Relationship and effect of miR-1-3p expression and BDNF level in patients with primary hypertension complicated with depression

Jihong Ding[1], Chunyu Jiang[2], Le Yang[3], Xianyan Wang[4, *]

[1]*The Outpatient Department of Psychology, The Third Affiliated Hospital of Qiqihar Medical University, Qiqihar 161000, Heilongjiang, P.R. China*

[2]*The Third Department of Cardiovascular Medicine, The Third Affiliated Hospital of Qiqihar Medical University, Qiqihar 161000, Heilongjiang, P.R. China*

[3]*The Department of Infection Control, The Third Affiliated Hospital of Qiqihar Medical University, Qiqihar 161000, Heilongjiang, P.R. China*

[4]*The Department of Basic Pathology, Pathology College, Qiqihar Medical University, Qiqihar 161006, Heilongjiang, P.R. China*

ARTICLE INFO

ABSTRACT

This experiment was designed to explore the relationship and effect of miR-1-3p expression and BDNF level in patients with primary hypertension complicated with depression. The subjects of the study were 145 patients with hypertension with a small fluctuation range of blood pressure in recent three months. Within 48 hours after admission, patients were evaluated with the Hospital Anxiety and Depression Scale (HADS) and Hamilton Depression Rating Scale (HAMD). After fasting for 12 hours, enrolled subjects were subject to blood collection (5 ml) in the morning for detecting blood lipid levels, miR-1-3p expression and BDNF by using an automatic biochemical analyzer, real-time fluorescence quantitative PCR (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA), respectively. Results showed that compared with the normal control group, while miR-1-3p expression increased obviously in patients with hypertension, while the level of BDNF decreased significantly; and compared with patients with simple hypertension, the expression of miR-1-3p in hypertension patients with depression was significantly increased, while BDNF level was decreased evidently (All $P < 0.05$). miR-1-3p expression in patients with hypertension complicated with depression was negatively correlated with serum BDNF level (r=-0.302, $P < 0.05$). In relative to the normal control population, the area under the curve (AUC) of ROC produced by serum miR-1-3p and BDNF in patients with primary hypertension complicated with depression was 0.971 (95% CI = 0.945-0.998, P < 0.0001) and 0.875 (95% CI = 0.808-0.942, P < 0.0001); and in relative to primary hypertension patients without depression, the AUC of ROC produced by serum miR-1-3p and BDNF in patients with primary hypertension with depression was 0.957 (95% CI = 0.925-0.989, P < 0.0001) and 0.883 (95% CI = 0.821-0.944, P < 0.0001), respectively. HADS-D score, HAMD score, course of the disease, miR-1-3p expression and BDNF level showed statistical differences in primary hypertension patients with and without depression (All $P < 0.05$). It was concluded that **t**here are high miR-1-3p expression and low serum BDNF levels in patients with primary hypertension complicated with depression. miR-1-3p has a negative correlation with BDNF, and it may play a role by negatively regulating the expression of BDNF. Detecting miR-1-3p and BDNF in patients with primary hypertension can indicate the occurrence of depression to some extent.

Introduction

Hypertension is a systemic disease in which the pathological increase of arterial pressure in patients at rest can lead to the lesions of important target organs such as the heart, brain, kidney and blood vessels (1,2). Clinically, it is divided into primary hypertension and secondary hypertension, of which the former one is more common (3,4). Hypertension is a clinical syndrome characterized by elevated systemic arterial pressure (5). Its pathogenesis is the result of the joint action of many factors, and it has complex pathogenesis (6). Family history of hypertension, high sodium and low potassium diet, obesity, excessive drinking and long-term mental stress is its traditional risk factors (6.7); In addition, clinically, some patients without the above risk factors can still get sick (8). Among them, new risk factors of hypertension, such as cysteine, tumor

necrosis factor, C-reactive protein and microRNAs (miRNAs, hot-spot recently), have attracted increasingly more attention in the medical community (9-12). In recent years, with the acceleration of population aging, hypertension has become the main risk factor for cardiovascular and cerebrovascular diseases (13). Owing to its high disability rate, lethality and the need for lifelong medication, it brings heavy psychological burden and economic pressure to hypertension patients (14,15). The incidence of hypertension complicated with depression is increasing year by year (16). Hypertension and depression affect each other and are inseparable (17). However, the pathogenesis of hypertension complicated with depression is not clear at this stage.

