Received: 24 June 2019 | Revised: 27 August 2019 | Accepted: 2 September 2019

DOI: 10.1111/jch.13704

ORIGINAL PAPER

WILEY

Down-regulation of miRNA 145 and up-regulation of miRNA 4516 may be associated with primary hypertension

Gülsüm Özkan MD[1] | Şükrü Ulusoy MD[2] | Elif Geyik PhD[3] | Yunus Erdem MD[4]

[1]Department of Nephrology, School of Medicine, Namık Kemal University, Tekirdağ, Turkey

[2]Department of Nephrology, School of Medicine, Karadeniz Technical University, Trabzon, Turkey

[3]Department of Biology and Genetics, Genometri Biotech, İzmir, Turkey

[4]Department of Nephrology, School of Medicine, Hacettepe University, Ankara, Turkey

Correspondenec
Gülsüm Özkan, Department of Nephrology, School of Medicine, Namık Kemal University, 59000 Tekirdağ, Turkey.
Email: gulsumozkan78@hotmail.com

Abstract

Complex mechanisms including genetic factors have been proposed in the pathogenesis of primary hypertension (HT). Micro RNAs (miRNAs) are single-stranded RNA molecules that are not converted into protein products. However, it has been established that genes regulate conversion into protein products. The primary aim of this study was to investigate the roles of miRNA 4516, miRNA 145, miRNA 24, and miRNA 181a in the pathogenesis of HT. The secondary aim was to investigate the relation between these miRNAs and renin, aldosterone, norepinephrine, renalase, and NOS. Fifty-two hypertensive and 51 control normotensive individuals under observation in the Cappadocia cohort were included in the study. miRNA 4516, miRNA 181a, miRNA 24, and miRNA 145 levels were measured using the ddPCR method. miRNA 4516 and norepinephrine levels were significantly higher in the HT group ($P < .005$ for both), while miRNA 145 levels were significantly lower (<.05). miRNA 4516 up-regulation ($P < .05$) and miRNA 145 down-regulation ($P < .05$) were identified as independent predictors of HT. Renalase exhibited negative correlation with miRNA 4516 and positive correlation with miRNA 145 in the patient and control group. In addition, negative correlation was present between miRNA 24 and NE and NOS and between miRNA 181a and NOS in the patient group. Our study identified, for the first time in the literature, miRNA 4516 up-regulation and miRNA 145 down-regulation as independent determinants of HT. Further studies performed in the light of our findings may lead to a better understanding of the pathogenesis and new therapeutic possibilities.

1 | INTRODUCTION

Hypertension (HT) is a widespread disease affecting approximately 25% of the adult population worldwide.[1] In addition to its ubiquitous character, it is also an independent risk factor for diseases with high morbidity and mortality, such as stroke, coronary artery disease, heart failure, and chronic kidney disease.[2]

Complex mechanisms are involved in the pathogenesis of HT. In the light of studies performed to date, neurohormonal mechanisms, such as sympathetic nervous system activation and renin-angiotensin-aldosterone symptom activation, together with vascular reactivity and insulin resistance, have been implicated in the pathogenesis of primary HT.[3,4] There has also been a recent increase in studies involving the genetics of primary HT, and abnormal expression of some genes has been linked to its development. However, the genetic mechanisms in the pathogenesis of primary HT have still not been fully elucidated.[5,6]

MicroRNAs (miRNAs) are single-stranded RNA molecules 20-25 nucleotides in length coded by genes transcribed by DNA. Translation to protein does not occur, and they are therefore known

as "non-coding RNA." While miRNAs are not converted to protein products, they have been observed to regulate the conversion of genes to protein products. miRNAs have recently been thought to be capable of involvement in the pathogenesis of several diseases, and considerable research into this has taken place. Experimental and clinical studies have investigated the role of miRNAs in the pathogenesis of HT. They have been observed to regulate the conversion of genes to protein products.[5-8] The role of miRNA 4516, miRNA 145, miRNA 24, and miRNA 181a in the pathogenesis of primary HT has only been investigated in very few clinical and experimental studies, and various hypothetical results have been obtained.[9-16] The primary aim of this clinical study was therefore to investigate the roles of miRNA 4516, miRNA 145, miRNA 24, and miRNA 181a in the pathogenesis of HT. The secondary aim was to investigate the relations between these miRNAs and renin, aldosterone, norepinephrine (NE), renalase, and NOS.

2 | METHOD

2.1 | Patient selection

Individuals in the Cappadocia cohort,[17] aged over 18, who had been informed about the study and expressed verbal willingness to take part, and with sufficient intellectual capacity to provide a medical history, for measuring BP at home and for performing 24-h ABPM, were enrolled. Pregnant women, patients with known heart failure, kidney failure or chronic liver disease, or using antihypertensive drugs, and subjects refusing to provide contact details were excluded. Ethical committee approval was obtained from the Namık Kemal University School of Medicine Ethical Committee. Patients were enrolled after providing verbal and written consent.

