a and miR-483-5p significantly increased and miR-142-3p and miR-125b significantly reduced in the serum of MCI patients compared to controls. The expressed miRNAs significantly correlated with severe cognitive decline, measured by MMSE, MoCA, ADL, and memory scores. The expression of Bax, and caspase-3 apoptotic inducing genes significantly increased and Bcl-2 antiapoptotic gene significantly reduced in MCI subjects compared to controls. In addition, the plasma levels of MDA, NO, and DPP4 activity significantly increased, and the levels of SOD, CAT, BDNF, and SIRT1 significantly reduced in MCI subjects compared to controls. The expressed miRNAs correlated positively with NO, MDA, DPP4 activity, BDNF, and SIRT-1, and negatively with the levels of CAT, SOD, *Bcl-2*, *Bax*, and *caspase-3* genes.

Conclusion: Circulating miR-124a, miR-483-5p, miR-142-3p, and miR-125b significantly associated with severe cognitive decline, cellular oxidative stress, and apoptosis in patients with MCI. Thus, it could be potential non-invasive biomarkers for the diagnosis of MCI with high diagnostic performance.

Keywords: circulating miRNAs, MCI, biomarkers, cellular oxidative stress, apoptosis, real-time RT-PCR

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Introduction

Older ages clinically manifested by mild cognitive impairment (MCI) which is considered as an intermediate stage between the expected cognitive decline of

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normal aging and the more serious progressive decline of dementia. Subjects with MCI had a higher risk to progress to dementia compared with healthy controls.[1–3] Previous studies showed that the progression rates from MCI to dementia significantly increased from 5.4% and 11.7% per year.[4–7]

The incidence of MCI has no significant factors even it was linked in most cases by heart problems, blood pressure, and diabetes.[7] However, problems with memory, language, thought, routine MCI diagnosis, hypertension, and judgment were significantly increased in older adults with MCI.[8]

However, routine MCI diagnosis including clinical observation neuropsychological exam, neuroimaging, genetic testing, and neurochemical bodily may be supportive to postpone or prevent the subsequent progression to dementia.[8–10] However, their routine use is unfeasible in the clinical setting due to its difficulty, invasiveness, and inconvenience to obtain data. Thus, the search for rapid and non-invasive diagnostic biomarkers is required to improve MCI diagnosis.

Previous research studies showed that increased dipeptidyl peptidase-4 (DPP4) activity and reduced both brain-derived neurotrophic factor (BDNF) and Sirtuin 1 (SIRT1) in peripheral circulation might all play pathogenetic roles in subjects with MCI.[11–18] The changes in these physiological biomarkers are significantly associated with cellular oxidative stress and inflammation of MCI patients.[11] In MCI, both DPP4 activity and BDNF significantly correlated with cellular oxidative stress and inflammation,[19–21] oxidative stress imbalance and inflammation lead to cellular mitochondrial dysfunction, disruption of cellular homeostasis, and progressive neurodegeneration by cell death or apoptosis.[22–26] Previous studies showed that aging promotes neuronal apoptosis via increasing the expression of caspase-3, Bax, and reduction in the expression of *Bcl-2* genes.[27,28] Thus, oxidative stress and inflammation accelerated aging and faster progression of neurodegenerative diseases particularly MCI and dementia.[22–31] In addition to, with aging, brain parenchyma was impacted by cellular oxidative stress and potentially by TNF alpha deleterious action which significantly affects upon cognitive function.[23–25]

Circulating miRNAs are short non-coding RNAs showed to be associated with many physiological, cellular, and molecular developments, which occurred in normal and diseased cells, including MCI and dementia.[32–34]

The release of miRNAs in tissue fluids; serum or plasma or saliva inactive state significantly recommends its use as non-invasive diagnostic biomarkers for MCI.[32–35] Several miRNAs were reported to be significantly associated with MCI and were potential biomarkers for the diagnosis of MCI.[34–39] However, its association with cellular oxidative stress, apoptosis, and metabolic MCI parameters remains to be elucidated. The present study aimed to evaluate the potential role of circulated miRNAs in the pathogenesis of MCI and its association with cellular oxidative stress, apoptosis, and circulated BDNF, 1 (SIRT1, and dipeptidyl peptidase-4 (DPP4) in older adults with MCI.

Materials and Methods

Subjects

A total of 160 subjects aged 65.4±3.7 years were invited to this study. Only 150 of the subjects agreed to participate and classified according to the diagnosis of mild cognitive impairment (MCI) into two groups; healthy normal (n=80) and MCI (n=70). Elders with severe psychiatric illness, endocrine, immune, eating disorders, poor hearing and vision, and nervous system diseases, and taking glucocorticoid medication that could interfere cognitive ability measurements were already excluded from both cases and controls by investigating their past medical history. Ten subjects were excluded from this study (four refused participation, three with nervous system diseases, and three received glucocorticoid medication). The study protocol was reviewed according to the ethical guidelines of the 1975 Declaration of Helsinki and approved by the ethical committee of Rehabilitation Research Chair (RRC), King Saud University, Kingdom of Saudi Arabia, under file number (ID: RRC-2019-028) and signed informed consent forms were received from all subjects prior data collection.

Assessment of Cognitive Performance

A well-trained research neurologists performed the cognitive and functional status of all participants according to Petersen's criteria (Table-1) [38] and by using the Activity of Daily Living scale (ADL), MiniMental State Examination (MMSE), and Montreal Cognitive Assessment (MoCA) as previously reported.[39–46] The MMSE and the MoCA are the most widely used cognitive screening instruments for MCI which covers various areas of cognitive domains.[47,48] The score of the MMSE (≤27) and that of the MoCA (<26) were taken together to evaluate cognitive impairment[48]. Thus, recruited participants were divided into normal control (n=80) and the MCI (n=70) group. The demographics and baseline characteristics of participants are shown in (Table-2).