miRNA is a non-coding RNA molecule with a length of about 19 ~ 23 NT, which regulates gene expression at the post-transcriptional level (18). In cells, miRNA regulates gene expression by mediating the formation of RNA silencing complex and binding to the 3 'untranslated region (3'-UTR) of completely (plant) or incompletely (animal) matched mRNA, so as to degrade mRNA or through other approaches to inhibit translation (19,20). There are thousands of human miRNAs included in the miRBase database. miRNAs may control the expression of one-third of human genes and can exist in a stable form in the blood (21). In recent ten years, the research of miRNA has gradually become a research hotspot in the field of molecular biology (22-24). It plays an important regulatory role in the pathophysiological processes such as cell differentiation, development, proliferation and apoptosis (25,26). In view of its high stability, wide distribution and good repeatability, exploring the changes of miRNAs in the circulatory system is commonly studied as a non-invasive diagnostic marker of diseases (27-29). As a potential marker, miRNAs have also been studied in cardiovascular diseases, endocrine diseases, tumors, etc. (30-32). Depression is one of the most common mental diseases in the clinic (33). However, the research on depression still faces difficulties such as no clinical index with accurate early diagnostic significance and the shortage of effective agents for the treatment of depression. By detecting the changes of serum miR-1-3p content, this study analyzed the relationship between the changes of serum miR-1-3p and primary hypertension complicated with depression and studied the practical significance of serum miR-1-3p in patients with primary hypertension complicated with depression.

Materials and methods
Subjects of study

In this study, 145 patients with hypertension with a small fluctuation range of blood pressure in recent three months were randomly selected, and some basic data were collected from the enrolled patients by using the pre-designed general situation questionnaire, including gender, age, smoking history, course of the disease, etc. Within 48 hours after admission, patients were tested immediately with the Hospital Anxiety and Depression Scale (HADS) (34) and Hamilton Depression Rating Scale (HAMD) (35). According to the suggestions of the scale maker, patients with HADS-D $\geq$ 8 and HAMD $\geq$ 8 were diagnosed as depression, and those with HADS-D < 8 or HAMD < 8 were diagnosed as no depression. According to the results of the scale, the participants were divided into depression group and non-depression group. At the same time, 70 normal subjects who visited the hospital for general physical examination were included as the normal control group. Eligible subjects for the normal control group were those aged 18 years old, without cardiovascular diseases, diabetes, liver and kidney dysfunction, etc. after a careful medical history and physical examination, routine blood test, liver and kidney function test, blood sugar and blood lipids, chest X-ray, electrocardiogram and echocardiography.

According to the 2018 Chinese Guidelines for Prevention and Treatment of Hypertension (36), the diagnostic criteria of hypertension were systolic blood pressure (SBP) $\geq$ 140mmHg and/or diastolic blood pressure (DBP) $\geq$ 90mmHg. For the patients, corresponding inclusion criteria were: patients who met the diagnostic criteria for hypertension specified in the 2018 Chinese Guidelines for Prevention and Treatment of Hypertension, or who had been previously diagnosed with hypertension in hospitals above class II; and patients with depression based on HADS-D and/or HAMD. Exclusion criteria: Patients with diabetes, coronary heart disease and other cardiovascular and cerebrovascular diseases; patients

with secondary hypertension; patients with severe hepatic and renal insufficiency; patients undergoing or preparing to participate in other clinical trials; patients allergic to multiple drugs; patients with important organ damage or other serious system diseases in the medical history; patients with infectious diseases; patients with autoimmune diseases; patients with malignant tumors; patients with thyroid dysfunction; patients with severe trauma and surgical history in recent 6 months; patients with positive past or family history of mental disorders; patients with other serious mental diseases except depression; patients with clear suicidal tendency; patients with language impairment, cognitive impairment, or unable to complete the self-assessment scale due to factors such as educational level; and patients whose follow-up is interrupted for any reason and the curative effect cannot be judged. All subjects signed informed consent.

