European Journal of Clinical and Experimental Medicine

ONLINE FIRST

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Article type: Original Article

Received: 9 May 2026

Accepted: 29 June 2026

Published online: 23 July 2026

eISSN: 2544-1361

Eur J Clin Exp Med

doi:10.15584/ejcem.2026.3.9

Expression of the miR-155, miR-326 and IL-17/IL-23 axis in patients with essential hypertension – a case-control study

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ABSTRACT

Introduction and aim. Essential hypertension (EH) is a major cardiovascular risk factor worldwide. The interleukin (IL)-17 and IL-23 axis is a key driver of Th17-mediated immune response; however, the involvement of microRNAs in modulating this axis in patients with essential hypertension remains poorly understood. This study aimed to evaluate expression profiles of miR-155 and miR-326 together with the serum of IL-17A and IL-23 in patients with EH.

Material and methods. This case-control study enrolled 100 participants (50 patients with essential hypertension and 50 healthy controls). The expression of miR-155 and miR-326 was determined by (RT-qPCR), whereas serum IL-17A and IL-23 concentrations were measured using (ELISA).

Results. IL-17A and IL-23 levels were significantly elevated in patients with essential hypertension compared to healthy controls ($p<0.001$). Additionally, miR-155 was significantly overexpressed, whereas miR-326 was significantly downregulated in patients with essential hypertension ($P<0.001$ for both). Exploratory receiver operating characteristic analysis showed preliminary discriminatory performance for miR-155, miR-326, IL-17A and IL-23 with AUC values of 0.988, 0.927, 0.974 and 0.946, respectively. No direct correlation between miRNA expression and cytokine levels was identified.

Conclusion. Patients with essential hypertension exhibit dysregulation of miR-155 and miR-326, IL-17A, and IL-23. Lack of significant correlations suggests that these biomarkers may reflect distinct aspects of hypertension-associated inflammation. Further prospective and longitudinal investigations are needed to validate these findings and clarify underlying mechanisms.

Keywords. hypertension, interleukin-17, interleukin-23, microRNAs, miR-155, miR-326

Introduction

Essential hypertension is a chronic elevation of blood pressure and is considered both a disease itself and a major risk factor for other diseases leading to global public health challenge. While hemodynamic, renal and neurohormonal mechanisms have traditionally defined the pathophysiological framework of essential hypertension, accumulating evidence highlights low-grade chronic inflammation as a critical driver of vascular dysfunction and sustained blood pressure elevation.[1] Patients with hypertension exhibit elevated circulating inflammatory markers, oxidative stress, and altered immune cell phenotypes.[2,3] The renin–angiotensin–aldosterone system interacts closely with immunity via angiotensin II, which not only constricts blood vessels but also serves as an immunomodulator, stimulating dendritic cell activation and promoting inflammatory cell recruitment.[4]

Among the immune pathways implicated in essential hypertension, the Interleukin-17 and Interleukin-23 (IL-17/IL-23) axis has been recognized as an important contributor to inflammation in essential hypertension.[5] Within the axis, IL-23 stabilizing the Th17 phenotype and sustaining IL-17A production.[6] IL-17A promotes endothelial dysfunction by impairing nitric oxide (NO) bioavailability, promoting oxidative stress and increased renal sodium reabsorption, collectively leading to elevated blood pressure.[7–9] Although most mechanistic evidence arises from preclinical models, human studies support the relevance of these inflammatory cytokines in the pathogenesis of essential hypertension.[5,10–12]

MicroRNAs (miRNAs) are small conserved non-coding RNA molecules that regulate gene expression at the post-transcriptional level. They are increasingly recognized as major regulators of immune cell differentiation, cytokine production, and vascular homeostasis. Among miRNAs with established roles in immune regulation, miR-155 has been reported to enhance Th17 differentiation by inhibiting suppressor of cytokine signaling 1 (SOCS1), a key inhibitor of cytokine signaling, thereby enhancing IL-17 production and perpetuating inflammatory cascades.[13] Conversely, miR-326 has been reported to regulate Th17 differentiation by targeting negative regulator (Ets-1), thereby promoting the expansion of Th17 cells and potentially augmenting IL-17A-driven immune responses.[14] In particular, these two miRNAs are thought to play distinct regulatory roles within the same Th17 pathway.