2.2 | Study protocol

Fifty-two patients with HT and 51 normotensive controls under monitoring as normotensive or hypertensive in the Cappadocia cohort and not receiving antihypertensive therapy were included in the study. Subjects' demographic data were recorded at the beginning of the study. Detailed physical examinations were performed, and office BP measurement was carried out in line with the appropriate guidelines.[18] Office BP was measured using a UA-651SL monitor (A&D Co., 1-243 Asahi), a validated device. Before the procedure, all patients were asked to rest for at least 5 minutes in a relaxed position in a quiet room at a comfortable temperature. BP was measured by a physician from both arms using a cuff of a suitable size for the patient's forearm, with the forearm held at heart level, with the back and the forearm supported, and with the patient sitting upright. We were careful to ensure that patients did not cross their legs or speak during the procedure. Once BP had been measured from both arms, subsequent BP measurements were carried out using the arm eliciting the highest value. BP was measured five times at 1-min intervals. The first measurement was excluded from the analysis. The mean value of the next four measurements was recorded as office BP. Patients were then equipped with 24-h ABPM devices and asked to collect 24-h urine. Patients with an office average BP measurement ≥140/90 mm Hg, and a 24-h ABPM all-day average ≥130/80 mm Hg were diagnosed with HT. We think that our mean office BP values were lower than the 24-h ABPM values since mean office SBP and DBP were calculated based on the mean of four out of five consecutive BP measurements. However, based on the two measurement results (office and 24 h-ABPM), all subjects in our patient group had BP values consistent with a diagnosis of HT. Blood samples were collected for miRNA 4516, miRNA 145, miRNA 24, miRNA 181a, renin, aldosterone, NE, renalase, and NOS investigation. Twenty-four-hour urine sodium was measured using a Roche Cobas C 501 (Roche Diagnostics GmbH, D-68305 - Mannheim) autoanalyzer with an ion-selective electrode. Next, 24-h sodium excretion values (mmol/d) were calculated as the concentration of sodium in urine (mmol/L) × urinary volume (L/d).

2.3 | Ambulatory blood pressure measurement

Twenty-four-hour BP (24-h ABPM) was measured using a Mobil-O-Graph NG 24 h ABPM Classic (IEM GmbH) device. Monitoring was performed from the non-dominant arm. Patients were asked to record their hours of sleeping, waking and eating, and their daily activities. Sleeping-waking periods were evaluated accordingly. Patients were instructed to keep the relevant arm immobile during BP measurement. Daytime BP measurement was performed once every 15 minutes and night-time measurement once every half hour. Measurements with at least 70% validity from day- and night-time measurements in 24-h ABPM records were included in the analysis.

2.4 | Sample collection

Peripheral blood was collected and divided into two tubes, one for serum and the other for whole blood. The whole blood was collected to Paxgene Blood RNA Tubes (Qiagen) to stabilize RNA for long term storage. 2,5 mL of whole blood was drawn to Paxgene Blood RNA Tubes which contains special preservation solution for RNA and stored in line with the manufacturer's instructions. Serum collected in gel-containing tubes with gels was centrifuged and transferred to new dry tubes and stored at −20°C until laboratory analysis.

2.5 | RNA extraction and measurement of concentration of RNA

RNA extraction was performed using PAXgene Blood miRNA kits (Qiagen) according to the manufacturer's instructions. The isolated RNA quantity was measured by Promega Quantifluor instrument and Quantiflour RNA System Kit. RNA concentrations and purity were measured with a NanoDrop Lite Spectrophotometer by applying 2 µL of RNA to the instrument probe.

2.6 | cDNA synthesis

For quantitation of miRNA 24, miRNA 145, and miRNA 181a in the project, ready to use Thermo Fisher TaqMan miRNA Assays (Thermo) were used and for reverse transcription, specific RT primers which were in the content of the assay were used with Bioline Sensifast cDNA Synthesis Kit (Bioline), to produce cDNA for each miRNA. For miRNA 4516, TaqMan Advanced miRNA Assay was used because of only that type of kit was produced for quantitation of miRNA 4516. For reverse transcription of that miRNA, a special TaqMan Advanced miRNA cDNA Synthesis Kit which is compatible with Advanced miRNA Assays was used. This cDNA synthesis kit contains universal RT primers.

The RT primers, which were utilized in the study, were selected according to the instructions of producers of miRNA assays. Reverse transcription is achieved by primers obtained by the probes and a Bioline Sensifast cDNA Synthesis Kit (Bioline), with 100 ng RNA being used as the starting quantity. The mix contains 4 μL 5xTransAmp Buffer, 1 μL Reverse Transcriptase, 1 μL RT primers, 1 μL dNTP mix (10 mmol/L each) 7 μL DNase, RNase-free water, and 100 ng RNA.

The thermal cycling conditions were 16°C-30 minutes, 42°C-30 minutes, 48°C-15 minutes, and 85°C-5 minutes.

A different quantification assay was employed for miR-4516. The reverse transcription of miR-4516 was performed with the components of TaqMan Advanced miRNA Assays (Thermo), following the manufacturer's protocol.