Collection of serum samples

After fasting for 12 hours, enrolled subjects were subject to blood collection (5 ml) in the morning, which were preserved in the disposable vacuum blood collection tubes. Within 2 hours, the serum was separated and transferred to the RNase-free EP tube y centrifugation at 3500rpm 4℃ for 15 minutes. The above samples were centrifuged at 14000rpm for 15min again, and the supernatant was collected with the possible residual cell fragments removed, which was regarded as the experimental serum sample, stored in the refrigerator at - 80℃ for further use.

Detection of miR-1-3p in serum by real-time fluorescence quantitative PCR (qRT-PCR)

The total serum RNA of the above subjects was taken respectively, and the specific steps were carried out according to the kit operation manual. RNA was reverse transcribed into cDNA by a reverse transcription kit. The reaction conditions were 42 ℃ for 20 min, 99 ℃ for 5 min and 4 ℃ for 5 min. Using cDNA as a template, qRT-PCR was carried out by PCR kit. PCR reaction solution was prepared according to the following system: 2 μl stock solution after reverse transcription reaction; 6 μl RNase Free H2O; 1 μl sequence-specific upstream primer (10 pmol/μl); 1 μl sequence-specific downstream primer (10 pmol/μl); and 10 μl 2×Taq PCR Master Mix, in a total of 20 μl. The reaction procedure was: 94 °C for

the 90s; and 40 cycles of 94 °C for 30s, 60 °C for 30s; and 72 °C for 1 min. With U6 as an internal reference, PCR primers were synthesized by Invitrogen Trading (Shanghai) Co., Ltd. Primers, templates and enzymes were added in proportion, and qRT-PCR was performed with 7300 quantitative PCR instruments. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression of miR-1-3p in each sample.

Index detection in the serum

As for the detection of BDNF level in the serum, another serum sample was taken for detection by using the human brain-derived neurotrophic factor (BDNF) kit according to enzyme-linked immunosorbent assay (ELISA). In addition, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured by enzymatic determination with an automatic biochemical analyzer with the collected venous blood as the sample.

Statistical analysis

SPSS 19.0 software (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. Continuous variables with normal distribution were expressed by mean ± standard deviation, categorical variables were expressed by percentage, and non-normal distribution variables were expressed by median (interquartile spacing). The continuous variables were compared with one-way ANOVA, and the categorical variables were compared with the chi-square test or Fisher exact test. Pearson correlation analysis and ROC curve analysis were performed at the same time. In addition, univariate analysis confirmed the influencing factors of hypertension complicated with depression. $P < 0.05$ was used to present that the difference was statistically significant.

Results and discussion

Baseline characteristics of enrolled subjects

Based on the scoring criteria of HADS-D and HAMD scales, 145 patients with hypertension were included and divided into the depression group (N=66) and the non-depression group (N=79). The baseline characteristics of all subjects are shown in Table 1. There was no significant difference in age, gender and other general data among the three groups.

Furthermore, the levels of TC, TG, LDL-C and Hcy in patients from the depression group and non-depression group were significantly higher than those in the normal control group, and the level of HDL-C was significantly lower than that in the normal control group (All $P < 0.05$). In addition, the HADS-D and HAMD scores of patients with depression were significantly higher than that of patients without depression ($P < 0.05$).