Table 1 Assessment of Cognitive Performance According to Petersen's Criteria

Criteria of MCI	Control Group (n=80)	MCI Group (n=70)
Memory complaint by participant or family (Yes/No)	No	Yes
Normal activities of daily living (20 items; score <26)	>26	<26
Normal general cognitive function (A: MMSE score between 20 and 27 [cutoff points for illiterate (≤ 20), primary school (≤23) and secondary school and above (≤ 27)]; B: MoCA score <26)	MMSE:> 27 MoCA: >26	MMSE: ≤27 MoCA: <26
Objective impairment scores	>1.5 SD	<1.5 SD
The Clinical Dementia Rating scale	No	0–0.5

Table 2 The Demographics and Baseline Characteristics of Participants

Parameters	Control Group	MCI Group
N	80 (53.33%)	70 (46.66%)
Male/Female	50/30	40/30
Age (years)	65.3 ± 3.5	64.9 ± 4.1
BMI (kg/m^2)	19.9± 2.6	24.1± 3.6 [b]
Waist (cm)	77.3± 9.3	98.1 ± 6.3 [b]
Hips (cm)	86.9 ± 7.5	92.6 ± 18.3 [b]
WHR	0.89±0.11	1.4±0.16 [b]
Education (years)	10.2 ± 0.8	5.3 ± 0.6[b]
Lifestyle factors, %		
Working	96.5	89.5[a]
Exercising regularly	88.4	76.1[a]

Notes: Values are expressed as mean ±SD; Significance at $p < 0.05$. [a]$p < 0.01$, [b]$p < 0.001$.

Assessment of Nitric Oxide (NO)

Serum accumulated NO was estimated as the stable end products nitrite and nitrate as previously reported.[43,46] In this experiment, cadmium reagent performed to convert nitrate to nitrite which then measured by spectrophotometric assay using the Griess reagents sulfanilamide, HCl and *N*-naphthylethylenediamine.[47–51] The absorbance of nitrite concentrations was taken at 545 nm.[47–51] The concentration of the accumulated NO calculated according to the following formula:

NO concentration (μmol/L.) = {[At-Ab/As-Ab× Cons of S/V.serm used ×1000]}

Assessment of Plasma Malonaldehyde (MDA)

Plasma Lipid peroxide MDA was estimated as a measure of cellular lipid peroxidation in all subjects by using a reversed-phase high-performance liquid chromatography (HPLC/PDA Shimadzu®) using an analytic column C-18 (Phenomenex×150mm×4.6 mm, 10 μm) as previously reported.[49–52] In this method, acidic thiobarbituric acid reacted with plasma MDA at 90◦C for 1 h, protein removal by centrifugation, filtered, and finally, the colored complex detected spectrophotometrically at 532 nm. MDA levels were expressed as nmol of MDA/mg protein.[49–52]

Assessment of Antioxidant Enzymes

In this test, catalase (CAT) and superoxide dismutase (SOD) activity were estimated by a spectrophotometer analysis as previously reported.[49–56] All enzymatic assays were conducted in triplicate, corrected by hemoglobin content, and expressed as U/g of hemoglobin.[51]

Assessment of Plasma DPP4 Activity, BDNF, Sirtuin 1 (SIRT1) Levels

Plasma DPP4 activity was performed as previously described.[11,32,54–57] In addition, brain-derived neurotrophic factor (BDNF) and sirtuin 1 (SIRT1) were estimated in the serum of all participants by immune assay (ELISA) technique,[57] using the human BDNF Quantikine Kit (Catalog no: DBD00, R&D System, Minneapolis, MN, USA) and human SIRT1 ELISA Kit (Catalog no: E94912Hu, USCN Life Science, Wuhan, China). The results were performed in duplicates and were used for statistical analyses.[57]

Real-Time RT-PCR Analysis of Circulating miRNAs and Apoptotic Genes

Extraction of RNA and Synthesis of *cDNA*

For each participant, the miRNease isolation kit (Qiagen, Hilden, Germany) was used to extract total RNA from serum samples. A reverse-transcription polymerase chain reaction (RTPCR) was applied to analyze total RNA in all serum samples. Then, a complementary DNA (cDNA) was generated using reverse-transcription miScriptII RT kits

(Qiagen), and the levels of miRNAs were evaluated by optical density.[11]

Real-Time RT-PCR Analysis

The primers of circulating miRNAs; miR-124a, miR-483-5p, miR-142-3p, and miR-125b (Applied Biosystems, Foster City, CA, USA), in addition to primer sequences of the apoptotic genes (*Bax*, *Bcl-2*, and *caspase-3*) (Table 3), were used to screen the expression of miRNAs and apoptotic genes in the plasma of all participants by using a quantitative real-time RT-PCR.[11] The average copy number of the resultant PCR components was normalized according to the GAPDH gene which was used as an internal housekeeping gene.[32] In the PCR process, templets of respective cDNA subjected to four thermal phases; primary denaturation phase (I) (at 94°C for 2 minutes); denaturation phase (II) (at 94°C for 30 seconds); annealing phase (III) (at 59°C for 30 seconds), and amplification phase (IV) (at 72°C for 30 seconds). The PCR phases (II–IV) proceed for 45 cycles and all reactions were measured in a triplicated manner.[32]

Table 3 Neuropsychological Test Scores and the Cognitive Status of the Participants (n=15; Mean ±SD)

Parameters	Control Group	MCI Group	t	p-Value
MMSE	28.4 ± 3.3	21.3 ± 2.5	12.8	<0.001
MoCA	22.7±2.9	27.9±5.4	18.9	<0.001
ADL	22.1±3.7	28.6±10.3	19.7	<0.001
IADL	8.3±0.2	6.9±0.3	15.3	<0.001
Wechsler Memory Scale – Revised-				<0.001
VPA	10.3±2.8	6.9±3.1	12.5	
VR	12.8±4.3	8.5±2.7	10.4	
IM	7.2±2.9	4.5±3.1	11.9	
PR	17.0±3.9	12.9±2.9	16.9	
Wechsler Adult Intelligence scale – Chinese revision				<0.001
S	19.6±3.8	12.8±5.8	18.9	
R	14.1±3.7	8.1±2.8	16.9	
DSC	38.2±8.6	24.2±10.3	12.8	
PC	10.4±1.9	9.4±1.3	15.8	
BDs	79.3±24.3	89.8±31.6	14.7	
V	17.2±3.7	14.5±4.1	8.5	

Note: Values are expressed as mean ±SD.

