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Differential Expression of Hypertension-Associated MicroRNAs in the Plasma of Patients With White Coat Hypertension

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Abstract: White coat hypertension (WCH) is a high cardiovascular risk condition, and a fundamental understanding of the cause and pathophysiology of the disorder is still lacking. Recent studies demonstrated that microRNAs (miRNAs) are involved in hypertension; however, the roles of miRNAs in WCH are not known.

The expressions of selected 10 miRNAs were investigated independently in plasma samples from 30 hypertension (HT) patients, 30 WCH patients, and 30 normotensive (NT) subjects.

MiR-21, miR-122, miR-637, and let-7e expression levels were significantly upregulated in the HT group compared with the NT groups ($P = 0.017$, $P = 0.022$, $P = 0.048$, and $P = 0.013$, respectively). MiR-122 and miR-637 expressions were also significantly upregulated in the WCH group compared with the NT group ($P = 0.048$ and $P = 0.039$, respectively). MiR-296-5p expression level was significantly downregulated in HT patients and upregulated in the WCH patients compared with the NT group ($P = 0.049$ and $P = 0.039$, respectively).

Additionally, the ambulatory 24-hour and daytime systolic and diastolic blood pressures were negatively correlated with miR-296-5p. MiR-296 and miR-637 had area under the curve (AUC) values of 0.778 and 0.774, respectively, which demonstrates their sufficiency to distinguish WCH from NT individuals. MiR-296 and miR-637 had AUC values of 0.868 and 0.680, respectively, which shows their potential to distinguish WCH from HT individuals.

We report for the first time a plasma miRNA profile for WCH patients and demonstrate a novel link between miRNA and WCH. These findings may reveal crucial insights into the development of WCH.

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Abbreviations: ABPM = ambulatory blood pressure monitoring, AUC = area under the curve, cDNA = first-strand DNA, HT = hypertension, miRNA = microRNA, NT = normotensive, qRT-PCR = quantitative RT-PCR, ROC = receiver operating characteristic, WCH = white coat hypertension.

INTRODUCTION

Hypertension, as one of the most significant modifiable risk factors for cardiovascular, cerebrovascular, and renal diseases, is an important public health challenge worldwide due to its high prevalence and concomitant elevation in associated disease risk.[1] In addition, chronic exposure to elevated blood pressure induces ''target-organ damage,'' which is responsible for hypertension-related morbidity and mortality, and causes various structural and functional alterations in tissues and organ systems.[2] Several mechanisms have been implicated in the pathogenesis of hypertension including overactivation of the renin–angiotensin–aldosterone system, sympathetic nervous system overdrive, endothelial dysfunction, oxidative stress, and impaired angiogenesis; however, many molecular aspects of the essential hypertension development still remains to be clarified.

Pickering et al[3] first used the term white coat hypertension (WCH) in a 1988 publication for subjects who were not on treatment for hypertension with elevated office blood pressure and normal daytime blood pressure measured with ambulatory blood pressure monitoring (ABPM).[4] Despite several earlier reports, the ability of WCH to lead cardiovascular target organ damage similar to sustained hypertension remains controversial.[5]

The endothelial dysfunction, atherosclerotic processes, and target-organ damage were accompanied with WCH, as they were in the hypertension (HT) patients.[6,7] Patients with WCH, which is a high cardiovascular risk condition, have more tendencies to develop sustained hypertension, metabolic syndrome, and diabetes compared with NTs. The cardiovascular event was found to be intermediate between sustained hypertension and normotension.[8] WCH has unknown epigenetic infrastructure, which increases the cardiovascular risks including hypertension.

MicroRNAs (miRNAs) are small, noncoding RNA molecules ~21 to 23 bp long. MiRNAs induce messenger RNA (mRNA) degradation or inhibit their targets' translations through posttranscriptional targeting of mRNAs. The roles of miRNAs have been extensively elucidated in several diseases (diabetes mellitus, metabolic syndrome, and cancer); nonetheless, there is a paucity of data when it comes to cardiovascular disorders and hypertension. Research only recently focused on the association of miRNAs and hypertension, and some new insights are sure to follow. There are, however, a limited

number of reports in the literature that studied miRNAs in HT, whereas there is no data for WCH.