Table 1. Baseline characteristics of enrolled subjects

	Normal control group (N=70)	Non-depression group (N=79)	Depression group (N=66)
Age (years)	57±9	55±10	61±8
Female, n (%)	32 (45.71)	40 (50.63)	30 (48.48)
BMI (kg/m^2)	22.13±2.80	22.86±3.41	23.54±3.87
Hypertension, n (%)	0 (0)	79 (79)	66 (66)
Depression, n (%)	0 (0)	0 (0)	66 (66)
HADS-D	4.65±1.88	4.80±2.01	8.52±3.16
HAMD	5.32±1.12	5.64±1.30	22.35±1.79
Smoking, n (%)	29 (41.43)	36 (45.57)	27 (40.91)
Duration of hypertension (years)	0	9±6	14±5
TC level	4.53±0.48	5.27±0.51	5.33±0.59
TG level	1.28±0.41	3.21±0.39	3.33±0.47
HDL-C level	1.40±0.22	1.01±0.18	1.15±0.20
LDL-C level	2.41±0.35	3.78±0.36	3.98±0.40
Homocysteine level	11.52±1.26	17.89±1.76	18.25±1.84

Note: BMI, body mass index; HADS-D, Hospital Anxiety and Depression Scale-Depression; HAMD, Hamilton Depression Rating Scale; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

**Detection of miR-1-3p expression and BDNF level in the serum**

qRT-PCR and ELISA were used to detect the miR-1-3p expression and BDNF level, respectively. The results showed that there were significant differences in miR-1-3p expression and BDNF level among the three groups (All $P < 0.05$). Specifically, compared with the normal control group, there was no significant change in miR-1-3p expression and BDNF level in the non-depression group (All $P > 0.05$); while miR-1-3p expression increased obviously in patients with hypertension, while the level of BDNF decreased significantly (All $P < 0.05$). At the same time, compared with patients with simple hypertension, the expression of miR-1-3p in hypertension patients with depression was significantly increased, while BDNF level was decreased evidently (All $P < 0.05$). The results are shown in Figure 1.
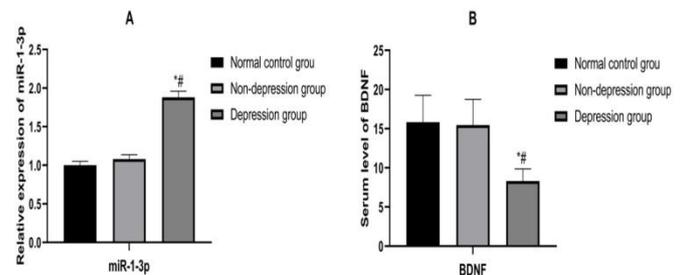
Figure 1. Detection of miR-1-3p expression and BDNF level in the serum by qRT-PCR and ELISA respectively. Note: A. Detection of miR-1-3p expression in the serum by qRT-PCR; B. Detection of BDNF level in the serum by ELISA; *compared with the normal control group, $P < 0.05$; #compared with the non-depression group, $P < 0.05$. miR-1-3p, microRNA-1-3p; BDNF, brain-derived neurotrophic factor

Correlation analysis of serum miR-1-3p and BDNF in patients with primary hypertension complicated with depression

As shown in Figure 2, Pearson correlation analysis showed that the expression of serum miR-1-3p in patients with hypertension complicated with depression was negatively correlated with the level of serum BDNF (r=-0.302, $P < 0.05$).
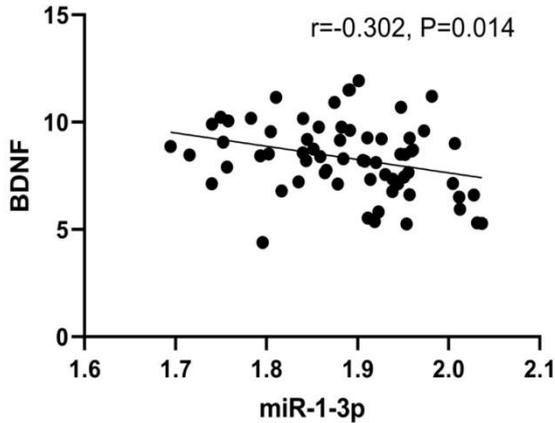
Figure 2. Correlation analysis of serum miR-1-3p and BDNF in patients with primary hypertension complicated with depression. Note: miR-1-3p, microRNA-1-3p; BDNF, brain-derived neurotrophic factor

Efficacy analysis of miR-1-3p and BDNF on patients with primary hypertension complicated with depression

In relative to the normal control population, the area under the curve (AUC) of ROC produced by serum miR-1-3p in patients with primary hypertension complicated with depression was 0.971 (95% CI = 0.945-0.998, P < 0.0001). The AUC of ROC produced by serum BDNF was 0.875 (95% CI = 0.808-0.942, P < 0.0001).

