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Association of miR-133a With Microalbuminuria in Patients With Resistant Arterial Hypertension and Chronic Kidney Disease

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Background: MicroRNAs (miRNAs) are important post-transcriptional regulators of gene expression and have emerged as potential biomarkers of cardiovascular and renal diseases. Resistant arterial hypertension (RAH) is frequently associated with chronic kidney disease (CKD) and microalbuminuria, reflecting early microvascular damage. However, the relationship between microRNA expression and microalbuminuria in patients with RAH remains insufficiently researched. This study aimed to evaluate the expression of selected miRNAs in patients with RAH, with and without CKD, and to assess their association with urinary albumin excretion.

Material/Methods: This cross-sectional study included 115 patients with RAH, of whom 42 (36.5%) had coexisting CKD. The expression levels of 11 selected miRNAs were assessed in peripheral blood mononuclear cells using quantitative real-time polymerase chain reaction. Urinary albumin-to-creatinine ratio (UACR) was used to assess albuminuria. Correlations between microRNA expression and UACR were analyzed using Spearman's rank correlation.

Results: In patients with RAH and microalbuminuria, a statistically significant positive correlation was observed between hsa-miR-133a expression and UACR (Spearman's r=0.38, *P*=0.024, n=35). No significant correlations were found between urinary albumin excretion and other analyzed microRNAs or in patients without microalbuminuria.

Conclusions: Expression of hsa-miR-133a was associated with urinary albumin excretion in patients with RAH and microalbuminuria. The findings suggest that hsa-miR-133a may be a potential noninvasive biomarker of microalbuminuria and early renal microvascular injury in a high-risk hypertensive population; however, further longitudinal studies are required to clarify the prognostic value and clinical utility of miR-133a in this patient population.

Keywords: Hypertension • MicroRNAs • Kidney Failure, Chronic

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Introduction

Resistant arterial hypertension (RAH) is a severe form of hypertension characterized by inadequate blood pressure control despite treatment with multiple antihypertensive agents, including a diuretic [1]. RAH frequently coexists with chronic kidney disease (CKD), and this combination is associated with a markedly increased risk of cardiovascular morbidity and mortality [2-5]. Moreover, the prevalence of hypertension increases with declining renal function, and RAH becomes more common as CKD progresses [2-4,6].

Microalbuminuria is a well-established marker of renal microvascular injury and systemic endothelial dysfunction and is strongly associated with increased cardiovascular risk and progression of CKD. Even low-grade increases in urinary albumin excretion are linked to adverse cardiovascular outcomes and accelerated renal decline [2,7]. In patients with arterial hypertension, microalbuminuria reflects early target-organ damage and provides prognostic information beyond conventional risk factors. In the context of RAH and CKD, the presence of microalbuminuria may indicate ongoing microvascular injury and heightened cardiovascular risk [6,7].

The pathogenesis of RAH in CKD is multifactorial and involves sodium and volume retention, dysregulation of the renin-angiotensin-aldosterone system (RAAS), activation of the sympathetic nervous system, vascular remodeling, and endothelial dysfunction [8]. Impaired renal sodium handling and intrarenal RAAS activation contribute to sustained blood pressure elevation and progressive renal injury [8,9]. These mechanisms promote microvascular damage and may contribute to the development of albuminuria, a marker of early renal target-organ injury.

MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression at the post-transcriptional level and play an important role in cardiovascular and renal pathophysiology [10]. Altered miRNA expression profiles have been reported in arterial hypertension, CKD, and hypertension-related target-organ damage. Increasing evidence suggests that circulating and cellular miRNAs may serve as potential noninvasive biomarkers reflecting vascular dysfunction, inflammation, and renal injury [11-14].

Among these, miR-133a has attracted particular attention due to its involvement in vascular function, myocardial remodeling, and renal pathophysiology. Experimental and clinical studies suggest that miR-133a may influence blood pressure regulation and renal sodium handling through modulation of intrarenal angiotensinogen expression and tumor necrosis factor-α-dependent pathways [15]. Through these mechanisms, miR-133a may affect intraglomerular pressure, endothelial function, and tubular sodium reabsorption, processes closely related to the development of albuminuria and hypertensive target-organ damage. Clinical studies also reported associations between circulating miR-133a levels and cardiovascular remodeling and hypertension-related organ damage [16-18].

Therefore, the aim of the present study was to evaluate the expression of selected circulating microRNAs in patients with RAH, with and without coexisting CKD, and to investigate the association between miRNA expression levels, particularly miR-133a, and urinary albumin excretion as a marker of renal microvascular injury.

The analyzed panel of microRNAs was selected a priori based on previously published experimental and clinical evidence indicating their involvement in blood pressure regulation, vascular remodeling, inflammation, and renal injury.