Abbreviations: MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; ADL, activity of daily living; IADL, instrumental activities of daily living; VPA, verbal paired associates; VR, visual reproduction; IM, immediate memory; PR, picture recall; S, similarities; R, arithmetic; DSC, digit symbol-coding; PC, picture completion; BDs, block design(s); V, vocabulary.

Statistical Analysis

Power calculations of the selected sample size of 150 subjects showed to give an estimated power of 95% and a significance level of 0.05 with an expected frequency of 10.5%.

An SPSS statistical program (SPSS, IBM Statistics V.17) was used to analyze all data produced in this study. The data of continuous variables are expressed as mean±SD. The frequency differences between the groups were analyzed by using a non-parametric test (Mann–Whitney–Wilcoxon test) and the $\chi 2$ test, respectively. In all groups, two independent sample *t*-tests were used for comparison between the studied variables such as cognitive score (dependent variable), expression levels of miRNAs, apoptotic genes, oxidative stress parameters, plasma DPP4 activity, BDNF, and sirtuin 1 (SIRT1) levels (independent variables). In addition, multiple stepwise regressions and Pearson's correlation analyses were used to estimate the associations between cognitive function status and the studied independent variables in older subjects with MCI and in controls. All tests were two-tailed; because of multiple assessments, results were only considered statistically significant at a value of $p < 0.05$.

Results

A total of 150 older adults were recruited in this study. Based on Petersen's criteria (Table 1), mild cognitive impairment (MCI) was predicted in 46.66% of the participants. The participants classified into two groups; healthy control (n=80) and MCI group (70). In subjects with MCI, adiposity parameters; BMI, Waist, hips, and WHR were significantly increased (p=0.001) compared to healthy controls (Table 2). In addition, education scores, and lifestyle factors (working and regular exercise) significantly reduced (P=0.001) in MCI compared to normal controls (Table 2).

Cognitive function and neuropsychological scores of all subjects were estimated in this study (Table 3). Mini-mental state scores (MMSE) and active daily living scores (ADL & IADL) significantly (P=0.001) increased and the Montreal cognitive assessment scores significantly (P=0.001) reduced in older adults with MCI compared to normal controls (Table 3).

Mean neuropsychological test scores measured by memory and adult intelligence scales are listed in Table 3. The *t*-test was used to compare neuropsychological status and cognition function between normal and MCI groups. The two groups were significantly different (P=0.001) in neuropsychological as well as cognitive scores as shown in (Table 3).

In this study, the effect of cellular oxidative stress on cognitive function was estimated (Figure 1). The results showed a significant increase (p=0.001) in the levels of cellular NO and MDA in older adults with MCI compared to healthy controls (A&B). In addition, a significant decrease (p=0.001) in the levels of antioxidant enzymes; CAT and SOD in older adults with MCI compared to healthy controls. The change in the cellular oxidative-defensive system closely correlated (P=0.001) with the status of cognitive function. In subjects with MCI, serum levels of NO and MDA correlated positively with MMSE, MoCA, ADL, and memory scores, and negatively correlated with reduced activity of antioxidant enzymes; CAT and SOD, respectively (Table 4).

In addition, the influence of cellular apoptosis on cognitive function was reported (Figure 2). In this experiment, the cellular expression of *Bcl-2*, *Bax*, and *caspase-3* genes was examined in all subjects. In Older adults with MCI, the expression levels of both Bax and caspase-3 genes significantly (p=0.001) increased, and the expression levels of the Bcl-2 gene significantly (p=0.01) reduced in comparison with the results of healthy controls (Figure 2D). The expression of cellular apoptotic genes correlated with the cognitive function. Cognitive function scores of MMSE, MoCA, ADL, and memory correlated positively with the expression of Bax and caspase-3, and negatively with the expressed Bcl-2 gene (Table 4).

The correlation between metabolic changes in the serum levels of DPP4 activity, BDNF and SIRT-1 were estimated in control and MCI subjects (Figure 2). As shown in Figure 2, the results showed that the levels of BDNF, SIRT-1 significantly reduced and the DPP4 activity significantly increased in older subjects with MCI compared to healthy controls (2A,2Band2C). The levels of DPP4 activity, BDNF and SIRT-1 were significantly associated with cognitive status. Cognitive function; MMSE, MoCA, ADL, and memory scores correlated positively (p=0.001) with serum BDNF and SIRT-1, and negatively with serum DPP4 activity as shown in Table (4). Similarly, in subjects with MCI physiological changes in the serum levels of DPP4 activity, BDNF and SIRT-1 were intercorrelated with cellular oxidative and apoptosis. The serum levels of DPP4 activity, BDNF, and SIRT-1 correlated