In this regard, for their function in gene expression regulation, the role of several epigenetic mechanisms is lately emerging as a possible step forward in the understanding of hypertension development. In this study, we evaluated the expression levels of circulating miR-21,[9] miR-122,[10] miR-125a,[11] miR-126,[9] miR-130,[12] miR-155,[13] miR-195,[14] miR-296-5p,[15] miR-637,[16] and let-7e,[11] which were previously demonstrated to be deregulated in the plasma of patients with hypertension, and analyzed their potential for identifying, evaluating, and distinguishing HT and WCH from NT healthy individuals.

MATERIALS AND METHODS

Informed Consent

The protocol for sample collection was approved by the ethics committee of Istanbul University's Cerrahpasa Medical School and was carried out according to the requirements of the Declaration of Helsinki. All patients were fully informed of the study procedures before they gave their consents.

Study Population

The study group consisted of 30 untreated WCH patients (10 male, 20 female; age 45.3±7.1 years) and 30 newly diagnosed and untreated HT patients (13 male, 17 female; age 47.2 ± 5.4 years) referred to our outpatient clinic over a 6-month period from January to June 2014. The study also included 30 NT healthy volunteers (10 male, 20 female; age 44.3 ± 5.7 years) who served as the control group. Patients were classified as WCH, HT, or NT before the study. Power analysis demonstrated that a total of at least 56 patients (28 patients in each group) are required to achieve 80% power at 2-sided 5% significance level for distinguishing WCH patients from healthy individuals or patients with hypertension.

The same doctor obtained brachial arterial pressures with a mercury sphygmomanometer, which was standardized in accordance with the approval of the American and British Hypertension Society and the World Health Organization. Measurements were performed on 3 occasions within a span of 5 days. The average of the 3 measurements was taken as the SBP and diastolic blood pressure (DBP).

Subjects with other risk factors for atherosclerosis (LDL > 130 mg/dL, diabetes mellitus, metabolic syndrome, body mass index (BMI) >27 kg/m^2, and smoking), subjects with signs or symptoms of atherosclerotic vascular disease, sleep apnea syndrome, chronic obstructive lung disease and/or asthma, endocrine diseases, or alcoholism were excluded. Patients using drugs for blood pressure (angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and β-blockers), lipid metabolism (statins and fibrates), and antioxidant substances within the previous 12 months were also excluded. All patients were free of concomitant vascular disease, malignancy, and connective tissue diseases.

Measurements of Blood Pressure

Patients with a blood pressure >140/90 mm Hg underwent a 24-hour ABPM with an ABPM device (BR-102 Plus, Schiller, Baar, Switzerland) approved by the European Hypertension Society.[17] Measurements were performed on the left arm as suggested by the British Hypertension Society.[18] Patients were classified into sustained HT (ABPM overall ≥130/80 mm Hg and/or daytime ≥135/85 mm Hg) and WCH (ABPM overall <130/80 mm Hg and/or daytime <135/85 mm Hg) groups according to the results of the ambulatory measurements before study.

Sample Collection and RNA Isolation

Whole blood samples were collected into ethylenediaminetetraacetic acid containing Vacutainers in the morning after 12 to 14 hours of fasting. Then the samples were immediately centrifuged for plasma separation at 3000 g for 10 minutes. After phase separation, plasmas were collected and divided into 2 aliquots, snap frozen and stored at −80°C until RNA isolation.

Total RNA from 30 HT, 30 WCH, and 30 NT blood samples were isolated using miRNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The purities and concentrations of RNA samples were determined spectrophotometrically using NanoDrop ND-2000c (Thermo Fisher Scientific, Inc, Wilmington, DE).

cDNA Synthesis and Quantitative Real-Time PCR

First-strand DNA (cDNA) synthesis was carried out with 2 μL of total RNA from each sample using "miScript II RT Kit" (Qiagen) following the manufacturer's protocol. Custom miScript miRNA PCR Array (Qiagen) was used to profile the expression levels of the following miRNAs, which were previously demonstrated to be associated with HT: miR-21, miR-122, miR-125a, miR-126, miR-130a, miR-155, miR-195, miR-296, miR-637, and let7e. RNU6B was used for normalization of miRNA expression levels. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was carried out using miScript SYBR Green PCR Kit (Qiagen) following the manufacturer's protocol in a Roche LightCycler 480-II real-time thermal cycler (Roche, Basel, Switzerland). Each experiment was performed in duplicate. The relative quantification analysis was done by delta-delta-CT method as described.[19] Raw data of the qRT-PCR experiments are provided as a supplementary material in Gene Expression Omnibus format.