2.7 | Absolute quantification of miRNAs by ddPCR

Absolute quantification of miRNAs, miRNA 4516: hsa-miR-4516, miRNA 181a: hsa-miR-181a-5p, miRNA 24: hsa-miR-24-3p, miRNA 145: hsa-miR-145-5p, is achieved with a BioRad Droplet Digital PCR QX200 system (Bio-Rad). Droplets consisting of the PCR mix and cDNA are generated for the absolute quantification of each miRNA. The PCR mix consisted of 12 μL ddPCR Supermix for Probes (No dUTP) (Bio-Rad), 1 μL TaqMan miRNA Assay (Thermo), 10 μL RNase, DNase-free water and 2 μL cDNA. The droplets were generated

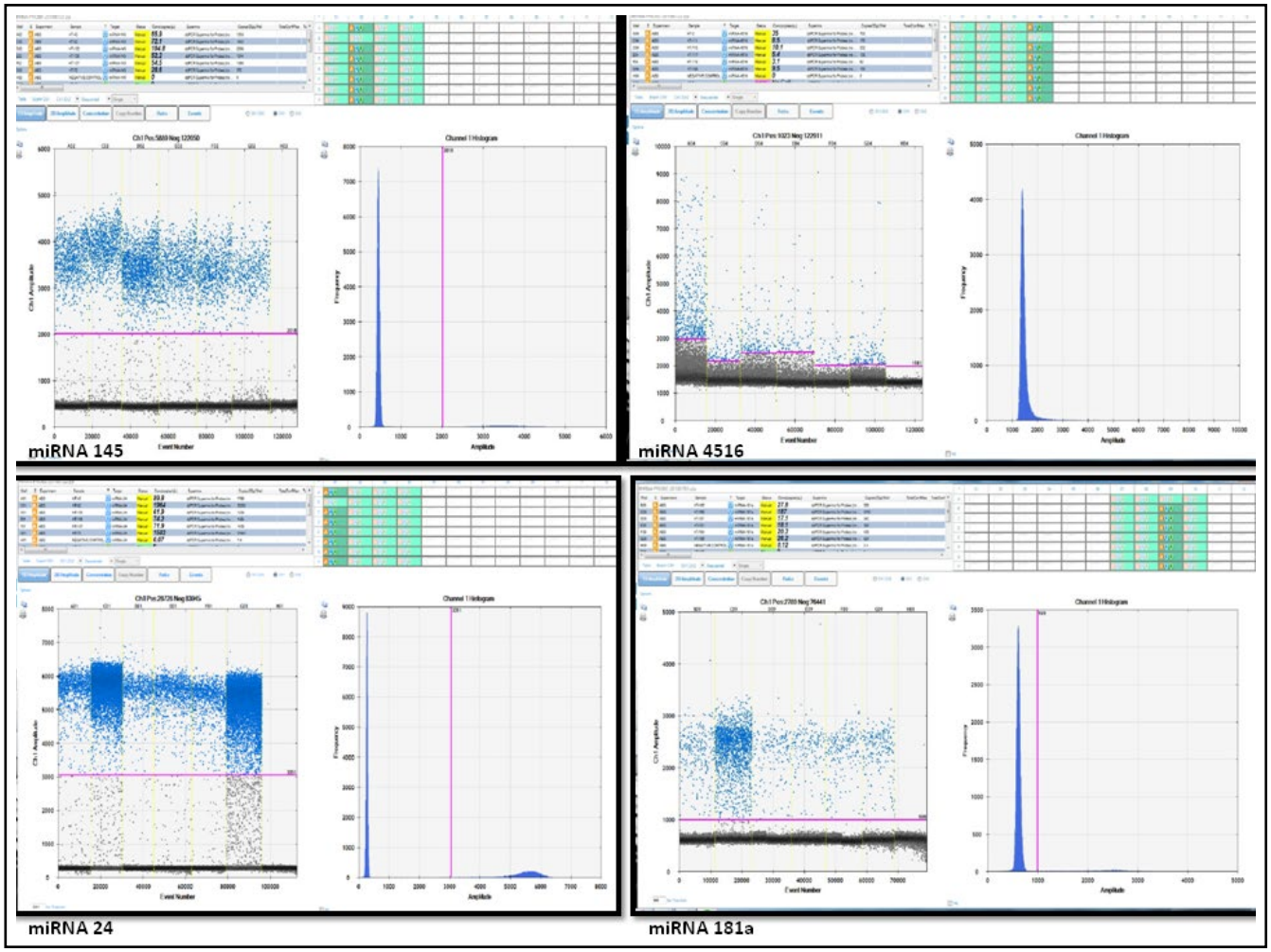

FIGURE 1 Droplet and copy numbers for miR-21, miR-145, miR-181a, and miR-4516. miRNA quantitation was performed under optimized ddPCR conditions using the TaqMan Assay. The results were presented as copy numbers per microliter. Samples with at least 15 000 droplets were included in the study. miRNA quantitation was calculated by separating negative (black) and positive (blue) droplets using a Poisson 95% confidence interval

with 70 µL of Bio-Rad Droplet Generation Oil (Bio-Rad) and 20 µL PCR mix using special cartridges and a Bio-Rad Droplet Generator (Bio-Rad). Next, 40 µL of generated droplets was applied to PCR plates for PCR. The thermal cycling conditions were 95°C-5 minutes, 35 cycles of 95°C-30 sec- 60°C-1 minutes, and 98°C-10 sec. The PCR products were loaded to QX200 Droplet Reader (Bio-Rad). Quantification of miRNAs was performed on QuantaLife Software (Bio-Rad) provided with the instrument. PCR products with more than 15 000 droplets included in the analysis. Otherwise, the experiment was repeated to obtain sufficient droplet numbers for absolute quantification (Figure 1).