Moreover, assessing miR-155 and miR-326 together with IL-17A and IL-23 may help clarify potential interactions between epigenetic regulation and inflammatory responses in essential hypertension. Most previous investigations have focused exclusively on studying IL-17/IL-23 axis components or miR-155 alone.[3,12,15–17] To our knowledge, only a few studies have comprehensively analyzed the combined expression pattern of circulating miR-326 and miR-155, IL-17A and IL-23 in patients with essential hypertension. Addressing this gap may improve our understanding of how specific miRNAs and the IL-17/IL-23 axis are associated with immune dysregulation in essential hypertension.

Aim

This study aimed to perform an exploratory analysis of the combined expression profiles of miR-155 and miR-326 together with circulating IL-17A and IL-23 levels in patients with essential hypertension. Secondary analyses examined the correlations among these biomarkers and explored their discriminatory performance using receiver operating characteristic analysis.

Material and methods

Study design and population

This study was designed to evaluate the expression profiles of specific microRNAs and the IL-17/IL-23 inflammatory axis in patients with essential hypertension. The study cohort was comprised of 100 participants: 50 patients with a confirmed diagnosis of essential hypertension (patients group; 26 men, 24 women; mean age, 48.74±7.83 years) and 50 healthy individuals (healthy control; 28 men, 22 women; mean age, 46.16±8.03 years).

Participants were consecutively enrolled from the outpatient clinics and physical examination unit of Al-Diwaniyah Teaching Hospital (Iraq, Al-Diwaniyah Governorate) between October 2025 and January 2026. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki (Number 41, Date 20/10/2025). The institutional ethics committee specifically approved the use of verbal informed consent as part of the approved study protocol because the study was observational and involved routine venous blood collection without any additional intervention.

Inclusion and exclusion criteria

Rigorous exclusion criteria were applied to minimize confounding variables. Participants were excluded if they fulfilled any of the following criteria: immunocompromised status, active malignancies, diabetes mellitus, active smoking, secondary hypertension of known etiology, autoimmune disorders or chronic inflammatory diseases. Healthy volunteers in the healthy control group were required to be strictly normotensive defined systolic blood pressure (SBP)<120 mmHg and diastolic blood pressure (DBP)<80 mmHg with no personal history of chronic inflammatory or cardiovascular diseases. Blood pressure was measured by physicians using a calibrated mercury sphygmomanometer with the participant in the seated position after at least 5 minutes of rest. Two consecutive measurements were obtained at 1–2-minute intervals, and the average of the two readings was recorded. Essential hypertension was confirmed as a (SBP) ≥140 mmHg and/or (DBP) ≥90 mmHg, confirmed on repeated measurements during separate clinical visits according to the current clinical guidelines.

Sample size calculation

The study enrolled 50 patients with essential hypertension and 50 healthy controls. Sample size was determined based on participant availability during the predefined study period. A formal a priori power analysis was not performed. Accordingly, this limitation should be taken into account when interpreting the study findings.

Blood collection, processing and storage

Each participant underwent peripheral venous blood collection (5 mL) after fasting overnight for 8–10 hours. A (2 mL) aliquot of whole blood was immediately transferred into RNase-free tubes to preserve RNA integrity. The remaining (3 mL) was transferred into clean sterile tubes, at room temperature allowed to clot for 20–30 minutes, and to isolate serum blood centrifuged for 10 minutes at 3000 rpm. Both whole-blood aliquots with serum were immediately stored at -80°C until downstream molecular and biochemical analyses. Before serum analysis, frozen samples were thawed to room temperature, mixed gently by vortexed for 3 seconds, then centrifuged at 10,000×g for 5 minutes at 4°C to precipitate any cellular debris. Visually hemolysis, lipemic, or icteric samples were rigorously discarded.