Data Analysis and Statistics

A sample size of n = 28 per group is required to provide 80% power to detect a difference in the mean relative expression levels with a significance of 0.001 (2-sided α). The normal distribution of data was tested by the 1-sample Kolmogorov–Smirnov test. All statistical comparisons were performed using the 2-sided student *t* test on log-transformed data. The unpaired *t* test was also validated using the nonparametric Mann–Whitney *U* test. An analysis of variance was utilized to compare multiple group means. The following post hoc evaluation was made by Bonferroni method. Categorical variables were compared by use of the χ^2 test or Fisher exact test for small samples. The values exhibiting continuity were given as mean or standard deviation. Pearson correlation was used for numerical data. Spearman correlation was used for nominal data. To evaluate the diagnostic accuracy, we carried out receiver operating characteristic (ROC) curve analysis. ROC curves were plotted to see the power of miRNAs to differentiate the HT, WCH, and NT groups from each other. The area under the curve (AUC) was then estimated with 95% confidence interval. $P < 0.05$ values were considered to be statistically significant. Statistical analyses were performed using SPSS 17.0 software for Windows (SPSS Inc, Chicago, IL).

RESULTS

The basic characteristics, and ambulatory and clinical blood pressure measurements of the studied groups are provided

in Table 1. Age, sex distribution, BMI, waist measurement, glucose, hemoglobin a1c, total cholesterol, HDL, LDL, triglyceride, thyroid stimulating hormone, creatinine, and creatinine clearance did not show difference among the groups. The clinical systolic blood pressure (SBP) and DBP measurements of the NT group were significantly lower than in the HT group ($P < 0.001$ and $P < 0.001$, respectively) and the WCH group ($P < 0.001$ and $P < 0.001$, respectively). The ambulatory 24-hour, daytime and nighttime SBP and DBP of the HT group were significantly higher than in the WCH and control groups ($P < 0.001$, all).

The relative expression levels of selected miRNAs in study groups are shown in Table 2. let-7e ($P = 0.013$), miR-21 ($P = 0.017$), miR-122 ($P = 0.022$), and miR-637 ($P = 0.048$) displayed increased relative expression levels in HT patients compared with those of NT group. Expression levels of miR-21 and let-7e did not show any alteration in WCH group in comparison with NT individuals. MiR-122 ($P = 0.048$) and miR-637 ($P = 0.039$) showed increased expression in the WCH group. On the contrary, miR-296-5p expression was significantly reduced in HT patients in comparison with NT individuals ($P = 0.049$). Interestingly, its expression is strongly upregulated in WCH group ($P = 0.039$). No significant difference was observed between the control and other groups with regard to miR-125a, miR-126, miR-130a, miR-155, and miR-195 expression levels (Figure 1).

The clinical SBP was positively correlated with miR-21 ($r = 0.215$, $P = 0.042$), miR-122 ($r = 0.219$, $P = 0.043$), miR-637 ($r = 0.251$, $P = 0.018$), and let-7e ($r = 0.220$, $P = 0.037$). The ambulatory 24-hour SBP and DBP were negatively correlated with miR-296-5p ($r = -0.531$, $P < 0.001$ and $r = -0.528$, $P < 0.001$, respectively) (Figure 2A and B). The ambulatory daytime SBP was positively correlated with miR-21 ($r = 0.256$, $P = 0.048$) and let-7e ($r = 0.308$, $P = 0.017$). MiR-296-5p was negatively correlated with ambulatory daytime SBP ($r = -0.523$, $P < 0.001$) and ambulatory daytime DBP ($r = -0.424$, $P = 0.001$). The ambulatory nighttime SBP was positively correlated with miR-21 ($r = 0.278$, $P = 0.032$) and let-7e ($r = 0.326$, $P = 0.011$). MiR-296-5p was negatively correlated with ambulatory nighttime SBP ($r = -0.416$, $P = 0.001$) and ambulatory nighttime DBP ($r = -0.457$, $P < 0.001$). There was no correlation between age, sex, and miRNAs in all groups. Correlation of miRNAs' expressions with each other are demonstrated in Table 3.