Material and Methods

Study Population

This observational, cross-sectional study was conducted at the Department of Cardiology, Medical University of Lublin, Poland, between 2021 and 2022. A total of 115 adult patients with idiopathic RAH were consecutively enrolled. RAH was defined according to current European Society of Hypertension guidelines as uncontrolled blood pressure despite treatment with at least 3 classes of antihypertensive drugs, including a diuretic, administered at maximally tolerated doses [19]. CKD was defined according to current Kidney Disease: Improving Global Outcomes guidelines as an estimated glomerular filtration rate (eGFR) lower than 60 mL/min/1.73 m^2 and/or the presence of albuminuria [2]. Patients were classified into 2 groups according to the presence or absence of coexisting CKD.

In addition, a group of healthy volunteers (n=33), without known chronic diseases and not receiving regular pharmacological treatment, was recruited as a reference population. Peripheral blood samples were collected from these individuals and used exclusively for calibration of relative miRNA expression levels (relative quantification [RQ] values). These individuals were not included in comparative analyses.

Statistical Considerations

Given the number of miRNAs analyzed, correlation analyses were considered exploratory. No formal correction for multiple comparisons was applied, and the results should be interpreted with caution and require validation in independent cohorts.

Ethics Approval

The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved

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by the Bioethics Committee at the Medical University of Lublin (decision No. KE-0254/141/2020; June 25, 2020). Written informed consent was obtained from all participants prior to enrollment.

Clinical and Laboratory Assessments

All participants underwent a standardized clinical evaluation, including medical history, physical examination, and assessment of cardiovascular comorbidities. The diagnosis of RAH was confirmed using ambulatory blood pressure monitoring and/or home blood pressure monitoring to exclude white-coat and masked hypertension. Office blood pressure measurements were performed according to current guidelines using validated automated devices, with patients in a seated position after an appropriate rest period.

Renal function was assessed by measuring serum creatinine concentration, and eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Urinary albumin excretion was evaluated using the urinary albumin-to-creatinine ratio (UACR) obtained from spot urine samples. Normoalbuminuria was defined as UACR less than 30 mg/g, microalbuminuria as UACR of 30 to 300 mg/g, and proteinuria as UACR greater than 300 mg/g.

Routine laboratory parameters were determined using standard methods at the central laboratory at the Medical University of Lublin.

Cell Isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using density gradient centrifugation with Ficoll-Paque Plus (Cytiva, Uppsala, Sweden). Whole blood samples were collected into 2.7-mL S-Monovette tubes (Sarstedt, Germany) containing K3-EDTA as an anticoagulant. Immediately after collection, blood samples were diluted 1: 1 with phosphate-buffered saline (PBS; without calcium and magnesium ions; Biomed-Lublin, Poland) in sterile 15-mL conical tubes (Eppendorf, Germany). The diluted blood was carefully layered over 3 mL of Ficoll-Paque Plus in a new sterile 15-mL conical tube and centrifuged at 400×*g* for 30 minutes at 20°C (5810 R Centrifuge, Eppendorf, Germany) with the brake turned off.

Following centrifugation, the mononuclear cell layer was carefully aspirated and transferred into fresh 15-mL conical tubes. Cells were washed with PBS by gentle resuspension and centrifuged at 450×*g* for 10 minutes at 20°C. The washing step was repeated twice to remove residual plasma and Ficoll. The resulting cell pellet was resuspended in 1 mL of PBS and distributed into sterile 1.5-mL DNA LoBind tubes (Eppendorf, Germany). After a final centrifugation at 400×*g* for 10 minutes at 4°C (5415 R Centrifuge, Eppendorf, Germany), the supernatant was discarded and PBMC pellets were stored at -80°C until RNA extraction.

Total RNA Isolation

Total RNA, including the small RNA fraction, was extracted from PBMC pellets using the mirVana miRNA Isolation Kit (Invitrogen, Thermo Fisher Scientific, Vilnius, Lithuania) according to the manufacturer's protocol. RNA concentration and purity were determined spectrophotometrically using a NanoDrop 2000c instrument (Thermo Fisher Scientific, Waltham, MA, USA). For all analyzed samples, the A260/A280 ratio ranged between 1.8 and 2.0, indicating acceptable RNA purity.

Reverse Transcription

Complementary DNA was synthesized using the TaqMan MicroRNA Reverse Transcription Kit, together with miRNA-specific stem-loop primers (Applied Biosystems, Vilnius, Lithuania), following the manufacturer's recommendations. Each reverse transcription reaction was performed in a total volume of 15 µL containing: 1.5 µL of 10× reverse transcription buffer, 0.15 µL of 100 mM dNTP mix (with dTTP), 3 µL of 5× RT primer, 0.19 µL RNase inhibitor (20 U/µL), 1 µL MultiScribe Reverse Transcriptase (50 U/µL), 4.16 µL nuclease-free water, and 5 µL RNA template, corresponding to 10 ng of total RNA. Reverse transcription was conducted using a Veriti Dx Thermal Cycler (Applied Biosystems, Foster City, CA, USA) under the following thermal profile: 16°C for 30 minutes, 42°C for 30 minutes, followed by enzyme inactivation at 85°C for 5 minutes. The resulting complementary DNA was subsequently used as a template for quantitative polymerase chain reaction (PCR) analysis.