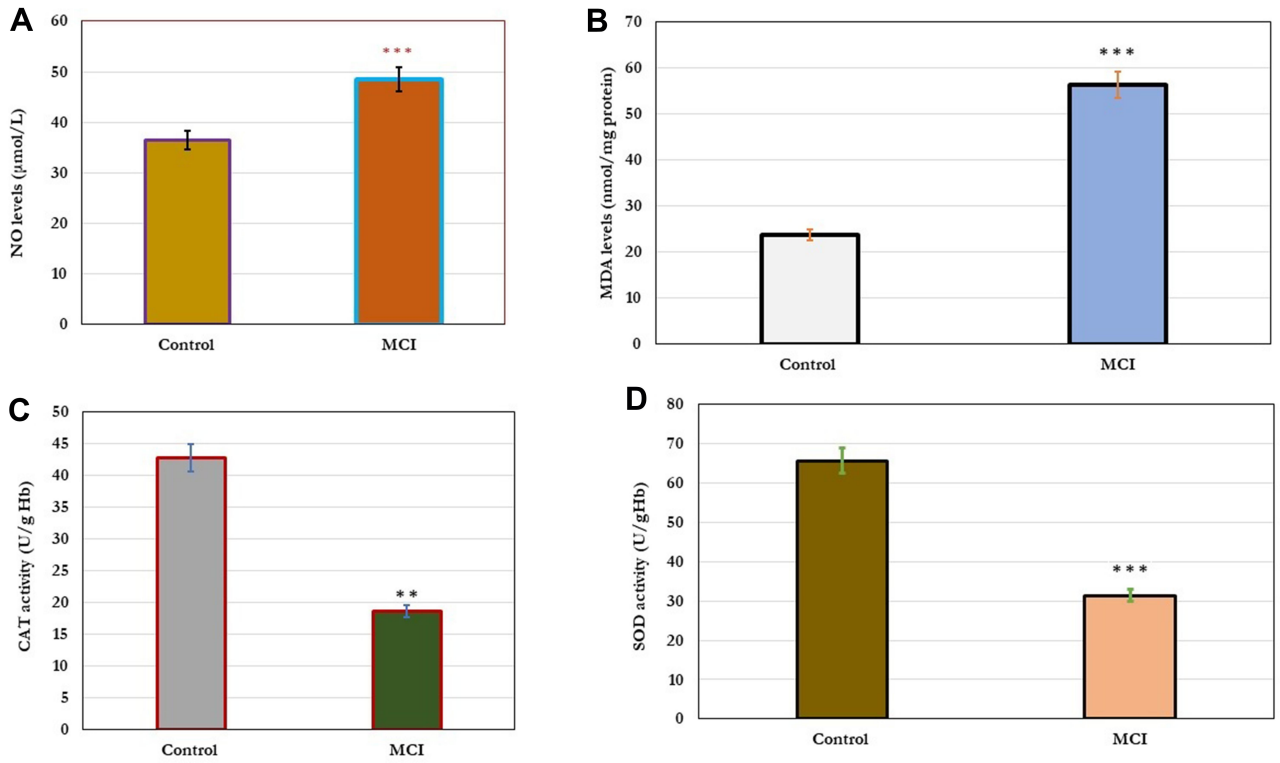

Figure 1 Changes in cellular oxidative stress; NO and MDA (**A** and **B**) and antioxidant enzymes; Cat and SOD the levels (**C** and **D**) in healthy control (n=80) and older adults with MCI (n=70). The results showed significant increase (p=0.001) in the levels of cellular NO and MDA in older adults with MCI compared to healthy controls (**A** and **B**). In addition, significant decrease (p=0.001) in the levels of antioxidant enzymes; CAT and SOD in older adults with MCI compared to healthy controls. Significance of the comparison was evaluated by Mann–Whitney-Wilcoxon test and sample *t*-test, ** p≤ 0.01, *** p≤ 0.001.
Abbreviations: MCI, mild cognitive impairment; NO, nitric oxide; MDA, malonaldehyde, SOD, superoxide dismutase, CAT, catalase enzyme.

Table 4 Correlations Between DPP4 Activities, BDNF, SIRT-1, Celluar Oxidative Stress, Apoptosis, and Relative Expression of miRNAs vs Cognitive Parameters

Characteristics	Cognitive Function (n=70)							
	MMSE[a]		MoCA[a]		ADL[a]		Memory Scores[a]	
	r	P	*r*	P	*r*	P	*r*	P
DPP4 activity (nmol/min/mL)	−0.320	<0.001	−0.291	< 0.001	−0.31	<0.001	−0.24	<0.001
BDNF (ng/mL)	0.192	<0.001	0.23	<0.001	0.18	<0.001	0.15	<0.001
SIRT1(ng/mL)	0.31	<0.001	0.45	<0.001	0.27	<0.001	0.34	<0.001
NO	0.35	<0.001	0.28	<0.001	0.29	<0.001	0.47	<0.001
MDA	0.23	<0.001	0.36	<0.001	0.51	<0.001	0.14	<0.001
CAT	−0.32	<0.001	−0.39	<0.001	−0.25	<0.001	−0.31	<0.001
SOD	−0.54	0.001	−0.48	<0.001	−0.37	<0.001	−0.43	<0.001
Bax	0.21	<0.001	0.18	<0.001	0.26	<0.001	0.26	<0.001
Bcl-2	−0.45	<0.001	−0.49	<0.001	−0.53	<0.001	−0.78	<0.001
Caspase-3	0.48	<0.001	0.21	<0.001	0.23	<0.001	0.19	<0.001
miR-124a	0.251	<0.001	0.362	<0.001	0.182	<0.001	0.135	<0.001
miR-483-5p	0.314	<0.001	0.217	<0.001	0.321	<0.001	0.158	<0.001
miR-142-3p	−0.242	<0.001	−0.342	<0.001	−0.124	<0.001	−0.314	<0.001
miR-125b	−0.254	<0.001	−0.227	<0.001	−0.351	<0.001	−0.318	<0.001

Note: [a]P-value determined by partial correlation analysis with respect to the DPP4 activity, BDNF, SIRT-1, cellular oxidative stress, apoptosis, and relative expression of miRNAs adjusted for age, BMI, gender, education level, exercise levels, and working.

positively with CAT and SOD activity, *Bcl-2* gene expression, and negatively with cellular NO, MDA, and expressed *Bax*, and *caspase-3* genes (Table 5).