The quantification data are getting by ddPCR which enables absolute quantification by detecting the droplets that contains the flourescently labeled amplicons via laser. The calculation of the fluorescently labeled droplets was performed by Poisson Diagram by internal normalization of results.

The uL in the copies/uL in miRNA abundance was referring to the loaded nucleic acid to the ddPCR reaction. In this study, it refers to cDNA product amount.

For validation of PCR results, miRNA 21 is used to control the protocol because it is the most abundant miRNA in all types of tissues. There was not special positive control because the normal quantities of miRNAs in the study were not definite in hypertension.

2.8 | Elisa

Levels of enzymes and hormones, such as renin, aldosterone, norepinephrine, renalase, and eNOS (Endothelial NOS, NOSIII), were calculated using the ELISA method. The tests were prepared with human ELISA kits (Human Nitric Oxide Synthase, Endothelial ELISA Kit-Cat. No: E0815h; General Aldosterone ELISA Kit-Cat. No: E0911Ge; Human Renin ELISA Kit-Cat. No: E0889h; General Noradrenaline ELISA Kit-Cat. No: E0907Ge; Human Renelase ELISA Kit-Cat. No: E1103h) (EIAab, CN) according to the manufacturer's instructions. A BioTek ELx800 absorbance reader (BioTek) was used to read the florescence of samples on ELISA plates. Calculations were performed on KC Junior Software adapted to the BioTek ELx800 absorbance reader.

2.9 | Statistical analysis

Compatibility with normal distribution of the study data was examined using the Kolmogorov-Smirnov test. The *t* test was used to

TABLE 1 A comparison of the demographic and biochemical parameters of the hypertensive and control groups

	Control group (n:51) mean ± sd or median (min-max)	Hypertensive group(n:52) mean ± sd or median (min-max)	*P* values
Age (y)	50.76 ± 7.99	53.50 ± 7.75	NS
Gender (F/M) (%)	66/34	60/40	NS
BMI(kg/m^2)	28.5 (21.3-42.1)	31.15(23.1-49.8)	NS
Office SBP(mm Hg)	117.82 ± 10.97	133.35 ± 14.21	<.001
Office DBP(mm Hg)	73.57 ± 9.89	84.42 ± 10.53	<.001
24 h-ABPM mean SBP(mm Hg)	113.10 ± 8.36	139.75 ± 10.43	<.001
24 h-ABPM mean DBP(mm Hg)	69.84 ± 6.87	86.15 ± 7.28	<.001
Micro RNA 145 (copy/uL)	72 (4-384)	56.55 (4.3-392)	<.05
Micro RNA 4516 (copy/uL)	14.65 (0.4-34.5)	20.50 (2.2-44.6)	<.005
Micro RNA 24 (copy/uL)	391 (8-5450)	156 (6-6370)	NS
Micro RNA 181a (copy/uL)	34.80 (0.1-244)	25.3 (0.1-274)	NS
Renin	0.07(0.03-0.75)	0.065 (0.02-0.26)	NS
Aldosterone	0.40 (0.09-1.18)	0.47 (0.13-1.25)	NS
Norepinephrine (ng/mL)	0.39 (0.09-1.22)	0.52 (0.12-1.58)	<.005
Renalase (µg/mL)	0.02 (0.008-0.26)	0.02 (0.009-0.066)	NS
NOS	0.079 (0.02-0.38)	0.10 (0.01-0.33)	NS
24 h-Urine Sodium	259.41 ± 122.38	236.34 ± 106.33	NS

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; NOS, nitric oxide synthase.

compare normally distributed data and the Mann-Whitney U test for non-normally distributed data. The chi-square test was used for analysis of demographic characteristics, and Pearson correlation analysis was applied to determine correlations. Logistic regression analysis was used to determine possible independent predictors of HT. $P < .05$ were considered statistically significant. All statistical analyses were performed on SPSS 20 software (IBM).

3 | RESULTS

Fifty-two newly diagnosed primary HT patients (mean age 53.50 ± 7.75 years) and 51 healthy controls (mean age 50.76 ± 7.99 years) were included in the study. No significant difference was determined between the groups in terms of age, sex, or BMI (Table 1). As shown in Table 1, miRNA 4516 and norepinephrine levels were significantly higher in the HT patient group than in the control group ($P < .005$ for both), while miRNA 145 levels were significantly lower (<0.05).