Quantitative Real-Time PCR

Quantitative real-time PCR (RT-qPCR) was performed using TaqMan Universal Master Mix II with UNG (Applied Biosystems, Vilnius, Lithuania) and specific TaqMan MicroRNA Assays. Each reaction contained 0.67 µL of RT product, 3.83 µL nuclease-free water, 0.5 µL miRNA-specific primer/probe mix, and 5 µL of TaqMan Universal Master Mix, resulting in a final reaction volume of 10 µL. Amplification reactions were carried out in triplicate using the StepOnePlus Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) in a 96-well MicroAmp Fast Optical reaction plate. A prespecified panel of 11 circulating microRNAs was selected prior to data analysis based on previously published experimental and clinical evidence demonstrating their involvement in blood pressure regulation, vascular remodeling, inflammatory pathways, and CKD. The selection was hypothesis-driven and predefined before statistical analysis. No additional microRNAs were screened beyond this predefined panel. The analyzed microRNAs included the following:

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Table 1. Demographic and clinical characteristics of patients with resistant arterial hypertension with or without chronic kidney disease.

Variable	RAH without CKD (n=73)	RAH with CKD (n=42)	*P* value
Age (years)	65 (57.0-71.0)	72.5 (66.0-76.0)	<0.001
Sex, n (%)			0.956
Male	40 (54.8)	22 (52.4)	
Female	33 (45.2)	20 (47.6)	
Body mass index (kg/m^2)	30.1 (27.0-33.1)	29.0 (27.4-33.4)	0.910
Duration of arterial hypertension (years)	8.0 (7.0-11.0)	12.0 (10.0-15.0)	0.001
Duration of resistant arterial hypertension (years)	4.0 (3.0-5.0)	4.0 (3.0-6.0)	0.028
Serum creatinine (mg/dL)	0.9 (0.80-1.10)	1.20 (1.00-1.40)	<0.001
Estimated glomerular filtration rate (mL/min/1.73 m^2)	79.6 (67.5-92.8)	54.2 (45.9-60.9)	<0.001
Urinary albumin-to-creatinine (mg/g)	21.8 (17.5-25.7)	117.6 (53.9-174.4)	<0.001

Values are presented as median (Q1-Q3). *P* values were calculated using the Mann-Whitney U test. Abbreviations: RAH, resistant arterial hypertension; CKD, chronic kidney disease.

hsa-miR-1, hsa-miR-21, hsa-miR-26b, hsa-miR-126, hsa-miR-133a, hsa-miR-143, hsa-miR-145, hsa-miR-155, hsa-miR-195, hsa-miR-208, and hsa-miR-320.

RNU48 was used as the endogenous control for normalization of miRNA expression levels. This small nucleolar RNA has been widely applied as a reference gene in circulating miRNA studies due to its relatively stable expression across different biological conditions. Although U6 is also commonly used, its expression variability in plasma samples has been reported, which may affect normalization accuracy. Therefore, RNU48 was selected as a reference gene in accordance with previously established protocols. Relative miRNA expression levels were calculated using the comparative Ct ($2^{-\Delta Ct}$) method, where ΔCt represents the difference between the Ct value of the target miRNA and the endogenous control (RNU48). For statistical analyses, log-transformed RQ values were used to improve data distribution [20]. Expression values were determined using Expression Suite Software version 1.0.3 (Life Technologies). The experimental procedures for sample processing and miRNA expression analysis were performed as previously described [21].

Statistical Analysis

Statistical analyses were performed using Statistica software (version 13.3; TIBCO Software Inc, Palo Alto, CA, USA). Data distribution was assessed using the Shapiro-Wilk test. Continuous variables with non-normal distribution are presented as median and interquartile range (IQR), whereas categorical variables are presented as counts and percentages.

Comparisons between patients with RAH with and without CKD were performed using the Mann-Whitney U test for continuous variables and the chi-square test or Fisher exact test for categorical variables, as appropriate. Correlations between miRNA expression levels and UACR were assessed using the Spearman rank correlation coefficient. Log-transformed relative expression (RQ) values were used in statistical analyses. A 2-sided *P* value <0.05 was considered statistically significant. Pairwise deletion of missing data was applied in correlation analyses.

Results

Baseline Characteristics

A total of 115 patients with RAH were included in the study, of whom 42 (36.5%) had coexisting CKD. Patients with RAH and CKD were significantly older than those without CKD (median age 72.5 [66.0-76.0] vs 65.0 [57.0-71.0] years; *P*<0.001) and had a longer duration of arterial hypertension (12.0 [10.0-15.0] vs 8.0 [7.0-11.0] years; *P*=0.001). As expected, serum creatinine levels were significantly higher in the CKD group (1.20 [1.00-1.40] vs 0.90 [0.80-1.10] mg/dL; *P*<0.001), whereas eGFR was significantly lower (54.2 [45.9-60.9] vs 79.6 [67.5-92.8] mL/min/1,73 m^2; *P*<0.001). UACR values were also markedly higher in patients with CKD (117.6 [53.9-174.4] vs 21.8 [17.5-25.7] mg/g; *P*<0.001). Sex distribution and body mass index were comparable between the study groups. Baseline demographic and clinical characteristics are presented in **Table 1**.