To test the power of the miRNAs for distinguishing NT, WCH, and HT groups, ROC analysis was performed. MiR-296 and miR-637 had AUC values of 0.778 and 0.774, respectively, which demonstrates their sufficiency to distinguish WCH from NT individuals (Figure 3A). MiR-122 had AUC values of 0.688, which demonstrates its potential to distinguish HT from NT individuals on its own (Figure 3B). MiR-296 and miR-637, with AUC values of 0.868 and 0.680, respectively, also had enough power to distinguish WCH from HT individuals on their own (Table 4 and Figure 3C).

DISCUSSION

Arterial hypertension is a crucial risk factor for myocardial infarction, congestive heart failure, stroke, and cardiovascular-induced morbidity and mortality. Primary or essential

TABLE 1. Demographic Characteristics, Clinical Blood Pressure, and ABPM of Study Groups

	NT Group (n = 30)	WCH Group (n = 30)	HT Group (n = 30)	*P*
Sex (F/M)	20/10	20/10	17/13	0.65
Age, y	44.3 ± 5.7	45.3 ± 7.1	47.2 ± 5.4	0.164
BMI, kg/m^2	23.6 ± 1.1	23.8 ± 1.3	23.7 ± 1.4	0.772
Waist measurement, mm	85.3 ± 6.7	85 ± 5.2	83.1 ± 4.5	0.239
Glucose, mg/dL	85.5 ± 5.2	88.2 ± 9.2	88.8 ± 7.1	0.158
Hemoglobin a1c, %	5.2 ± 0.4	5.3 ± 0.3	5.4 ± 0.3	0.134
T. cholesterol, mg/dL	176.8 ± 23	182.8 ± 16.1	181.9 ± 16.5	0.408
HDL, mg/dL	58.9 ± 13.2	53.6 ± 10.7	53.2 ± 11.2	0.117
LDL, mg/dL	105.6 ± 20.9	111.8 ± 10.4	110.8 ± 17	0.302
Triglyceride, mg/dL	91.1 ± 29.1	103.2 ± 28.7	96.3 ± 34.1	0.319
Thyroid stimulating hormone, μIU/mL	2.2 ± 1.1	1.9 ± 1.1	1.9 ± 0.9	0.523
Creatinine, mg/dL	0.7 ± 0.2	0.8 ± 0.1	0.8 ± 0.2	0.439
Creatinine clearance, mL/min	111.4 ± 31.4	123.1 ± 29.4	117.4 ± 27.6	0.320
Clinical SBP, mm Hg	121.7 ± 6.9	148.8 ± 4.3	149.6 ± 5.3	**<0.001**[†]
Clinical DBP, mm Hg	75.2 ± 5.5	94.3 ± 3.6	94.7 ± 3.8	**<0.001**[‡]
Overall SBP, mm Hg[*]	119.8 ± 8.7	118.1 ± 8.8	145.2 ± 4.4	**<0.001**[§]
Overall DBP, mm Hg[*]	71.6 ± 6.4	74.3 ± 5.7	91.7 ± 3.9	**<0.001**[§]
Daytime SBP, mm Hg[*]	122.1 ± 9.9	121.8 ± 9.3	151.4 ± 4.5	**<0.001**[§]
Daytime DBP, mm Hg[*]	78 ± 8.4	77 ± 7.6	96.5 ± 3.5	**<0.001**[§]
Nighttime SBP, mm Hg[*]	111.8 ± 8.8	110.1 ± 8.2	127.4 ± 6.8	**<0.001**[§]
Nighttime DBP, mm Hg[*]	67.1 ± 4.6	66.6 ± 4.1	78.6 ± 6.6	**<0.001**[§]

ABPM = ambulatory blood pressure monitoring, BMI = body mass index, DBP = diastolic blood pressure, F = female, HT = hypertension, M = male, NT = normotensive, SBP = systolic blood pressure, WCH = white coat hypertension.