Analysis of the HT patient group revealed negative correlation between miRNA 4516 and renalase ($r = -.277$, $P < .05$) (Figure 2), positive correlation between miRNA 145 and renalase ($r = .307$, $P < .05$) (Figure 3), and negative correlation between miRNA 24 and NE ($r = -.291$, $P < .05$) and NOS ($r = -.302$, $P < .05$) (Figure 4A,B), and negative correlation between miRNA 181a and NOS (Figure 5) ($r = -.303$, $P < .05$). Negative correlation was determined between miRNA 4516 and renalase ($r = -.367$, $P < .05$) in the control group, and positive correlation was determined between miRNA 145 and renalase ($r = .365$, $P < .05$). No significant correlation was observed between patient and control group BP values and miRNA 4516, miRNA 145, miRNA 24, miRNA 181a, renin, aldosterone, norepinephrine (NE), renalase, or NOS.

At logistic regression analysis, we identified miRNA 4516 up-regulation (exp ß: 1.06 CI: 1.008-1.117) ($P < .05$) and miRNA 145 down-regulation (exp ß: 0.98 CI: 0.97- 0.99) ($P < .05$) as independent predictors of HT.

4 | DISCUSSION

miRNA 4516 and NE levels were higher, while miRNA 145 levels were lower, in our HT patient group than in the control group. In addition, we identified miRNA 4516 up-regulation and miRNA 145 down-regulation as predictors of HT. In addition, correlation analysis revealed negative correlation between miRNA 4516 and renalase and positive correlation between miRNA 145 and renalase in the patient and control groups, while in the patient group, negative correlation was observed between miRNA 24 and NE and NOS and between miRNA 181a and NOS. However, these correlations were weak.

Studies in which miRNAs were detected in human serum and plasma first appeared in the early 2000s.[7] The role of miRNAs in the pathogenesis of primary HT began being investigated in the years that followed. This first involved the determination and comparison of miRNA expression in normotensive and hypertensive individuals using the microarray method, while another method was the investigation of miRNAs with quantitative polymerase chain reaction (qPCR).[8] Circulating miRNA concentrations are very low, thus requiring a highly sensitive method for quantification. qPCR for miRNA concentration is still the best method for application and analysis of the results. ddPCR is a novel method permitting accurate quantitation of targets, especially those present at low levels or about which little is known. PCR is performed in partitions which include 2-3 copies of DNA in each droplet, resulting in high sensitivity even in low amounts of sample. This overcomes the DNA quantity problem in techniques such as microarray, NGS, and Sanger sequencing.

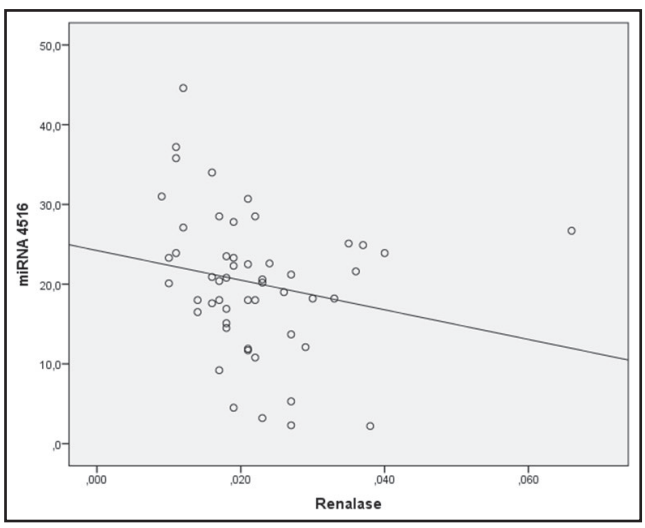

FIGURE 2 Correlation analysis of miRNA 4516 and renalase. Negative correlation was determined between miRNA 4516 and renalase in the patient group ($r = -.277$, $P < .05$)

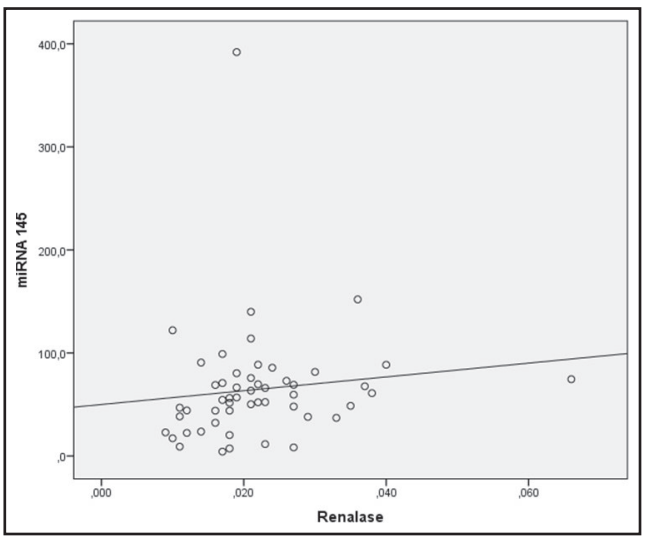

FIGURE 3 Correlation analysis of miRNA 145 and renalase. Positive correlation was determined between miRNA 145 and renalase in the patient group ($r = .307$, $P < .05$)