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Table 2. Laboratory parameters of patients with resistant arterial hypertension with and without chronic kidney disease.

Parameter	RAH without CKD (n=73)	RAH with CKD (n=42)	*P* value
White blood cell count (×10^9/L)	7.0 (5.9-8.0)	6.6 (5.0-8.1)	0.084
Platelet count (×10^9/L)	240.0 (214.0-284.0)	236.5 (194.8-274.2)	0.235
Uric acid (mg/dL)	6.4 (5.6-7.4)	7.1 (6.1-8.1)	0.032
Fasting glucose (mg/dL)	101.0 (97.0-121.0)	109.0 (97.2-151.8)	0.209
Glycated hemoglobin HbA1c (%)	6.2 (5.9-7.0)	7.1 (6.2-8.1)	0.002
Total cholesterol (mg/dL)	202.0 (170.0-225.9)	194.5 (171.5-215.5)	0.401
LDL cholesterol (mg/dL)	137.0 (102.0-154.0)	130.0 (94.8-148.0)	0.489
HDL cholesterol (mg/dL)	47.0 (41.0-55.0)	45.0 (39.0-51.2)	0.339
Triglycerides (mg/dL)	109.0 (94.0-160.0)	102.5 (98.0-142.5)	0.743

Values are presented as median (Q1-Q3). P values were calculated using the Mann-Whitney U test. Abbreviations: RAH, resistant arterial hypertension; CKD, chronic kidney disease; HbA1C, glycated hemoglobin; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table 3. Echocardiographic parameters of patients with resistant arterial hypertension with and without chronic kidney disease.

Variable	RAH without CKD (n=73)	RAH with CKD (n=42)	*P* value
LVEF (%)	62.0 (57.0-65.0)	59.0 (55.0-63.8)	0.165
LA dimension in LAX (mm)	39.0 (37.0-41.0)	41.0 (38.0-43.0)	0.050
LA surface area in Ap4CH (cm^2)	20.7 (18.3-22.5)	22.4 (20.6-23.5)	0.004
LA volume (mL)	89.0 (76.0-108.0)	100.5 (90.8-109.6)	0.003
LAVI (mL/m^2)	42.6 (37.0-50.0)	48.0 (43.0-55.0)	0.003
LV systolic diameter (mm)	45.0 (42.0-47.0)	45.0 (41.0-47.8)	0.974
LV diastolic diameter (mm)	49.0 (47.0-52.0)	49.5 (46.0-52.8)	0.733
Interventricular septum thickness (mm)	12.0 (11.0-12.0)	11.5 (11.0-12.0)	0.196
Posterior wall thickness (mm)	11.0 (11.0-12.0)	11.0 (11.0-12.0)	0.514
LVMI (g/m^2)	104.0 (96.0-125.0)	107.0 (99.0-117.5)	0.862
LVMM (g)	207.3 (182.0-241.0)	213.5 (180.2-239.2)	0.628
Right ventricular diameter (mm)	28.0 (26.0-29.0)	28.5 (27.0-30.0)	0.108
RWT	0.5 (0.4-0.5)	0.5 (0.4-0.5)	0.338

Values are presented as median (Q1-Q3). P values were calculated using the Mann-Whitney U test. Abbreviations: LVEF, left ventricular ejection fraction; LA, left atrium; LAX, long-axis view; Ap4CH, apical four-chamber view; LAVI, left atrial volume index; LV, left ventricle; IVS, interventricular septum; RV, right ventricle; LVMI, left ventricular mass index; LVMM, left ventricular muscle mass; RWT, relative wall thickness.

Laboratory Parameters

Patients with RAH and CKD exhibited significantly higher serum uric acid levels compared with patients without CKD (7.1 [6.1-8.1] vs 6.4 [5.6-7.4] mg/dL; *P*=0.032). Glycated hemoglobin levels were also significantly higher in the CKD group (7.1 [6.2-8.1] vs 6.2 [5.9-7.0]%; *P*=0.002). No significant differences were observed in fasting glucose concentrations or lipid profile parameters, including total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides, between the groups. Laboratory parameters are summarized in **Table 2**.

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Table 4. Cardiovascular morbidity in patients with resistant arterial hypertension with and without chronic kidney disease.