Also, the correlation between MicroRNAs' differential expression with cognitive function in older adults was studied (Figure 3). The results showed that the relative expression of miR-124a and miR-483-5p significantly increased (P=0.001), and miR-142-3p and miR-125b significantly reduced (P=0.01) in older adults with MCI compared to healthy controls (Figure 3). Cognitive function scores of MMSE, MoCA, ADL, and memory correlated positively with the expression levels of miR-124a and miR-483-5p, and negatively with the expressed miR-142-3p, and miR-125b, respectively (Table 4).

Expressed microRNAs positively correlated with cellular oxidative stress parameters; NO and MDA and negatively with deficient activities of antioxidative enzymes; CAT and SOD, respectively (Table 6). In subjects with MCI, the relative expression of microRNAs correlated positively with cognitive physiological changes in the serum levels of DPP4 activity, BDNF, and SIRT-1 (p=0.001) and negatively with the expressed cellular apoptosis genes *Bcl-2*, *Bax*, and *caspase-3* (Table 6).

Discussion

Mild cognitive impairment (MCI) was predicted in 46.66% of the study population. Molecular-based assays confirmed that circulating miR-124a, miR-483-5p, miR-142-3p, and miR-125b were significantly associated with severe cognitive decline, oxidative stress, and apoptosis in patients with MCI.

In this study, participants with MCI recorded higher MMSE, ADL, IADL, and lower MoCA scores compared to healthy controls. In addition, neuropsychological status, especially poor memory and intelligence, was significantly reported among MCI subjects.

MCI was reported in the aged people, whereas 5.4% and 11.7% per year of MCI significantly at higher risks to develop to severe dementia.[4–6] Many problems in memory, language, thought, and judgment were significantly reported in patients with MCI which affects on their daily activities.[2,8]

Cellular oxidative stress was significantly associated with the severity of cognition impairment particularly in older adults with MCI.[32,58–62] DNA damage (8-oxo-2′-deoxyguanosine; 8-oxodGuo), lipid peroxidation (malonaldehyde; MDA), and other cellular oxidative parameters significantly reported in higher ranges in the brain tissues, serum, plasma, and cerebrospinal fluid (CSF) patients with MCI.[58–61]

In the current study, oxidative stress markers MDA and NO significantly increased, and CAT and SOD antioxidant defense activity significantly reduced in MCI compared to healthy-aged individuals. The deficient cellular antioxidant activity and increased free radical oxidative stress