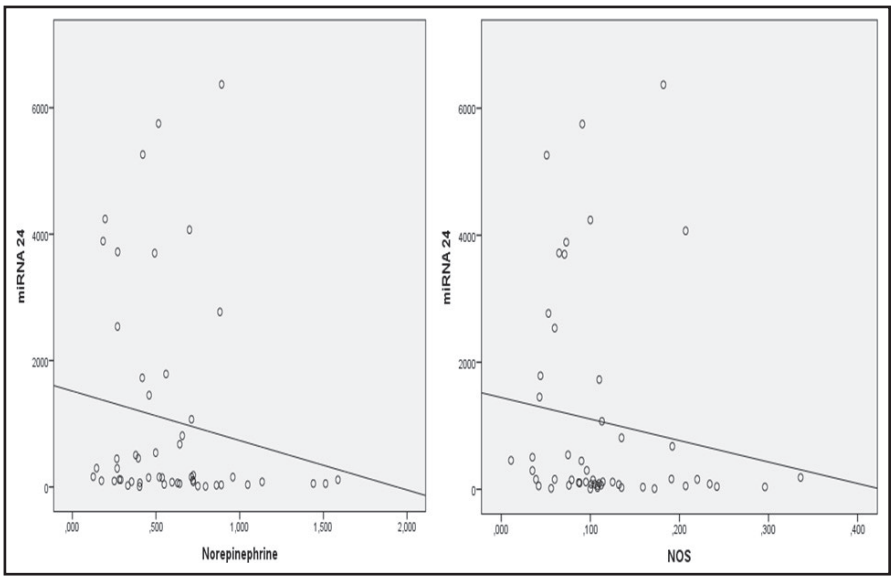

FIGURE 4 A, Correlation analysis of miRNA 24 and NE. Negative correlation was determined between miRNA 24 and NE in the patient group ($r = -.291$, $P < .05$). B, Correlation analysis of miRNA 24 and NOS. Negative correlation was determined between miRNA 24 and NOS in the patient group ($r = -.302$, $P < .05$)

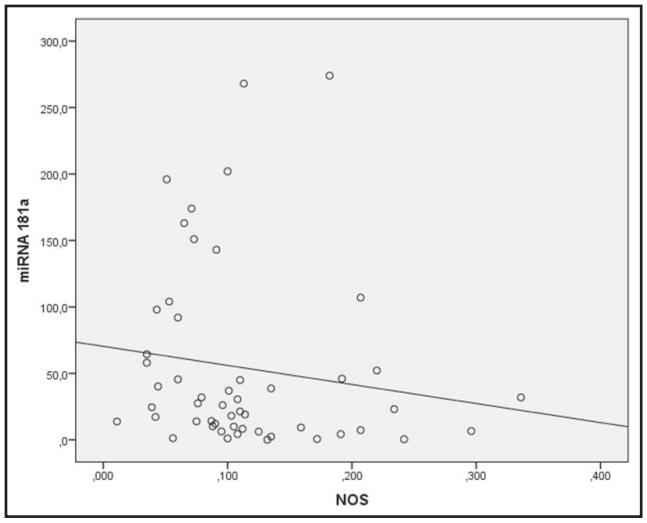

FIGURE 5 Correlation analysis of miRNA 181a and NOS. Negative correlation was determined between miRNA 181a and NOS in the patient group ($r = -0.303$, $P < .05$)

Another feature of ddPCR is that it does not require endogenous control, housekeeping genes, and standard curves for absolute quantification, making it superior to real-time PCR for qPCR in absolute quantification.[19,20] Our use of the ddPCR method in miRNA analysis increases the reliability of our results.

The first primary HT microarray study was performed by Li et al, who screened 1700 microRNAs in the plasma of 13 patients with primary HT and five healthy controls and determined 27 miRNAs, 14 of which were confirmed with qPCR.[21] Numerous studies investigating probable miRNAs with qPCR have been published following that study. However, this still produces inconsistent results.[7,8]

Marques et al[9] determined various miRNAs in kidney tissue from 15 hypertensive and seven normotensive individuals using the microarray method and showed that miRNA 181a was associated with renin expression. In another study, Marques et al[10] showed an association between miRNA 181a and blood pressure in two separate populations, but that this was independent of renin. In our study, we determined lower miRNA 181a levels in the patient group than in the control group, although the difference was not statistically significant. In addition, we detected weak negative correlation between miRNA 181a and NOS in the patient group. This correlation, which was not present in the control group, suggested that there may be a relation between miRNA 181a and NOS in hypertensive patients. However, the absence of any significant difference between 181a levels in the patient and control groups, and the correlation between miRNA 181a and NOS being weak, prevented us from drawing any definitive conclusion regarding this.

Studies have shown associations between miRNA 145 and the differentiation of cardiac fibroblasts in myofibroblasts and vascular smooth muscle contraction.[11,12] A few studies have investigated the role of miRNA 145 in the pathogenesis of primary HT. Santovito et al[13] examined miRNA 145 levels in atherosclerotic plaques in 15 HT patients with carotid endarterectomy and seven control individuals and determined significantly higher miRNA 145 levels in hypertensive atherosclerotic plaques. In one recent experimental study, Wang et al[14] determined significantly higher miRNA 145 levels in the spontaneous hypertensive rat aorta compared to a control group. A few studies have investigated the role of miRNA 145 in the pathogenesis of primary HT. Ours is the first study to investigate miRNA 145 levels in newly diagnosed primary HT patients under cohort follow-up. In contrast to previous studies, we determined significantly lower miRNA 145 levels in the HT group. We thought that this difference might derive from our patient group consisting of subject with newly diagnosed primary HT and to the absence of a history of atherosclerosis. Low miRNA 145 in the patient group and this downregulation being a factor associated with HT suggest a possible role in the pathogenesis of primary HT.