Parameter	RAH without CKD (n=73)	RAH with CKD (n=42)	*P* value	Test
Atrial fibrillation (AF, any type)	**14 (19.2%)**	**14 (33.3%)**	**0.041**	χ^2
Paroxysmal AF	9 (12.0%)	12 (30.0%)	–	
Persistent AF	2 (2.7%)	1 (2.5%)	–	
Permanent AF	3 (4.0%)	1 (2.5%)	–	
Heart failure (any phenotype)	**62 (84.9%)**	**38 (90.5%)**	**0.031**	**Fisher**
HFrEF	1 (1.3%)	0 (0.0%)	–	
HFmrEF	3 (4.0%)	2 (5.0%)	–	
HFpEF	58 (77.3%)	36 (90.0%)	–	
Previous stroke history	**9 (12.3%)**	**8 (19.0%)**	**0.311**	**Fisher**
Previous ischemic stroke	7 (9.6%)	6 (14.3%)	–	
Previous hemorrhagic stroke	2 (2.7%)	2 (4.7%)	–	
Previous TIA	**12 (16.4%)**	**9 (21.4%)**	**0.512**	χ^2
CAD	**48 (64.0%)**	**32 (80.0%)**	**0.017**	χ^2
Stable CAD without prior MI or revascularization	18 (24.0%)	12 (30%)	------	
Previous PCI	14 (18.7%)	10 (25.0%)	–	
Previous myocardial infarction	12 (16.0%)	5 (12.5%)	–	
Previous CABG	4 (5.3%)	5 (12.5%)	–	
Type 2 diabetes mellitus	**30 (40.0%)**	**23 (57.5%)**	**0.022**	χ^2

Values are presented as n (%). *P* values were calculated using the chi-square test or Fisher's exact test, as appropriate. Subcategories of coronary artery disease are not mutually exclusive, as patients with a history of myocardial infarction may also have undergone PCI or CABG. Stable CAD refers to diagnosed coronary artery disease without prior myocardial infarction or coronary revascularization. Abbreviations: AF, atrial fibrillation; HFrEF, heart failure with reduced ejection fraction; HFmrEF, heart failure with mildly reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; TIA, transient ischemic attack; CAD, coronary artery disease; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting.

Echocardiographic Findings

Patients with RAH and CKD demonstrated more pronounced left atrial remodeling. Left atrial surface area in apical 4-chamber view was significantly larger in the CKD group (22.4 [20.6-23.5] vs 20.7 [18.3-22.5] cm^2; *P*=0.004), as were left atrial volume (100.5 [90.8-109.6] vs 89.0 [76.0-108.0] mL; *P*=0.003) and left atrial volume index (48.0 [43.0-55.0] vs 42.6 [37.0-50.0] mL/m^2; *P*=0.003). Left atrial dimension measured in the parasternal long-axis view showed a borderline difference between groups (41.0 [38.0-43.0] vs 39.0 [37.0-41.0] mm; *P*=0.050). No significant differences were observed in left ventricular ejection fraction or left ventricular structural parameters (**Table 3**).

Cardiovascular Comorbidities

Atrial fibrillation was significantly more common in patients with RAH and CKD than in those without CKD (33.3% vs 19.2%; *P*=0.041). Coronary artery disease was also more prevalent in the CKD group (80.0% vs 64.0%; *P*=0.017), as was type 2 diabetes mellitus (57.5% vs 40.0%; *P*=0.022). Heart failure was observed in the majority of patients in both groups but was slightly more common in patients with CKD (90.5% vs 84.9%; *P*=0.031). A history of stroke and transient ischemic attack was also more common in patients with CKD; however, these differences did not reach statistical significance. Cardiovascular comorbidities in the studied populations are summarized in **Table 4**.

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Table 5. Correlation between circulating microRNA expression levels and UACR in patients with resistant arterial hypertension.

microRNA (logRQ)	RAH without microalbuminuria (r)	RAH with CKD and Mmicroalbuminuria (r)
hsa-miR-1	0.089	0.323
hsa-miR-126	0.136	0.300
hsa-miR-133a	0.143	0.380*
hsa-miR-143	0.171	0.307
hsa-miR-145	0.128	0.311
hsa-miR-155	0.171	0.228
hsa-miR-195	0.189	0.291
hsa-miR-208	0.161	0.324
hsa-miR-21	0.152	0.234
hsa-miR-26b	0.116	0.192
hsa-miR-320	0.164	0.294

Spearman correlation coefficients are shown. Correlations were calculated using pairwise complete observations (n=69-71 for "without microalbuminuria"; n=34-35 for "CKD and microalbuminuria"). * *P*<0.05. Abbreviations: UACR, urinary albumin-to-creatinine ratio; RAH, resistant arterial hypertension; CKD, chronic kidney disease; RQ, log-transformed relative expression.