Significant differences are indicated as bold.

[*] ABPM.

[†] WCH and control, $P < 0.001$; HT and control, $P < 0.001$.

[‡] WCH and control, $P < 0.001$; HT and control, $P < 0.001$.

[§] HT and WCH, $P < 0.001$; HT and control, $P < 0.001$.

TABLE 2. Relative Expression Levels of miRNAs in Study Groups

	NT Group (n = 30)	WCH Group (n = 30)	HT Group (n = 30)	*P*
miR-21	33.7 ± 45.6	37.4 ± 46.9	79.2 ± 91.2	**0.013**[*]
miR-122	39.5 ± 28.7	88.1 ± 121.7	214.5 ± 384.6	**0.019**[†]
miR-125a	15.7 ± 16.6	13.2 ± 11.7	15.4 ± 11.8	0.572
miR-126	13.2 ± 19.8	14.8 ± 19.2	13.6 ± 10.1	0.248
miR-130a	22.6 ± 24.7	22.3 ± 13.9	18.9 ± 16.1	0.271
miR-155	8.5 ± 8.9	10.2 ± 8.2	8.4 ± 8.4	0.284
miR-195	16.9 ± 21.4	11.8 ± 10.2	17.2 ± 13.5	0.372
miR-296-5p	8.7 ± 7.5	12.9 ± 7.1	5.6 ± 3.3	**<0.001**[‡]
miR-637	5.1 ± 5.7	8.7 ± 7.3	9.3 ± 9.5	**0.009**[§]
let-7e	43.7 ± 43.5	41.9 ± 43.8	114.8 ± 145.4	**0.003**[‖]

HT = hypertension, NT = normotensive, WCH = white coat hypertension.
Significant differences are indicated as bold.
[*] HT and NT, $P = 0.024$; HT and WCH, $P = 0.043$.
[†] HT and NT, $P = 0.021$.
[‡] WCH and NT, $P = 0.044$; WCH and HT, $P < 0.001$.
[§] HT and NT, $P = 0.023$.
[‖] HT and NT, $P = 0.01$; WCH and HT, $P = 0.008$.

hypertension is affecting >1 billion adults worldwide.[20] The strategies available to prevent or treat hypertension may be ineffective due to lack of a fundamental understanding of the cause and pathophysiology of the disorder. 'WCH' or 'isolated clinic hypertension' refers to the condition in which blood pressure is elevated in the office at repeated visits and normal out of the office, either on ABPM or home blood pressure monitoring.[21] In this study, we investigated plasma miRNA expression pattern in HT and WCH patients, compared with healthy subjects. We are the first to report a link between miRNA and WCH.

MiRNAs are potential biomarkers and therapeutic targets in cardiovascular disease and hypertension development. MiR-21 plays a role in angiogenesis, apoptosis, cardiac fibrosis, and renal fibrosis.[9,22,23] Continuous pressure overload of myocardium gives rise to cardiac fibrosis, and miR-21 is upregulated in response to cardiac stress. Thum et al[22] demonstrated that miR-21 is overexpressed in cardiac fibroblasts in the failing heart. Fernandes et al[9] demonstrated that miR-21 levels

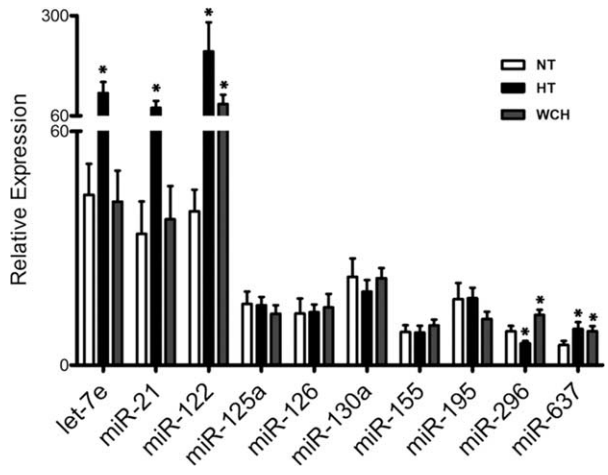

FIGURE 1. Relative expression levels of miRNAs, $*P < 0.05$. HT = hypertension, NT = normotensive, WCH = white coat hypertension.