Our search of the literature revealed no previous studies of miRNA 24 levels in primary HT. However, in their experimental study Robertson et al[15] showed that miRNA 24 plays a role in the

transcriptional modification of aldosterone synthase genes and 11β-hydroxylase involved in the synthesis of cortisol and aldosterone. Based on that study, we investigated the role of miRNA 24 in the pathogenesis of primary HT and its relations with renin and aldosterone levels. We determined lower miRNA 24 levels in our patient group compared with the control group, although the difference was not statistically significant. We also identified no significant association between miRNA 24 levels and renin or aldosterone. We determined negative correlation between miRNA 24 and NE and NOS in this study. This weak correlation that was present only in the patient group, and not in the control group, suggests a possible relation between miRNA 24 and NE and NOS. We think that further studies with wider patient populations will assist in confirming this finding.

Our review of the literature revealed a small number of previous studies investigating miRNA 4516 levels in individuals with primary HT. Gildea et al[16] investigated the association with salt sensitivity of miRNAs using the microarray method in urine specimens in salt-sensitive, salt-resistant, and inverse salt-sensitive hypertensive patients. They showed that 45 miRNAs, including miRNA 4516 might be associated with salt reaction. Based on that study, we investigated miRNA 4516 levels in primary HT patients, for the first time in the literature. We determined significantly higher miRNA 4516 levels in the patient group compared with the control group, that miRNA 4516 up-regulation together with miRNA 145 down-regulation associated with HT, and that miRNA 4516 up-regulation was positively correlated renalase levels.

Renalase is a flavoprotein intensely expressed in the heart and kidneys and that effectively metabolizes catecholamines, using nicotinamide adenine dinucleotide as a cofactor.[22] Desir et al[23] achieved a decrease in blood pressure with the administration of renalase and showed that this decrease was associated with epinephrine breakdown. Plasma epinephrine, L-DPOA, and dopamine were metabolized by 82%, 63%, and 31%, respectively, following renalase administration, but NE was not metabolized. We determined no significant difference between renalase levels in the patient and control groups, although renalase levels were positively correlated with miRNA 4516 levels and negatively correlated with miRNA 145 levels. The absence of any significant difference between renalase levels in the patient and control groups, and the presence of similar correlation in both groups between miRNA 4516 and miRNA 145 suggests that miRNA 4516 and miRNA 145 are associated with the pathogenesis of HT independently of renalase.

One of the mechanisms implicated for many years in the pathogenesis of primary HT is sympathetic nervous system activation. Potential mechanisms include increased central sympathetic outflow, impaired NE neuronal reuptake, and blunted arterial baroreflex of sympathetic nerve traffic.[24] Activation of the sympathetic nervous system can be identified by measuring heart rate, plasma epinephrine, and NE levels. Plasma NE levels are frequently used as markers of sympathetic nervous system activation.[25] There have been few previous studies of the relation between sympathetic nervous system activation and miRNA. In one experimental study, Jackson et al showed that increased renin production by means of sympathetic stimulation was associated with miRNA 181 down-regulation in spontaneously hypertensive mice.[26] In our study, we measured plasma NE levels as a marker of sympathetic nervous system activation. NE levels were significantly higher in the patient group than in the control group. However, there was no relation between NE and other miRNAs except for miRNA 24, and the correlation between miRNA 24 and NE was also quite weak. We think that further studies are needed to investigate the relation between miRNA and NE levels. In addition, we determined no significant relation between renalase and NE. As Desir et al emphasized in their study, the low NE-metabolizing effect of renalase may be responsible for the lack of association between NE levels and renalase.

In conclusion, the pathogenesis of primary HT has still not been explained. In our study, we determined, for the first time, that miRNA 4516 up-regulation and miRNA 145 down-regulation may be an independent predictor of HT. New studies performed in the light of our findings may lead to a better understanding of the pathogenesis and new therapeutic possibilities.

ACKNOWLEDGMENTS

This study was supported by the Turkish Society of Hypertension and Renal Diseases, the Turkish Society of Internal Medicine, and the Scientific Researches Fund of Namık Kemal University.

CONFLICT OF INTEREST

We report no conflict of interest.