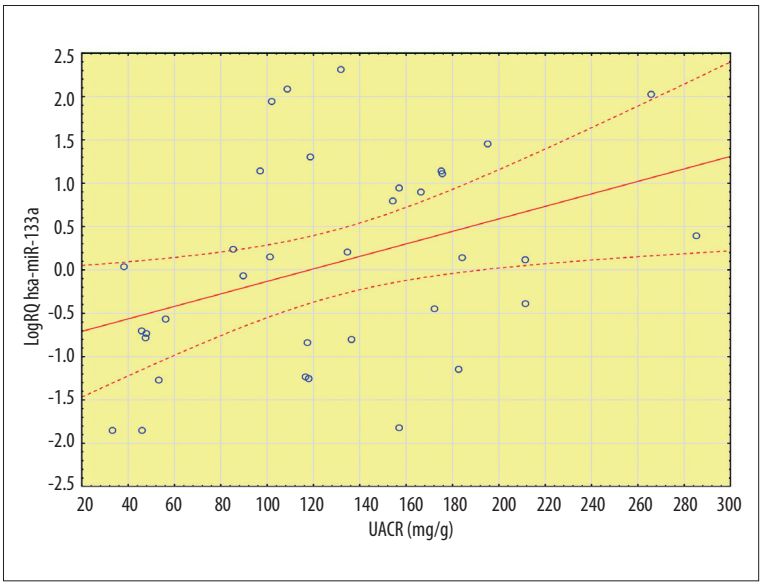

Figure 1. Scatterplot showing the correlation between circulating hsa-miR-133a expression (logRQ) and urinary albumin-to-creatinine ratio (UACR) in patients with chronic kidney disease and microalbuminuria (Spearman's r=0.38, *P*=0.024, n=35; pairwise complete observations).

Correlation Between miRNA Expression and Microalbuminuria

No significant differences in the expression levels of the analyzed miRNAs were observed between patients with and without CKD, suggesting that global miRNA expression profiles were comparable between the groups. Correlation analyses were performed for all analyzed miRNAs in both study groups. No statistically significant correlations were observed between the expression levels of the analyzed microRNAs and UACR in patients with RAH without microalbuminuria. In contrast, in patients with CKD and microalbuminuria (n=35), a statistically significant positive correlation was identified between hsa-miR-133a expression and UACR (Spearman's r=0.38, *P*=0.024). No significant correlations with UACR were found for the remaining analyzed microRNAs in either study group (**Table 5**). The correlation between hsa-miR-133a expression and UACR in patients with CKD and microalbuminuria is illustrated in **Figure 1**.

Correlations Between miRNA Expression and Clinical and Echocardiographic Parameters

Additional exploratory analyses were performed to evaluate potential associations between miRNA expression and selected clinical and echocardiographic parameters. Weak negative

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correlations were observed between hsa-miR-133a expression and left ventricular systolic diameter (r=-0.23, *P*=0.018), posterior wall thickness (r=-0.20, *P*=0.042), and left ventricular muscle mass (r=-0.19, *P*=0.047; n=106). Given the exploratory nature of these analyses and the modest strength of the associations, the findings should be interpreted with caution.

Discussion

The present study provides novel insights into the relationship between microRNA expression and renal involvement in patients with RAH. In a well-characterized cohort of patients with idiopathic RAH, those with coexisting CKD demonstrated a higher burden of cardiovascular comorbidities, more pronounced left atrial remodeling, and worse renal function parameters. The key finding of this study is the identification of a significant positive association between hsa-miR-133a expression and UACR in patients with CKD and microalbuminuria. Notably, among the analyzed panel of 11 microRNAs, only miR-133a demonstrated a significant association with UACR, suggesting a specific rather than global role of miRNA dysregulation in renal microvascular injury. Importantly, no significant differences in overall miRNA expression levels were observed between patients with and without CKD, indicating that the observed association is not driven by global alterations in miRNA expression but reflects a more specific relationship with renal microvascular injury. Additionally, weak negative correlations were observed between miR-133a expression and selected echocardiographic parameters reflecting left ventricular structure, which may suggest a potential relationship between miR-133a expression and cardiac remodeling in patients with RAH.

Our findings are consistent with those of previous studies reporting associations between miR-133a expression and markers of cardiovascular or renal target-organ damage. Experimental data indicate that miR-133a is involved in blood pressure regulation and renal sodium handling, particularly in models of salt-sensitive hypertension [15,22,23]. In clinical settings, altered circulating miR-133a levels have been reported in patients with arterial hypertension and left ventricular hypertrophy, supporting a link between miR-133a dysregulation and hypertensive organ damage [16]. Moreover, Parthenakis et al demonstrated an association between miR-133a expression and urinary albumin excretion in patients with newly diagnosed, untreated arterial hypertension [17].

Importantly, these prior studies were conducted predominantly in populations without advanced renal impairment or resistant hypertension. In contrast, our study extends these observations to a high-risk cohort with RAH and coexisting CKD, suggesting that the association between miR-133a expression and microalbuminuria persists in more advanced stages of hypertensive disease.

The biological mechanisms linking miR-133a expression with microalbuminuria are not fully understood; however, several pathways can be considered. Preclinical studies have identified miR-133a as a salt-sensitive microRNA involved in the regulation of intrarenal angiotensinogen expression and renal sodium handling, mediated in part by tumor necrosis factor-α–dependent pathways [15-17]. Through these mechanisms, miR-133a may influence intraglomerular pressure, endothelial function, and tubular sodium reabsorption—processes closely related to the development of albuminuria. Moreover, microalbuminuria reflects generalized endothelial dysfunction and increased vascular permeability, suggesting that altered miR-133a expression may represent a systemic epigenetic response to microvascular injury rather than a kidney-specific phenomenon. These mechanistic considerations remain speculative, as they were not directly assessed in the present study.