are increased in HT rats compared with NT rats, and exercise training lowered its expression in HT rats. They have suggested that exercise training restored the levels of miR-21 associated with revascularization in hypertension. In another study, elevated miR-21 level has been detected in HT patients compared with controls and correlations of miR-21 expression level with 24-hour DBP and the dipping status have been observed.[24] In our study, we found that miR-21 levels are significantly upregulated in HT patients in addition to slightly upregulated miR-21 levels in WCH patients, but this difference was not significant. The clinical SBP and ambulatory daytime and nighttime SBP were positively correlated with miR-21. These data support a strong relationship between miR-21 and hypertension, suggesting miR-21 as a potential regulator and target in hypertension.

In a recent study, miR-122 has been also shown to contribute to endothelial dysfunction in HT individuals.[10] WCH is associated with glucose dysregulation, insulin resistance, and metabolic syndrome. In the Pressioni Arteriose Monitorate E Loro Associazioni study, participants with WCH had more tendencies to develop future diabetes and impaired fasting glucose at 10 years.[25] In the present study, miR-122 levels were significantly higher in the HT group compared with the control group and the WCH group. MiR-122 levels were also higher in the WCH group compared with the control group ($P = 0.048$). Our results showed that miR-122 is differentially expressed in both HT and WCH patients in comparison with NT individuals, and this miRNA can be used as a diagnostic marker for distinguishing HT and WCH patients from NT individuals.

MiR-296 was proven to be involved in many biochemical processes such as glucose metabolism, cell growth, and angiogenesis.[26] Recent studies showed that miR-296-5p expressions were downregulated in HT patients in comparison with NT.[15] In the present study, miR-296-5p levels were the lowest in the HT group, and interestingly its expression in WCH group was higher compared with both control and HT groups. Additionally, the ambulatory 24-hour and daytime SBP and DBP were negatively correlated with miR-296-5p.

A frequently observed polymorphism of the *ATP6V0A1* is related with Chromogranin A (CHGA)/catestatin secretion and systemic blood pressure.[16] It has been found that T3246C conversion disturbs the binding of hsa-miR-637 to 3'

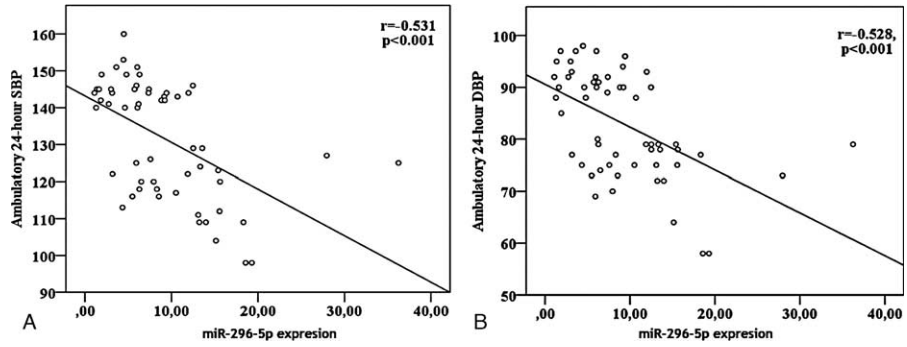

FIGURE 2. Relationship between ambulatory 24-hour SBP with miR-296-5p levels (A), ambulatory 24-hour DBP with miR-296-5p levels (B). DBP = diastolic blood pressure, SBP = systolic blood pressure.

Untranslated Region of ATP6V0A1 mRNA and prevents its translation, which consequently impairs the secretion of CHGA/catestatin.[16] In our study, miR-637 expression levels were upregulated in HT and WCH groups than in the NT group. The clinical SBP was mild and positively correlated with miR-637. This finding supported the fact that WCH is a moderate form between HT and NT.

Association of let-7e and hypertension by means of impaired endothelial dysfunction has been proposed, although a direct connection could be established only very recently.[15] Many studies found that let-7e expressions were upregulated in essential hypertension patients.[15] Similarly, in our study, let-7e levels were significantly higher in the HT group compared with both WCH and NT. The clinical SBP and ambulatory daytime and nighttime SBPs were positively correlated with let-7e.