While in relative to primary hypertension patients without depression, the AUC of ROC produced by serum miR-1-3p in patients with primary hypertension with depression was 0.957 (95% CI = 0.925-0.989, P < 0.0001). In addition, the AUC of ROC produced by serum BDNF was 0.883 (95% CI = 0.821-0.944, P < 0.0001). Serum miR-1-3p and BDNF may be indicative of primary hypertension complicated with depression and can be used as new indexes to judge primary hypertension complicated with depression from those of normal population and patients without depression.

Analysis of influential factors in patients with primary hypertension complicated with depression

Based on the occurrence of depression or not, this part of the study further explored the effects of gender, age, BMI, HADS-D score, HAMD score, blood lipid level, homocysteine level, smoking history, course of the disease, miR-1-3p expression and BDNF level on patients with primary

hypertension. As shown in Table 2, HADS-D, HAMD score, course of the disease, miR-1-3p expression and BDNF level showed statistical differences in primary hypertension patients with and without depression (All *P* < 0.05). These results indicated that patients with higher HADS-D and HAMD scores, longer duration of hypertension, increased expression of miR-1-3p, and lower level of BDNF would promote the occurrence of depression in primary hypertension patients.

Table 2. Influential factors in patients with primary hypertension complicated with depression

	Non-depression group	Depression group	*P*-value
HADS-D score	4.80±2.01	8.52±3.16	< 0.001
HAMD score	5.64±1.30	22.35±1.79	< 0.001
Duration of hypertension (years)	9±6	14±5	< 0.001
miR-1-3p expression	1.08±0.06	1.95±0.24	< 0.001
BDNF level	16.21±6.46	8.33±1.71	< 0.001

Note: HADS-D, Hospital Anxiety and Depression Scale-Depression; HAMD, Hamilton Depression Rating Scale; miR-1-3p, microRNA-1-3p; BDNF, brain-derived neurotrophic factor.

The comorbidity rate of hypertension with anxiety and/or depression is increasing year by year (16, 37). Patients with hypertension are prone to anxiety and depression (17, 38). At present, the common pathogenesis of hypertension with anxiety and/or depression may be related to autonomic nerve dysfunction, activation of the immune system or neuronal damage caused by inflammatory reaction (39,40). Despite the deepening understanding of the occurrence and development of hypertension with anxiety and/or depression and the continuous improvement of the treatment of double-cardiac diseases (cardiovascular diseases and psychological disorders) (41,42), the comorbidity rate of hypertension with anxiety and/or depression is still quite high, which indicates that the treatment of anti-anxiety and depression requires to be improved. At present, it is particularly important to find out new therapeutic targets for hypertension complicated with depression. This study detected the changes of serum miR-1-3p expression and BDNF level in patients with primary hypertension complicated with depression, and analyzed the relationship of the changes of serum

miR-1-3p and BDNF with primary hypertension complicated with depression, so as to identify the practical significance of serum miR-1-3p and BDNF in the diagnosis of primary hypertension complicated with depression.