Total RNA extraction

Total RNA was isolated from the processed whole blood samples using TRIzol® reagent (Invitrogen, Thermo Fisher Scientific, USA) in strict accordance with the protocol of manufacturer. The concentration and purity of the extracted RNA were evaluated using a NanoDrop spectrophotometer (Thermo Scientific, USA), only samples with an absorbance (A260/280) between 1.8 to 2.0 were acceptable for downstream processing. RNA structure integrity was further evaluated by 1.5% agarose gel electrophoresis through visual assessment of 28S and 18S ribosomal RNA bands. Only samples showing intact ribosomal RNA bands with an approximate 28S:18S ratio of 2:1 and no evidence of degradation were included for subsequent analyses.

DNase treatment and cDNA synthesis

To remove residual genomic DNA contamination, total RNA was treated with DNase I (Promega, Madison, WI, USA) for 30 minutes at 37°C, followed by enzyme inactivation for 10 minutes at 65 °C. For microRNA-specific complementary DNA (cDNA) synthesis, a stem-loop reverse transcription method was employed. A universal RT primer was utilized alongside the M-MLV Reverse Transcriptase kit (Bioneer, Republic of Korea) with 100 ng/µL of template RNA. The RNA-primer mix was denatured at 70°C for 10 minutes and instantly chilled on ice. Reverse transcription was conducted at 42°C for 60 minutes followed by enzyme inactivation for 5 minutes at 65°C. For the amplification of the housekeeping reference gene (GAPDH),

cDNA synthesis was performed under analogous conditions using random hexamer primers, initiated with a denaturation step for 10 min at 65°C.

Quantitative real-time polymerase chain reaction (RT-qPCR)

The expression of the selected miRNAs was quantified by (RT-qPCR). Specific qPCR primers for hsa-miR-155 (MIMAT0000646) and hsa-miR-326 (MIMAT0000756) were designed using the Sanger Center miRNA database (miRBase) registry to select the appropriate mature miRNA sequences, with assistance from a specialized miRNA primer design tool. Primers for the GAPDH reference gene (NM_001256799.3) were generated using the NCBI database and Primer3Plus online software.[18] Quantitative PCR was performed using GoTaq® qPCR Master Mix (Promega, USA) on a MiniOpticon Real-Time PCR Detection system (Bio-Rad, USA) and each amplification was prepared in a final total volume of 20 µL, containing cDNA template, reverse primer, forward primer, GoTaq qPCR Master Mix, and DEPC-treated water. Thermocycling parameters were: start denaturation for 5 min at 95°C, followed by 45 amplification cycles including denaturation at 95°C for 20 s and 60°C for 30 s. The sequences of all primers used in this study are presented in Table 1.

Table 1. The primer sequences used for RT-qPCR

Primer	**Sequence**	
RT primer (universal)	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTT TTTTTTTTTVN	
hsa-miR-155 qPCR primer	F	AACACGCTTAATGCTAATCGTGATA
	R	GTCGTATCCAGTGCAGGGT
hsa-miR-326 qPCR primer	F	AACAATCCTCTGGGCCCTTC
	R	GTCGTATCCAGTGCAGGGT
GAPDH qPCR primer	F	TCACCAGGGCTGCTTTTAAC
	R	TGACGGTGCCATGGAATTTG

RT-qPCR quality control

Relative gene expression levels ware calculate via the comparative 2^−ΔΔCt method, using healthy normotensive group serving as the calibrator.[19] GAPDH was used as the endogenous reference gene. It is stability was assessed by comparing Ct values between patients with essential hypertension and healthy controls. Amplification specificity was evaluated by melt curve analysis which conformed to a single distinct melting peak for each target, indicating specific amplification. Amplification efficiencies were determined using 10-fold serial cDNA dilutions, yielding acceptable ranges between 90% and 110% ($(R^2)>0.99$). Intra-assay and inter-assay variability were assessed by running samples in triplicate within

the same plate and across three independent runs, respectively, with coefficients of variation (CV) consistently <5%. Although small RNA reference genes like U6, miR-16, SNORD44, and SNORD48 are generally recommended for circulating miRNA normalization according to the MIQE guidelines, these controls were not incorporated during the original study design. Consequently, retrospective re-normalization was not feasible. GAPDH was retained as the endogenous control because it demonstrated highly stable Ct values across all study samples under the present experimental conditions. Nevertheless, the use of GAPDH represents a methodological limitation, and future studies should validate normalization using multiple endogenous reference controls.