Beyond its association with albuminuria, miR-133a has been implicated in several cardiovascular and renal processes relevant to CKD. Previous studies have demonstrated altered miR-133a expression in conditions such as left ventricular hypertrophy and vascular calcification, suggesting a broader role in cardiovascular remodeling and CKD-related complications [16,24,25]. In patients with advanced CKD and end-stage renal disease, miR-133a has been linked to mechanisms regulating vascular calcification through modulation of osteogenic signaling pathways [24].

RAH represents a particularly high-risk clinical phenotype characterized by severe blood pressure dysregulation, advanced target-organ damage, and frequent coexistence of CKD. In this context, the observed association between miR-133a expression and microalbuminuria may reflect cumulative microvascular injury and systemic vascular dysfunction. Additionally, altered miR-133a expression has been associated with myocardial remodeling and fibrotic processes, suggesting a potential link with structural cardiovascular changes observed in this population [25,26].

Although previous studies have reported associations between albuminuria and other microRNAs, including miR-29, miR-21, miR-126, and miR-135a, suggesting the involvement of complex and overlapping epigenetic pathways [27-33], only miR-133a demonstrated a significant association with urinary albumin excretion in the present study.

From a clinical perspective, the identification of noninvasive biomarkers reflecting early renal and cardiovascular target-organ damage remains an important unmet need in patients with RAH. Microalbuminuria is a well-established marker of

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increased cardiovascular risk; however, it often reflects already established microvascular injury. The observed association between miR-133a expression and UACR suggests that miR-133a may provide complementary information to conventional clinical markers, potentially improving risk stratification in patients with RAH and CKD.

The assessment of miR-133a expression in PBMCs may reflect systemic epigenetic alterations associated with microvascular dysfunction, supporting its potential role as a circulating cellular biomarker. Nevertheless, the clinical utility of miR-133a requires confirmation in prospective and longitudinal studies.

Several limitations of this study should be acknowledged. First, the cross-sectional design precludes conclusions regarding causality or temporal relationships between miR-133a expression and microalbuminuria. Second, the relatively small sample size and single-center nature of the study may limit generalizability. Third, miR-133a expression was assessed in PBMCs rather than renal tissue, and mechanistic pathways were not directly investigated. Fourth, potential effects of pharmacological treatment on miRNA expression cannot be excluded. Finally, given the number of analyzed microRNAs, the possibility of type I error cannot be excluded. Additionally, the group of healthy volunteers was used solely for calibration of miRNA expression levels and was not included in comparative analyses, which limits the ability to assess differences between patients and healthy individuals.

Conclusions

This study demonstrated a positive correlation between hsa-miR-133a expression and urinary albumin excretion in patients with CKD and microalbuminuria. Among the analyzed microRNAs, miR-133a was the only molecule significantly associated with UACR, suggesting a specific role in microvascular renal injury. These findings indicate that miR-133a may represent a potential noninvasive biomarker of early renal injury in a high-risk hypertensive population. However, the cross-sectional design of the study precludes conclusions regarding causality or temporal relationships. Prospective longitudinal studies are required to determine whether increased miR-133a expression precedes or predicts worsening albuminuria and CKD progression and whether it may respond to therapeutic interventions.

Department and Institution Where Work Was Done

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Data Availability Statement

The research data supporting the findings of this study are openly available in the Polish Platform of Medical Research (PPM) repository (DOI: 10.71709/wga2-rv40). The dataset "Resistant arterial hypertension, co-morbidity and selected miRNAs Database v1.0" includes anonymized clinical and molecular data relevant to miRNA expression and is accessible through the PPM research data portal.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Bioethics Committee at the Medical University of Lublin (decision No. KE-0254/141/2020; June 25, 2020).

Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

References:

1. Mancia G, Kreutz R, Brunström M, et al. 2023 ESH Guidelines for the management of arterial hypertension The Task Force for the management of arterial hypertension of the European Society of Hypertension: Endorsed by the International Society of Hypertension (ISH) and the European Renal Association (ERA). J Hypertens. 2023;41(12):1874-2071
2. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2024 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney Int. 2024;105(4 Suppl.):S117-S314
3. Collins AJ, Foley RN, Vhavers B, et al. United States Renal Data System 2011 Annual Data Report: Atlas of chronic kidney disease & end-stage renal disease in the United States. Am J Kidney Dis. 2012;59(Suppl. 1):A7,e1-420
4. Tanner RM, Calhoun DA, Bell EK, et al. Prevalence of apparent treatment-resistant hypertension among individuals with CKD. Clin J Am Soc Nephrol. 2013;8(9):1583-90
5. Wolley MJ, Stowasser M. Resistant hypertension and chronic kidney disease: A dangerous liaison. Curr Hypertens Rep. 2016;18(5):36
6. Crahim V, Verri V, De Lorenzo A, Tibirica E. Reduced systemic microvascular function in patients with resistant hypertension and microalbuminuria: An observational study. J Hum Hypertens. 2024;38(12):806-13
7. Inker LA, Coresh J, Levey AS, et al. Estimated GFR, albuminuria, and complications of chronic kidney disease. J Am Soc Nephrol. 2011;22(12):2322-31
8. Campese VM. Patophysiology of resistant hypertension in chronic kidney disease. Semin Nephrol. 2014;34(5):571-76
9. Kwakernaak AJ, Krikken JA, Binnenmars SH, et al. Effects of sodium restriction and hydrochlorothiazide on RAAS blockade efficacy in diabetic nephropathy: A randomised clinical trial. Lancet Diabetes Endocrinol. 2014;2(5):385-95
10. Leitão AL, Enguita FJ. A structural view of miRNA biogenesis and function. Noncoding RNA. 2022;8(1):10
11. Leimena C, Qiu H. Non-coding RNA in the pathogenesis, progression and treatment of hypertension. Int J Mol Sci. 2018;19(4):927
12. Carmona A, Guerrero F, Jimenez MJ, et al. Inflammation, senescence and microRNAs in chronic kidney disease. Front Cell Dev Biol. 2020;8:739
13. Zou C. Advances in the study of miRNAs in chronic kidney disease with cardiovascular complications. Front Physiol. 2023;14:1283597