BDNF is a member of the neurotrophin family that has a high content in the hippocampus and cortex (43). By binding with its receptor, BDNF activates the intracellular signal transduction pathway, starts gene transcription, and participates in physiological activities such as promoting neuronal growth and regulating synaptic plasticity (44). Furthermore, miRNA is a kind of small non-coding RNA that plays an important role in life activities (21, 24). Our understanding of the pathophysiological mechanisms of psychological disorders such as depression has significantly increased over the years, there is still a shortage in the diagnosis and treatment of this type of disease, especially when it is acting as comorbidity (45). For this reason, research on miRNAs develops rapidly recently, which has been explored for psychological disorders. In this study, in patients with primary hypertension complicated with depression, the expression of serum miR-1-3p was significantly higher and the level of BDNF in serum was much lower than those in healthy controls and primary hypertension patients without depression; but there was no significant difference in the levels of miR-1-3p and BDNF between hypertension patients without depression and healthy subjects. It is suggested that miR-1-3p and BDNF may be involved in the process of primary hypertension complicated with depression. Meanwhile, in our previous research, we searched the online miRanda website and found that there were binding sites between miR-1-3p and BDNF. Significantly, in our study, through Pearson analysis, it was found that there was a negative correlation between miR-1-3p and BDNF in the serum of patients with primary hypertension complicated with depression. It is suggested that the high expression of miR-1-3p may participate in the process of depression in patients with primary hypertension by down-regulating the level of BDNF, but the specific mechanism has not been clarified that requires further identification. Further ROC curve analysis shows that in relative to the normal control population and primary hypertension patients without depression, the AUC of ROC predicted by serum miR-1-3p in

patients with primary hypertension was 0.971 and 0.957; and that of BDNF was 0.875 and 0.883, respectively. Both indexes showed relatively higher diagnostic value, especially miR-1-3p, suggesting that both miR-1-3p and BDNF can be used for the diagnosis of hypertension patients with depression or not, and BDNF can be regarded as an auxiliary prediction index.

Moreover, through the analysis of clinicopathological parameters, in addition to HADS-D, HAMD score, and course of the disease, the feasibility of both miR-1-3p and BDNF was confirmed in the diagnosis of primary hypertension complicated with depression. It can further improve the early diagnosis rate of patients with primary hypertension complicated with depression, so as to achieve the purpose of early treatment, and lay a research foundation for clarifying the gene regulation and corresponding mechanism of miR-1-3p in the development of primary hypertension complicated with depression. Therefore, our study proposed that both miR-1-3p and BDNF may be new indicators of primary hypertension complicated with depression.

In conclusion, there is a high expression of serum miR-1-3p and a low level of serum BDNF in patients with primary hypertension complicated with depression. miR-1-3p has a negative correlation with BDNF, and it may play a role by negatively regulating the expression of BDNF. Detecting the levels of serum miR-1-3p and BDNF in patients with primary hypertension can predict the occurrence of primary hypertension complicated with depression. However, there are some deficiencies in this study, such as few research samples. In order to early detect and diagnose primary hypertension complicated with depression, it is necessary to further expand the samples to verify and screen the specific serum biomarker combination of primary hypertension complicated with depression. In addition, this study focuses on clinical exploration. In the future, it is necessary to carry out in vivo and in vitro experiments to verify the specific relationship, roles and relevant mechanisms of the miR-1-3p/BDNF axis.

Acknowledgments

Medical Sciences of Qiqihar Medical University (Code: QMSI2019L-31)

Compliance with ethical standards
None.

Conflict of interest
The authors declare that they have no conflicts of interest concerning this article.