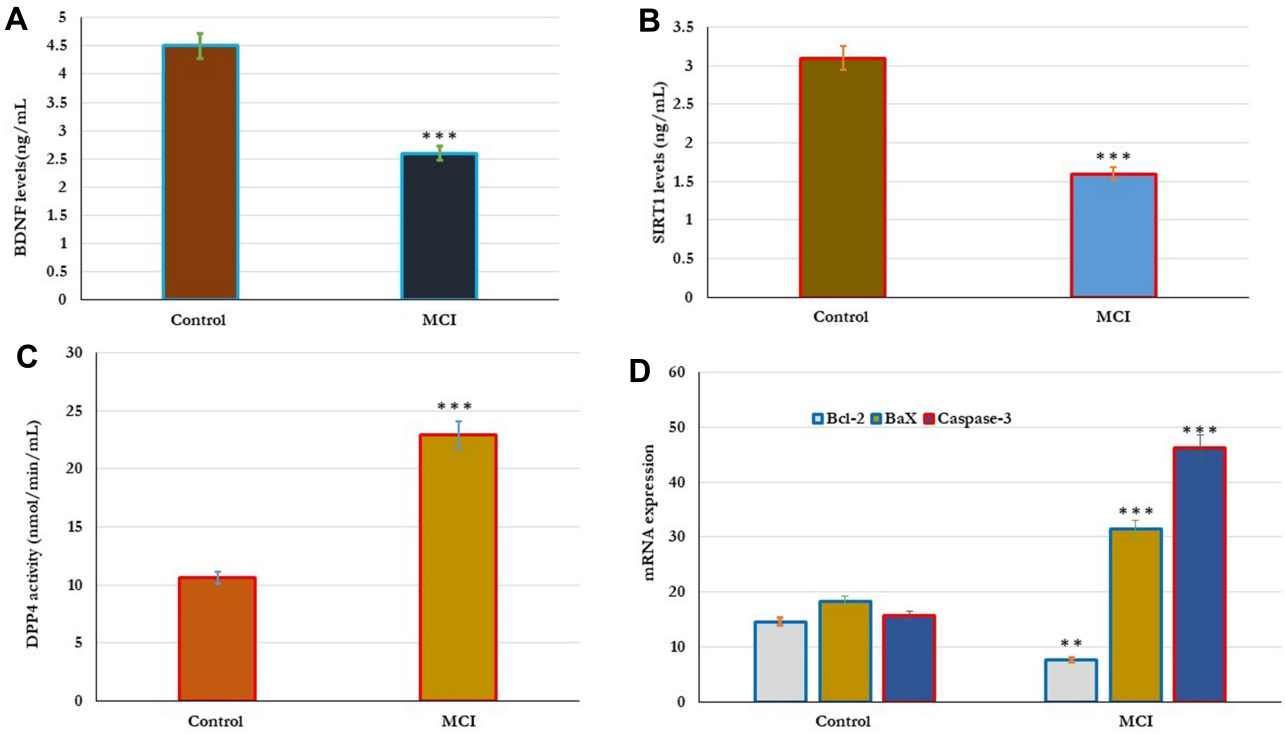

Figure 2 Metabolic changes in the DPP4 activity, BDNF, SIRT-1, and apoptotic genes *Bcl-2*, *Bax*, and *caspase-3* in healthy control (n=80) and older adults with MCI (n=70). (**A**–**D**). The results showed that the levels of BDNF, SIRT-1 significantly reduced and the levels of DPP4 activity significantly increased in older subjects with MCI compared to healthy controls (**A**–**C**). In addition, the expression of apoptotic genes was significantly reported in all subjects. In Older adults with MCI, the expression levels of both *Bax* and *caspase-3* genes significantly (p=0.001) increased, and the expression levels of *Bcl-2* gene significantly (p=0.01) reduced in comparison with the results of healthy controls (**D**). Significance of the comparison was evaluated by Mann–Whitney–Wilcoxon test and sample *t*-test, $^{**}p \leq 0.01$ $^{***}p \leq 0.001$.
Abbreviation: MCI, mild cognitive impairment.

significantly associated with memory loss, language, thought, and judgment of subjects with MCI. The results showed that low cognitive performance was associated with both elevated MDA and NO levels and decreased SOD and CAT activity in subjects with MCI.

Previous reports showed that accumulation of cellular oxidative free radicals (ROS) significantly produces degeneration of brain neurons,[61–65] vascular lesions,[66] which consequently lead to cognitive decline and dementia in old age. It was reported that in brain tissue, ROS generated from microglia and astrocytes are proposed to control synaptic and nonsynaptic communications between neurons and glia. Thus, the release of ROS radicals in higher quantity promotes neurodegeneration and memory loss via processes

Table 5 Correlations Between DPP4 Activities, BDNF, and SIRT-1 vs Celluar Oxidative Stress and Apoptosis in Older Adults with MCI

Characteristics	Cognitive-Related Metabolic Parameters[a]					
	DPP4 Activity		BDNF		SIRT1(ng/mL)	
	r	P	*r*	P	*r*	P
NO	−0.128	<0.01	−0.32	<0.01	−0.56	<0.01
MDA	−0.18	<0.001	−0.23	<0.001	−0.58	<0.001
CAT	0.89	<0.001	0.76	<0.001	0.81	<0.001
SOD	0.48	<0.001	0.71	<0.001	0.56	<0.001
Bax	−0.31	<0.001	−0.43	<0.001	−0.48	<0.001
Bcl-2	0.36	<0.001	0.51	<0.001	0.42	<0.001
Caspase-3	−0.87	<0.001	−0.96	<0.001	−0.74	<0.001

Note: [a]P-value determined by partial correlation analysis with respect to the DPP4 activity, BDNF, DBR, cellular oxidative stress, and apoptosis adjusted for age, BMI, gender, education level, exercise levels, and working among older adults.

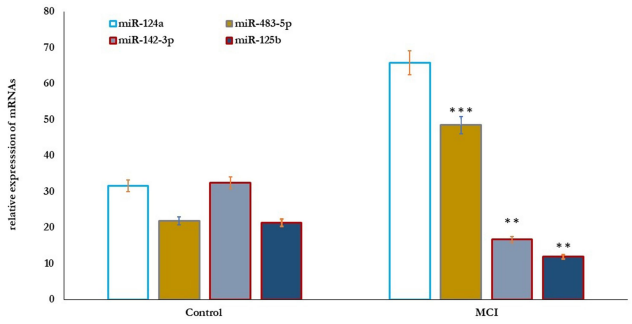

Figure 3 MicroRNAs' differential expression profile in healthy control (n=80) and older adults with MCI (n=70). The results showed that the relative expression of miR-124a and miR-483-5p significantly increased (P=0.001), and miR-142-3p, and miR-125b significantly reduced (P=0.01) in older adults with MCI compared healthy controls. Significance of the comparison was evaluated by Mann–Whitney–Wilcoxon test and sample *t*-test, **p≤ 0.01, ***p≤ 0.001.
Abbreviation: MCI, mild cognitive impairment.

of neuroinflammation and cell death.[62] Consistent with our results, MDA as lipid peroxide product was significantly reported in the serum of subjects with brain disorders such as MCI.[36,63] Also, it was reported that MDA and related lipid peroxides considered promising peripheral biomarkers during brain cases with white matter abnormalities. This may be related to higher lipid contents in both the axonal membranes and myelin sheaths of the brain.[63,64]

NO synthesized by the enzyme NOS from neurons of the brain and spinal cord.[63–67] It functions to maintain cellular vascular tone, neurotransmitter function, and mediation of cellular defense in normal cases.[68] However, NO was considered a neurotoxic agent when it produced at higher levels for brain microglia and might play a role in neurodegeneration.[68–72] Thus, in subjects with brain disorders such as MCI, Alzheimer's, and Parkinson's disease thought to be associated with higher production of cellular NO induced by NOS enzyme activity.[73]

In addition, like our results, dysregulation of antioxidant enzymes such as CAT and SOD was proposed to play a role in cellular oxidative stress associated with age-related pathologies, especially cognitive decline.[71–75] Whereas, a combined measurement of oxidative status with antioxidant potential was prospectively associated with the process of neurodegeneration and could be estimated as signs of cognitive decline among older ages.[76–82]

Previous research studies showed that the accumulation of ROS free radicals activates neural cell apoptosis during brain development.[77–80] A set of caspases enzymes such as caspase-3 and Bax were expressed to induce apoptosis and stimulate inflammation in the nervous system which leads to brain neurodegeneration.[79,80]