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

14. Shaheen N, Shaheen A, Diab RA, Desouki MT. MicroRNAs (miRNAs) role in hypertension: Pathogenesis and promising therapeutics. Ann Med Surg (Lond). 2023;86(1):319-28
15. Hao S, Salzo J, Zhao H, et al. MicroRNA-133a-dependent inhibition of proximal tubule angiotensinogen by renal TNF (tumor necrosis factor). Hypertension 2020;76(6):1744-52
16. Koval S, Snihurska S, Yushko K, et al. Plasma microRNA-133a level in patients with essential arterial hypertension. Georgian Med News. 2019;(290):52-59
17. Parthenakis F, Marketou M, Kontaraki J, et al. Comparative microRNA profilling in relations to urinary albumin excretion in newly diagnosed hypertensive patients. J Hum Hypertens. 2016;30:685-89
18. Szyszka M, Skrzypczyk P. MicroRNA-133a and microRNA-145 may be involved in the development of hypertension-mediated organ damage in children with primary hypertension – A preliminary study. J Clin Med. 2024;13(22):6929
19. Williams B, Mancia G, Spiering W, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. Eur Heart J. 2018;39:3021-104
20. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods. 2011;25:402-8
21. Błaszczyk R, Petniak A, Bogucki J, et al. Association between resistant arterial hypertension, type 2 diabetes and selected microRNAs. J Clin Med. 2024;13(2):542
22. Hao S, Hao M, Ferreri NR. Renal-specific silencing of TNF (tumor necrosis factor) unmasks salt-dependent increases in blood pressure via an NKCC2A (Na^+-K^+-$2Cl^-$ cotransporter isoform A)-dependent mechanism. Hypertension. 2018;71(6):1117-25
23. Hao S, Salzo J, Hao M, Ferreri NR. Regulation of NKCC2B by TNF-α in response to salt restriction. Am J Physiol Renal Physiol. 2020;318(1):F273-82
24. Li S, Zhi F, Hu M, et al. MIR-133a is a potential target for arterial calcification in patients with end-stage renal disease. Int Urol Nephrol. 2022;54(1):217-24
25. Wen P, Song D, Ye H, et al. Circulating MIR-133a as a biomarker predicts cardiac hypertrophy in chronic hemodialysis patients. PLoS One. 2014;9(10):e103079
26. Wang Y, Guo YF, Fu GP, et al. Protective effect of miRNA-containing extracellular vesicles derived from mesenchymal stromal cells of old rats on renal function in chronic kidney disease. Stem Cell Res Ther. 2020;11(1):274
27. Huang YQ, Huang C, Li J, et al. The association of miR-29a with proteinuria in essential hypertension. J Hum Hypertens. 2018;32(11):775-80
28. Huang C, Huang YQ. The correlation of circulating miR-29b and inflammatory markers with albuminuria in hypertensive patients. Clin Exp Hypertens. 2020;42(8):743-47
29. Tang PM, Zhang YY, Mak TS, et al. TGF-beta signalling in renal fibrosis: From Smads to non-coding RNAs. J Physiol. 2018;596(16):3493-503
30. Yildirim E, Ermis E, Allahverdiyev S, et al. Circulating miR-21 levels in hypertensive patients with asymptomatic organ damage. Medicine (Baltimore). 2019;98(39):e17297
31. Al-Kafaji G, Al-Mahroos G, Al-Muhtaresh HA, et al. Decreased expression of circulating microRNA-126 in patients with type 2 diabetic nephropathy: A potential blood-base biomarker. Exp Ther Med. 2016;12(2):815-22
32. He F, Peng F, Xia X, et al. MIR-135a promotes renal fibrosis in diabetic nephropathy by regulating TRPC1. Diabetologia. 2014;57(8):1726-36
33. Chen K, Jin X, Li Q, et al. Association of TRPC1 gene polymorphisms with type 2 diabetes and diabetic nephropathy in Han Chinese population. Endocr Res. 2013;38(2):59-68

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