References
1. Baltatu OC, Amaral FG, Campos LA, et al. Melatonin, mitochondria and hypertension. Cell Mol Life Sci. 2017;74(21):3955-3964.
2. Di Daniele N, Marrone G, Di Lauro M, et al. Effects of Caloric Restriction Diet on Arterial Hypertension and Endothelial Dysfunction. Nutrients. 2021;13(1):274.
3. Tziomalos K. Secondary Hypertension: Novel Insights. Curr Hypertens Rev. 2020;16(1):11.
4. Manosroi W, Williams GH. Genetics of Human Primary Hypertension: Focus on Hormonal Mechanisms. Endocr Rev. 2019;40(3):825-856.
5. Pedersen J, Hedegaard ER, Simonsen U, et al. Current and Future Treatments for Persistent Pulmonary Hypertension in the Newborn. Basic Clin Pharmacol Toxicol. 2018;123(4):392-406.
6. Thenappan T, Ormiston ML, Ryan JJ, et al. Pulmonary arterial hypertension: pathogenesis and clinical management. BMJ. 2018;360:j5492.
7. Vallianou NG, Geladari E, Kounatidis D. Microbiome and hypertension: where are we now? J Cardiovasc Med (Hagerstown). 2020;21(2):83-88.
8. Vasdev S, Stuckless J. Role of methylglyoxal in essential hypertension. Int J Angiol. 2010 Summer;19(2):e58-65.
9. Lu Y, Sun X, Peng L, et al. Angiotensin II-Induced vascular remodeling and hypertension involves cathepsin L/V- MEK/ERK mediated mechanism. Int J Cardiol. 2020;298:98-106.
10. Mehaffey E, Majid DSA. Tumor necrosis factor-α, kidney function, and hypertension. Am J Physiol Renal Physiol. 2017;313(4):F1005-F1008.
11. Kansui Y, Matsumura K, Morinaga Y, et al. C-reactive protein and incident hypertension in a worksite population of Japanese men. J Clin Hypertens (Greenwich). 2019;21(4):524-532.
12. Li X, Wei Y, Wang Z. microRNA-21 and hypertension. Hypertens Res. 2018 Sep;41(9):649-661.
13. Turana Y, Tengkawan J, Chia YC, et al. Hypertension and stroke in Asia: A comprehensive review from HOPE Asia. J Clin Hypertens (Greenwich). 2021;23(3):513-521.
14. Okeahialam BN, Adeniyi MA. Economic benefit of back titration in the treatment of hypertension in Jos, Nigeria. Clinicoecon Outcomes Res. 2017;9:207-210.
15. Zhai Z, Zhou X, Zhang S, et al. The impact and financial burden of pulmonary arterial hypertension on patients and caregivers: results from a national survey. Medicine (Baltimore). 2017;96(39):e6783.
16. Zhang Y, Chen Y, Ma L. Depression and cardiovascular disease in elderly: Current understanding. J Clin Neurosci. 2018;47:1-5.
17. Valladares-Garrido MJ, Soriano-Moreno AN, Rodrigo-Gallardo PK, et al. Depression among Peruvian adults with hypertension and diabetes: Analysis of a national survey. Diabetes Metab Syndr. 2020;14(2):141-146.
18. Ko NY, Chen LR, Chen KH. The Role of Micro RNA and Long-Non-Coding RNA in Osteoporosis. Int J Mol Sci. 2020;21(14):4886.
19. Huang Y. The novel regulatory role of lncRNA-miRNA-mRNA axis in cardiovascular diseases. J Cell Mol Med. 2018;22(12):5768-5775.
20. Correia de Sousa M, Gjorgjieva M, Dolicka D, Sobolewski C, Foti M. Deciphering miRNAs' Action through miRNA Editing. Int J Mol Sci. 2019;20(24):6249.
21. Reza AMMT, Yuan YG. microRNAs Mediated Regulation of the Ribosomal Proteins and its Consequences on the Global Translation of Proteins. Cells. 2021;10(1):110.
22. Kabekkodu SP, Shukla V, Varghese VK, et al. Cluster miRNAs and cancer: Diagnostic, prognostic and therapeutic opportunities. Wiley Interdiscip Rev RNA. 2020;11(2):e1563.
23. Wang H, Liu W, Luo B. The roles of miRNAs and lncRNAs in Epstein-Barr virus associated epithelial cell tumors. Virus Res. 2021;291:198217.
24. Cao Q, Wu J, Wang X, Song C. Noncoding RNAs in Vascular Aging. Oxid Med Cell Longev. 2020;2020:7914957.

25. Chen D, Hu S, Wu Z, et al. The Role of MiR-132 in Regulating Neural Stem Cell Proliferation, Differentiation and Neuronal Maturation. Cell Physiol Biochem. 2018;47(6):2319-2330.

26. Jin Y, Wang J, Zhang M, et al. Role of bta-miR-204 in the regulation of adipocyte proliferation, differentiation, and apoptosis. J Cell Physiol. 2019;234(7):11037-11046.