Our results showed that let-7e, miR-21, and miR-296-5p are differentially expressed in HT patients compared with WCH patients, and these miRNAs can be proposed as diagnostic markers. These miRNAs may play an important role in the distinguishing of patients with stage 1 hypertension and WCH. Moreover, miR-122, miR-296-5p, and miR-637 are differentially expressed in WCH patients compared with NT patients, and these miRNAs can be suggested as diagnostic markers.

MiR-125a inhibits endothelin-1 expression in vascular endothelial cells. Li et al[11] showed that HT rats had decreased expression of miR-125a, which were negatively associated with endothelin-1 expression. In our study, no significant difference was observed between the control and other groups in miR-125a expression level.

A recent study showed that the deletion of miR-126 in mice causes loss of vascular integrity and is partially embryonic lethal.[27] Fernandes et al[9] demonstrated the decreased miR-126 levels in HT rats compared with NT rats, and exercise training elevated the miR-126 levels in HT rats. They have suggested that exercise training restored the level of miR-126 associated with revascularization in hypertension. In our study, no significant difference was observed in miR-126 expression level between the control and other groups.

In a recent study, the expression of miR-130a was upregulated in the remodeled aorta and superior mesenteric artery of HT rats. They suggest that miR-130a is involved in the regulation of vascular smooth muscle cell proliferation, which may contribute to vascular remodeling in hypertension.[12] In this study, there was no significant difference between the plasma miR-130a expression level of control and other groups.

MiR-155 regulates endothelial nitric oxide synthase and suppresses type-1 angiotensin II receptor.[13,28] We found that the

TABLE 3. Correlation Between miRNAs in All Groups

		miR-122	miR-125a	miR-126	miR-130a	miR-155	miR-195	miR-296-5p	miR-637	let-7e
miR-21	R	0.568	0.717	0.730	0.162	0.820	0.600	0.228	0.611	0.853
	P	<0.001	<0.001	<0.001	0.141	<0.001	<0.001	0.038	<0.001	0.001
miR-122	R		0.472	0.254	0.018	0.590	0.384	0.001	0.547	0.463
	P		<0.001	0.025	0.876	<0.001	0.001	0.991	0.001	0.001
miR-125a	R			0.763	0.180	0.861	0.717	0.558	0.523	0.749
	P			<0.001	0.110	<0.001	<0.001	<0.001	<0.001	<0.001
miR-126	R				0.147	0.818	0.665	0.512	0.410	0.529
	P				0.198	<0.001	<0.001	<0.001	<0.001	<0.001
miR-130a	R					0.229	0.154	0.396	0.274	0.090
	P					0.046	0.187	<0.001	0.012	0.416
miR-155	R						0.644	0.551	0.612	0.807
	P						<0.001	<0.001	<0.001	0.001
miR-195	R							0.565	0.590	0.618
	P							<0.001	<0.001	<0.001
miR-296-5p	R								0.467	0.162
	P								<0.001	0.144
miR-637	R									0.586
	P									<0.001