In this study, we are trying to explore the potential mechanism of cellular apoptosis involved in neurologic injury associated with cognitive problems in patients with MCI. Thus, apoptotic genes, *Bcl-2*, *Bax*, and *caspase-3*, were estimated in all participants by using real-time PCR analysis. The results showed that the expression of *Bax* and *caspase-3* apoptotic genes significantly upregulated (increased) and the expression levels of the *Bcl-2* antiapoptotic gene significantly down-regulated (reduced) in the patients with MCI compared to healthy controls. The expressed apoptotic genes significantly correlated with the score of cognitive function among MCI patients. Cognitive function scores of MMSE, MoCA, ADL, and

Table 6 Correlations Between DPP4 Activities, BDNF, SIRT-1, Celluar Oxidative Stress, and Apoptosis vs Relative Expression of miRNAs in Older Adults with MCI

Characteristics	miRNAs Relative Expression[a]							
	miR-124a		miR-483-5p		miR-142-3p		miR-125b	
	r	P	*r*	P	*r*	P	*r*	P
DPP4 activity (nmol/min/mL)	0.12	<0.01	0.24	<0.002	0.38	< 0.002	0.145	<0.001
BDNF (ng/mL)	0.21	<0.01	0.31	<0.01	0.28	< 0.01	0.31	<0.001
SIRT1(ng/mL)	0.45	<0.01	0.57	<0.01	0.49	< 0.01	0.49	<0.01
NO	0.37	<0.001	0.42	<0.001	0.36	< 0.001	0.76	<0.001
MDA	0.18	<0.001	0.23	<0.001	0.42	< 0.001	0.27	<0.001
CAT	−0.51	<0.001	−0.62	<0.001	−0.54	< 0.001	−0.63	<0.001
SOD	−0.85	<0.001	−0.82	<0.001	−0.47	< 0.001	−0.84	<0.001
Bax	−0.45	<0.001	−0.36	<0.001	−0.56	< 0.001	−0.46	<0.001
Bcl-2	−0.17	<0.001	−0.42	<0.001	−0.68	< 0.001	−0.91	<0.001
Caspase-3	−0.75	<0.001	−0.53	<0.001	−0.47	< 0.001	−0.43	<0.001

Note: [a]P-value determined by partial correlation analysis with respect to the DPP4 activity, BDNF, DBR, cellular oxidative stress, and apoptosis adjusted for age, BMI, gender, education level, exercise levels, and working among older adults with MCI.

memory correlated positively with apoptotic genes *Bax* and *caspase-3*, and negatively with the expressed Bcl-2 antiapoptotic gene. The results signify the role of apoptosis in the pathogenesis of MCI.

Previously, it was reported that caspase enzymes especially caspases 3 and 7 activate regular apoptosis during brain development, neurodegeneration, and progressive dismantling of neuronal circuits in brain regions which inturn mediate memory functions. Thus, an abnormal increase in apoptosis via oxidative stress or inflammation significantly leads to deficiency in memory functions in older ages.[81,82] Caspase 3 activation by intrinsic and extrinsic apoptotic pathways showed to be the most vital event associated with neuronal cell death in most chronic neurodegenerative conditions.[81,82] The hippocampus is an important brain region responsible for learning and memory and higher exposure to free radical oxidative stress and apoptosis leads to significant abnormality in learning and memory among MCI patients.[83–85]

In this study, it was found that the levels of BDNF and SIRT-1 were significantly reduced and the enzyme DPP4 activity significantly increased in the serum of MCI patients compared to healthy controls. The changes in these neurometabolic parameters were significantly associated with the scores of cognitive measurements; MMSE, MoCA, ADL, and memory scores.

In patients with dementia (AD) and brain disorders, the levels of BDNF in serum showed to be associated with cognitive decline.[86–89] Matched to our results, BDNF significantly decreased in patients with MCI and AD,[87] and that higher levels of BDNF are required to protect future recurrence of brain disorders such as AD.[86] Also, SIRT-1 was significantly reduced in patients with MCI in relation to healthy control subjects. The increased levels of human SIRT1 were significantly associated with neuroprotection and longevity.[90,91] Thus, both BDNF and SIRT1 were considered as conceivable candidate genes contributing to MCI and AD.[92,93]

In this study, the levels of DPP4 activity, BDNF, and SIRT-1 in the serum of MCI patients correlated positively with CAT and SOD activity, Bcl-2 gene expression, and negatively with cellular NO, MDA, and expressed Bax, and caspase-3 genes. The increased enzyme DPP4 activity among our MCI patients was significantly associated with others who reported an increase in the levels of plasma DPP4 activity in MCI patients and concluded that DPP4 activity was mutually influenced by increased cellular free radical oxidative stress.[94] Moreover, the lower levels of DPP4 activity significantly improved cognitive function via the enhancement of inflammation, oxidative stress and reducing or suppression of apoptosis.[16–18] It was reported previously that increased DPP4 activity promotes the development of oxidative stress and inflammation,[95] which was significantly involved in the progression and pathogenesis of cognitive dysfunction.[1,90] Thus, decreased BDNF and increased DPP4 activities in the blood circulation of our MCI patients showed to have a pathogenetic role in the development of cognitive impairment as previously reported, and then it could be used as a prognostic biomarker for MCI.[57,97,98]

miRNAs are non-coding short cellular RNAs significantly expressed with cells and freely liberated in peripheral blood circulation, and easily identified in urine, plasma, serum, and cerebrospinal fluids. It has multicellular functions particularly the regulation of gene expression. Previous studies reported the expression of miRNAs in the brain and associated with the regulation of neuronal plasticity, function, and development. It was reported previously that most neurodevelopment disorders or neurodegenerative diseases are significantly associated with abnormality or dysfunction in miRNAs transcription.[13,99,100] In general, about half of all protein-coding genes identified to be regulated by microRNAs which significantly reduce the abnormality or fluctuations in protein expression.[101,102]

Thus, in this study, we are trying to understand the role of miRNAs in the development of neuropsychiatric disorders associated with cognitive impairment in patients with MCI.