In silico target prediction analysis

In silico miRNAs target interactions were analysis using the publicly available miRDB and TargetScan databases to identify potential down-stream inflammatory pathways regulated by miR-155 and miR-326 relevant to inflammatory signaling pathways.

Measurement of circulating cytokines

Commercially available sandwich ELISA kits were used to measure serum interleukin-17A (IL-17A) and interleukin-23 (IL-23) concentrations. Human IL-17A was quantified using an ELISA kit (Cat. No. E0047Hu, Bioassay Technology Laboratory, China) with detection range (5–1000 ng/L) and assay sensitivity (2.38 ng/L), and IL-23 was measured using ELISA kit (Cat. No. E0074Hu, Bioassay Technology Laboratory, China) with detection range (3–9000 ng/L) with sensitivity (1.52 ng/L). The inter-assay coefficient of variation (CV) was <10% for both assays according to the manufacturer. All laboratory procedures strictly adhered to the manufacturer's protocols. Standard solutions were prepared to generate standard curves, and serum samples were analyzed in duplicate wells. After the required incubation and washing steps, the appropriate detection antibodies were added, then followed by substrate development. Absorbance was measured at the recommended wavelength using a microplate reader, and cytokine level were measured from the corresponding standard curves.

Sample quality and exclusion criteria

Samples were excluded based on predefined criteria: (1) visual evidence of hemolysis, lipemia, or icterus; (2) RNA purity outside the acceptable range (A260/280<1.8 or >2.0) or RNA degradation observed on agarose gel; (3) failure of the internal control gene (GAPDH) amplification with a cycle threshold (Ct) value >35; and (4) coefficient of variation (CV) >10% among technical replicates in RT-qPCR or ELISA assays. Only samples that satisfied all predefined quality control criteria were retained for the final statistical analysis.

Statistical analysis

Statistical analyses were demonstrated using the IBM SPSS Statistics software (version 26.0 and IBM Corp., Armonk, NY, USA). For normally distributed continuous variables, descriptive statistics are shown as the mean±SD accompanied by 95% confidence intervals (CIs), whereas categorical data are described using frequencies and percentages. To assess normality of data distribution, we used the Shapiro–Wilk test before applying parametric statistical analyses. To confirm homogeneity of variances among groups was assessed using Levene's test prior to independent t test ($P > 0.05$). Between-group comparisons were performed using the independent samples t-test for continuous variables with normal distribution and the chi-square test for categorical variables. The strength of the associations between continuous variables was evaluated by Pearson's correlation coefficient (r). Exploratory receiver operating characteristic (ROC) curve analysis was performed to evaluate the preliminary discriminatory value of IL-17A, IL-23, miR-155, and miR-326 in distinguishing patients with essential hypertension from healthy controls. Sensitivity, specificity, optimal cutoff values, and the area under the curve (AUC) were calculated. Multivariable binary logistic regression analysis was performed to for potentially confounding variables including BMI and age. A statistically significant difference was considered at $P<0.05$. Because the study was performed in a single-center cohort without external validation, the finding of the ROC analysis should be considered exploratory.

Results

The clinical and demographic profiles of the study population are shown in Table 2. Statistical analysis demonstrated that the groups were comparable with respect to age and sex distribution, with no significant differences observed (p=0.155 and p=0.688, respectively). In contrast, the essential hypertensive group had significantly higher BMI and obesity prevalence than the healthy controls group (p=0.001 and p=0.002, respectively). Additionally, 80% of patients with essential hypertension had a family history of hypertension.