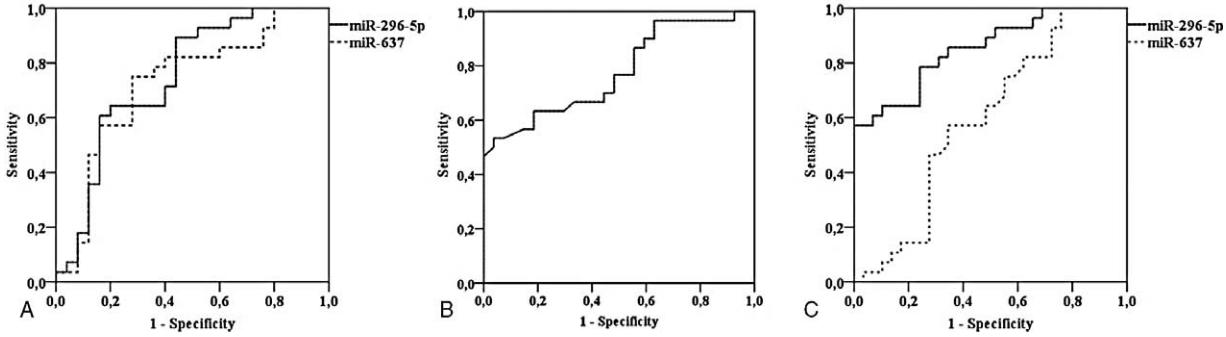

FIGURE 3. ROC analysis of miR-296-5p and miR-637, curves for individual miRNAs and their cooperative power to discriminate 2 sets of patients comprised NT and WCH (A). ROC analysis of miR-122, curves for individual miRNAs, and their cooperative power to discriminate 2 sets of patients comprised NT and HT (B). ROC analysis of miR-296-5p and miR-637, curves for individual miRNAs, and their cooperative power to discriminate 2 sets of patients comprised WCH and HT (C). HT = hypertension, NT = normotensive, ROC = receiver operating characteristic, WCH = white coat hypertension.

TABLE 4. Sensitivity, Specificity, AUC, Cutoff, and Asymptotic Significance of ROC Analysis of miRNAs

		Sensitivity (%)	Specificity (%)	AUC	Cutoff*	Asymptotic Significance
NT vs WCH	miR-296-5p	60.9	85.7	0.778	10.3	**0.002**
	miR-637	73.9	76.2	0.774	5.3	**0.002**
NT vs HT	miR-122	63.6	57.1	0.688	56.6	**0.035**
WCH vs HT	miR-296-5p	78.3	81.8	0.868	7.8	**<0.001**
	miR-637	60.9	63.6	0.680	5.7	**0.039**

AUC = area under the curve, HT = hypertension, NT = normotensive, ROC = receiver operating characteristic, WCH = white coat hypertension. Significant differences are indicated as bold.

* Relative expression level. Curves for individual miRNAs and their cooperative power to discriminate 2 sets of patients comprised NT and WCH, NT and hypertension, and WCH and hypertension.

HT group had the lowest plasma miR-155 levels. Yet there were no significant differences between the controls and other groups.

MiR-195 has been demonstrated to have proapoptotic role in cardiomyocytes.[14] Long et al[29] reported that plasma miR-195 level was upregulated at 8 and 12 hours after the onset of Acute myocardial infarction symptoms, and a significant correlation was observed with the plasma cardiac troponin I concentration.[29] In our study, we found that there were no significant differences between the controls and other groups, but the HT group had the highest miR-195 levels.

To our knowledge, we are the first to report a link between miRNAs and WCH. In many studies, WCH is a moderate form between HT and NT, and it is known as the premise of hypertension. In the literature, with some aspects, WCH is similar to both HT and NT.[6,30] WCH is a cardiovascular disease risk factor when compared with NT controls, but has a relatively benign outcome when compared with mild HT.[30]

This study suggests that the miRNAs may have important implications toward the understanding of hypertension and WCH. The expression trends of the investigated miRNAs in the HT patients are consistent in WCH patients. The clinical inference is that measurement, treatment, and monitoring of miRNAs may be valuable in the management of patients with WCH and HT in the future. WCH patients showed mild expressions between NT and HT groups. With emerging technologies such as next generation sequencing, a more direct relationship may be established between genetic and epigenetic factors and WCH. However, since our study population comprises relatively small population, further studies are required to understand the exact mechanisms underlying these observations in WCH and HT, and to confirm these results in larger populations. Moreover, although our results provided invaluable findings, the present study has some limitations. First of all, we could not profile all of the miRNAs detected in humans; we only investigated 10 miRNAs reported as related with hypertension in the literature. MiRNA profiling with microarray or RNA sequencing will help identifying new candidate biomarkers for both hypertension and WCH and will let confirmation of current markers. Furthermore, since it is a cross-sectional study and we could not investigate the patients after progressing to sustained HT longitudinally, we could not explain which of the miRNAs changed by the time and what kind of epigenetic alterations occurred. Besides, we investigated the deregulation of miRNA expressions in the plasma of patients as diagnostic indicators; however, the pathophysiologic potential of these miRNAs in relation to endothelial dysfunction and end-organ damage needs to be enlightened in further in in vitro and in vivo functional studies.

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