ORCID

Gülsüm Özkan https://orcid.org/0000-0003-0552-3911
Şükrü Ulusoy https://orcid.org/0000-0002-2541-255X
Elif Geyik https://orcid.org/0000-0002-9870-6890
Yunus Erdem https://orcid.org/0000-0002-5389-3143

REFERENCES

1. Kearney PM, Whelton M, Reynolds K, et al. Worldwide prevalence of hypertension: a systematic review. *J Hypertens*. 2004;22:11-19.
2. Lewington S, Clarke R, Qizilbash N, et al. Prospective studies collaboration. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet*. 2002;360:1903-1913.
3. Conceição-Vertamatti AG, Borghi F, Canova F, et al. History of vascular reactivity models and their involvement in hypertension pathogenesis. *Vasa*. 2017;46:431-439.
4. Wang Z, Peng X. Pathogenesis of essential hypertension: development of a 4-dimensional model. *Hypothesis*. 2013;11:1-7.
5. Nemecz M, Alexandru N, Tanko G, et al. Role of MicroRNA in endothelial dysfunction and hypertension. *Curr Hypertens Rep*. 2016;18:87.

6. Li X, Wei Y, Wang Z. microRNA-21 and hypertension. *Hypertens Res*. 2018;41:649-661.
7. Lawrie CH, Gal S, Dunlop HM, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. *Br J Haematol*. 2008;141:672-675.
8. Romaine SP, Charchar FJ, Samani NJ, et al. Circulating microRNAs and hypertension–from new insights into blood pressure regulation to biomarkers of cardiovascular risk. *Curr Opin Pharmacol*. 2016;27:1-7.
9. Marques FZ, Campain AE, Tomaszewski M, et al. Gene expression profiling reveals renin mRNA overexpression in human hypertensive kidneys and a role for microRNAs. *Hypertension*. 2011;58:1093-1098.
10. Marques FZ, Romaine SP, Denniff M, et al. Signatures of miR-181a on the renal transcriptome and blood pressure. *Mol Med*. 2015;21:739-748.
11. Wang YS, Li SH, Guo J, et al. Role of miR-145 in cardiac myofibroblast differentiation. *J Mol Cell Cardiol*. 2014;66:94-105.
12. Hu B, Song JT, Qu HY, et al. Mechanical stretch suppresses microRNA-145 expression by activating extracellular signal-regulated kinase 1/2 and upregulating angiotensin-converting enzyme to alter vascular smooth muscle cell phenotype. *PLoS One*. 2014;9:e96338.
13. Santovito D, Mandolini C, Marcantonio P, et al. Overexpression of microRNA-145 in atherosclerotic plaques from hypertensive patients. *Expert Opin Ther Targets*. 2013;17:217-223.
14. Wang Y, Jin L. miRNA-145 is associated with spontaneous hypertension by targeting SLC7A1. *Exp Ther Med*. 2018;15:548-552.
15. Robertson S, MacKenzie SM, Alvarez-Madrazo S, et al. MicroRNA-24 is a novel regulator of aldosterone and cortisol production in the human adrenal cortex. *Hypertension*. 2013;62:572-578.
16. Gildea JJ, Carlson JM, Schoeffel CD, et al. Urinary exosome miRNome analysis and its applications to salt sensitivity of blood pressure. *Clin Biochem*. 2013;46:1131-1134.
17. Unal S, Ascioglu S, Demirkazik A, et al. Baseline data of a prospective cohort study: Cappadocia cohort study, Turkey. *Turk J Public Health*. 2018;16(3):190-203. https://doi.org/10.20518/tjph.500196
18. Chobanian AV, Bakris GL, Black HR, et al. Joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. national heart, lung, and blood institute; national high blood pressure. Education program coordinating committee. 2003;42:1206-1252.
19. Miotto E, Saccenti E, Lupini L, et al. Quantification of circulating miRNAs by droplet digital PCR: comparison of EvaGreen- and TaqMan-based chemistries. *Cancer Epidemiol Biomarkers Prev*. 2014;23:2638-2642.
20. Li N, Ma J, Guarnera MA, et al. Digital PCR quantification of miRNAs in sputum for diagnosis of lung cancer. *J Cancer Res Clin Oncol*. 2014;140:145-150.
21. Li S, Zhu J, Zhang W, et al. Signature microRNA expression profile of essential hypertension and its novel link to human cytomegalovirus infection. *Circulation*. 2011;12(124):175-184.
22. Desir GV, Peixoto AJ. Renalase in hypertension and kidney disease. *Nephrol Dial Transplant*. 2014;29:22-28.
23. Desir GV, Tang L, Wang P, et al. Renalase lowers ambulatory blood pressure by metabolizing circulating adrenaline. *J Am Heart Assoc*. 2012;1:e002634.
24. Schlaich MP, Lambert E, Kaye DM, et al. Sympathetic augmentation in hypertension: role of nerve firing, NE reuptake, and Angiotensin neuromodulation. *Hypertension*. 2004;43:169-175.
25. Grassi G, Mark A, Esler M. The sympathetic nervous system alterations in human hypertension. *Circ Res*. 2015;116:976-990.
26. Jackson KL, Marques FZ, Watson AM, et al. A novel interaction between sympathetic overactivity and aberrant regulation of renin by miR-181a in BPH/2J genetically hypertensive mice. *Hypertension*. 2013;62:775-781.

How to cite this article: Özkan G, Ulusoy Ş, Geyik E, Erdem Y. Down-regulation of miRNA 145 and up-regulation of miRNA 4516 may be associated with primary hypertension. *J Clin Hypertens*. 2019;21:1724–1731. https://doi.org/10.1111/jch.13704