27. Ghalehnoei H, Bagheri A, Fakhar M, et al. Circulatory microRNAs: promising non-invasive prognostic and diagnostic biomarkers for parasitic infections. Eur J Clin Microbiol Infect Dis. 2020;39(3):395-402.

28. Erdmann K, Salomo K, Klimova A, et al. Urinary MicroRNAs as Potential Markers for Non-Invasive Diagnosis of Bladder Cancer. Int J Mol Sci. 2020;21(11):3814.

29. Ghamarnia, H., Mousabeygi, F., Rezvani, S. Water Requirement, Crop Coefficients of Peppermint (*Mentha piperita* L.) and Realizing of SIMDualKc Model. Agrotech Ind Crops 2021; 1(3): 110-121. doi: 10.22126/atic.2021.6791.1019.

30. Zhao X, Wang Y, Sun X. The functions of microRNA-208 in the heart. Diabetes Res Clin Pract. 2020;160:108004.

31. Lima CR, Gomes CC, Santos MF. Role of microRNAs in endocrine cancer metastasis. Mol Cell Endocrinol. 2017;456:62-75.

32. Tömböl Z, Turai PI, Decmann Á, Igaz P. MicroRNAs and Adrenocortical Tumors: Where do we Stand on Primary Aldosteronism? Horm Metab Res. 2020;52(6):394-403.

33. Ostuzzi G, Matcham F, Dauchy S, et al. Antidepressants for the treatment of depression in people with cancer. Cochrane Database Syst Rev. 2018;4(4):CD011006.

34. Snaith RP. The Hospital Anxiety And Depression Scale. Health Qual Life Outcomes. 2003 Aug 1;1:29.

35. Williams JB, Kobak KA, Bech P, et al. The GRID-HAMD: standardization of the Hamilton Depression Rating Scale. Int Clin Psychopharmacol. 2008;23(3):120-9.

36. Joint Committee for Guideline Revision. 2018 Chinese Guidelines for Prevention and Treatment of Hypertension-A report of the Revision Committee of Chinese Guidelines for Prevention and Treatment of Hypertension. J Geriatr Cardiol. 2019;16(3):182-241.

37. Bussotti M, Sommaruga M. Anxiety and depression in patients with pulmonary hypertension: impact and management challenges. Vasc Health Risk Manag. 2018;14:349-360.

38. Takita Y, Takeda Y, Fujisawa D, et al. Depression, anxiety and psychological distress in patients with pulmonary hypertension: a mixed-methods study. BMJ Open Respir Res. 2021;8(1):e000876.

39. Vasyuk YA, Dovzhenko TV, Dubrovskaya TI, et al. Characteristics of arterial hypertension clinical course in patients with obesity and anxiety-depressive disorders.. Ter Arkh. 2021;93(1):94-99.

40. Oganov RG, Pogosova GV, Koltunov IE, Ret al. [Depressive symptoms worsen cardiovascular prognosis and shorten length of life in patients with arterial hypertension and ischemic heart disease]. Kardiologiia. 2011;51(2):59-66.

41. Cohen BE, Edmondson D, Kronish IM. State of the Art Review: Depression, Stress, Anxiety, and Cardiovascular Disease. Am J Hypertens. 2015;28(11):1295-302.

42. De Hert M, Detraux J, Vancampfort D. The intriguing relationship between coronary heart disease and mental disorders. Dialogues Clin Neurosci. 2018;20(1):31-40.

43. Sasi M, Vignoli B, Canossa M, Blum R. Neurobiology of local and intercellular BDNF signaling. Pflugers Arch. 2017;469(5-6):593-610.

44. Chen SD, Wu CL, Hwang WC, et al. More Insight into BDNF against Neurodegeneration: Anti-Apoptosis, Anti-Oxidation, and Suppression of Autophagy. Int J Mol Sci. 2017;18(3):545.

45. Fries GR, Zhang W, Benevenuto D, Quevedo J. MicroRNAs in Major Depressive Disorder. Adv Exp Med Biol. 2019;1118:175-190.