In this study, real-time PCR analysis was performed to estimate microRNAs' differential expression in control and patients with MCI. It was found that the relative expression levels of miR-124a and miR-483-5p significantly increased and miR-142-3p and miR-125b significantly reduced in the serum of older adults with MCI compared to healthy controls. The data also showed that relative expression of miRNAs; miR-124a, miR-483-5p, miR-142-3p, and miR-125b correlated with the scores of cognitive function among MCI patients.

Similarly, increased peripheral miR-146a and miR-483-5p levels were previously shown to associate with the severity of cognitive impairment in subjects with MCI and to predict the conversion to dementia,[31,36,103] thus both miR-486-5p and miR-483-5p were the most significant indicators of MCI among older adults.

Matched to our results, the decline in the levels of miR–125b and miR-142-3p significantly associated with the scores of cognitive impairment in patients with MCI and AD,[104–107] and could be used as a useful noninvasive biomarker for older adults with MCI. In addition, higher specificity (68.3%) and a sensitivity of 80.8% with quiet priority and significant correlation with the Mini-Mental State Examination (MMSE) were reported for expressed miR-125b in patients with dementia.[108,109]

In the current study, correlation analysis interestingly showed that expressed microRNAs were significantly associated with cellular oxidative stress and apoptotic inducing genes. The expression of microRNAs correlated positively with oxidative stress parameters, NO and MDA, and negatively with the reduced activities of antioxidative enzymes; CAT and SOD as well as expression levels of cellular apoptosis genes *Bcl-2*, *Bax*, and *caspase-3*. Previously, miR 125b, miR 146a, and other related miRNAs showed an association with neuropathology, apoptosis, oxidative stress, and other neurodegeneration of the human central nervous system.[35] In addition, the relative expression of miR-124a and miR 125b correlated with cellular aging processes such as oxidative stress, age-related antioxidant dysfunction, senescence, and apoptosis.[35,110]

In the previous differential correlation analysis, plasma miR-125b with multiple miRNAs pairs showed to have higher accuracy for MCI detection.[111] It was downregulated in the serum of patients with dementia.[112] miR-125b promotes cellular apoptosis via regulating the function of a tumor suppressor gene (p53) which significantly associated with controlling diseases, aging, and metabolism particularly in brain neurodegeneration in AD.[113–115] It was reported that miR 125b enhances neuronal apoptosis and Tau phosphorylation in patients with Alzheimer's disease.[116] Our results matched with others which showed the expression of miRNAs; miR-124a, miR-483-5p, miR-142-3p, and miR-125b significantly associated with more severe cognitive decline in patients with MCI via promoting cellular oxidative and apoptosis.[117]

The expression patterns of serum microRNAs; miR-124a, miR-483-5p, miR-142-3p, and miR-125b correlated positively with DPP4 activity, BDNF, and SIRT-1 in the serum of MCI patients. The proposed correlation may proceed via the indirect influence of expressed microRNAs on neuronal oxidative stress and apoptosis in patients with MCI. Whereas increased DPP4 activity and decline of both BDNF, and SIRT-1 in the serum of MCI patients resulted in the enhancement of inflammation, oxidative stress, and apoptosis which was significantly implicated in the pathophysiology of cognitive decline.[19,54,55,118] In addition, many expressed microRNAs showed to target SIRT-1; thus, dysfunction or abnormal transcription of these miRNAs by higher oxidative stress and apoptosis may lead to neurodegenerative disorders.[97,119,120]

In order to have a full understanding of differentially expressed miR-124a, miR-483-5p, miR-142-3p, and miR-125b and its exact correlation with DPP4 activity, BDNF, and SIRT-1 in the serum of MCI patients, bioinformatics analysis of their target genes are necessary. Moreover, DPP4 activity, BDNF, SIRT-1, and its related genes showed to be associated with apoptosis, oxidative stress, inflammatory, and neural differentiation.[16,32,121–123] More molecular-based tests such as luciferase assays are commonly used as a reporter to assess the transcriptional activity in intact cells. The most common applications of these gene assays are to examine the regulation of transcriptional activities by promoters and transcription factors. These assays have also been adapted for testing the effect of miRNA-mediated, posttranscriptional regulation on target genes. For many human genes, this test is achieved by engineering a luciferase gene construct containing the predicted miRNA targeting sequence from the target gene (often located in the 3-UTR).[17,18,124–128] Thus, in our study, the potential interaction between expressed miR-124a, miR-483-5p, miR-142-3p, and miR-125b and its exact correlation with DPP4 activity, BDNF, and SIRT-1 in the serum of MCI patients could be explained on the basis of miRNA targeting sequence from the target genes of DPP4 activity, BDNF, and SIRT-1 using luciferase assay; however, larger cohort sample size is required which could be evaluated in future studies.

Conclusion

Our results indicated that circulating miR-124a, miR-483-5p, miR-142-3p, and miR-125b were potential biomarkers for diagnosis of MCI and significantly associated with severe cognitive decline, oxidative stress, and apoptosis in patients with MCI. The detection of circulating miR-124a, miR-483-5p, miR-142-3p, and miR-125b might serve as a new non-invasive biomarker for MCI with high diagnostic performance. However,future experimental studies based upon bioinformatics analysis were required to confirm the diagnostic value of these

circulating miRNAs, as well as their regulation mechanisms in the pathogenesis of MCI in aged patients.

Acknowledgments

The authors are grateful to the Deanship of Scientific Research, King Saud University for funding through Vice Deanship of Scientific Research Chairs.

Disclosure

The authors declare that they have no competing interests.

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