Information regarding antihypertensive treatment status (treated or untreated) was collected from patient interviews. However, detailed information regarding the specific classes, doses, and duration of antihypertensive medications was not consistently available and therefore was not included in the analysis.

Table 2. Demographics and clinical profile of study groups*

Characteristic	**EH patients (n=50)**	**Healthy controls (n=50)**	**Test**	**p**
Age, Mean±SD (years)	48.74±7.83	46.16±8.03		0.155
Male, n (%)	26 (52.0%)	28 (56.0%)	χ^2= 0.160	0.688
Female, n (%)	24 (48.0%)	22 (44.0%)		

SBP (mmHg), Mean±SD	148.25±15.99	112.25±5.60	t=14.898	0.001
DBP (mmHg), Mean±SD	96.84±8.69	74.37±5.12	t=15.719	0.001
BMI (kg/m²), Mean±SD	31.47±6.72	26.75±5.45	–	0.001
Obese (BMI ≥30), n (%)	29 (58.0%)	12 (24.0%)	–	0.002
Positive family history, n (%)	40 (80.0%)	–	–	–
Negative family history	10 (20.0%)	–	–	–
Duration of disease ↓ 5 years, n (%)	28 (56.0%)	–	–	–
Duration of disease ↑ 5 years, n (%)	22 (44.0 %)	–	–	–
Treating, n (%)	16 (32.0 %)	–	–	–
Non-treating, n (%)	34 (68.0 %)	–	–	–

* Family history, disease duration, and treatment status were assessed only in patients with essential hypertension

Stability assessment of GAPDH

To assess the suitability of GAPDH as a reference gene, Ct values were compared between patients with essential hypertension and healthy controls. Gene reference expression demonstrated minimal variability across samples, with mean Ct values of 22.03±0.45 in patients with essential hypertension and 22.08±0.47 in healthy controls with (P>0.05), supporting its suitability as an internal control under the experimental conditions of the present study (Table 3).

Table 3. Stability assessment of GAPDH Ct values in study groups

Groups	n	Mean CT±SD	Minimum CT	Maximum CT
EH Patients	50	22.03±0.45	21.29	22.78
Healthy controls	50	22.08±0.47	21.26	22.68
p		0.710		

As presented in Table 4, miR-155 expression was significantly upregulated in patients with essential hypertension compared with healthy controls (9.26-fold upregulation, P<0.001). In contrast, miR-326 expression was significantly downregulated in patients with essential hypertension, relative expression level (0.092-fold compared with healthy controls (P<0.001).

Table 4. miR-155 and miR-326 expression in patients with EH and healthy controls

miRNA	EH patients (n=50) Mean±SD	Healthy controls (n=50) Mean±SD	Fold change (EH)	p

miR-155	9.26±2.71	1.0±0.31	9.26↑	<0.001
miR-326	0.092±0.031	1.0±0.23	0.092↓	<0.001

* Values are mean±SD, ↑ – upregulated. ↓ – downregulated

There were marked elevations in the concentrations of serum IL-17A (59.66±6.75 pg/mL vs. 38.93±6.41 pg/mL, p<0.001) and IL-23 (107.82±11.33 pg/mL vs. 77.21±8.56 pg/mL, p<0.001) in patients with essential hypertension relative to healthy subjects (Table 5).

Table 5. Serum IL-17A and IL-23 level in patients with EH and healthy controls*

Parameter	EH Patients (n=50)	Healthy Controls (n=50)	p
IL-17A (pg/mL)	59.66±6.75	38.93±6.41	<0.001
Range	45.96–74.12	23.69–56.79	
IL-23 (pg/mL)	107.82±11.33	77.21±8.56	<0.001
Range	86.87–131.12	54.31–102.40	

* Values are mean±SD

Correlation analysis between miR-155 and miR-326

Correlation analysis showed a weak association between miR-155 and miR-326 expression levels (r=-0.126). No significant correlation was detected in this relationship (p>0.05) as presented in Figure 1.

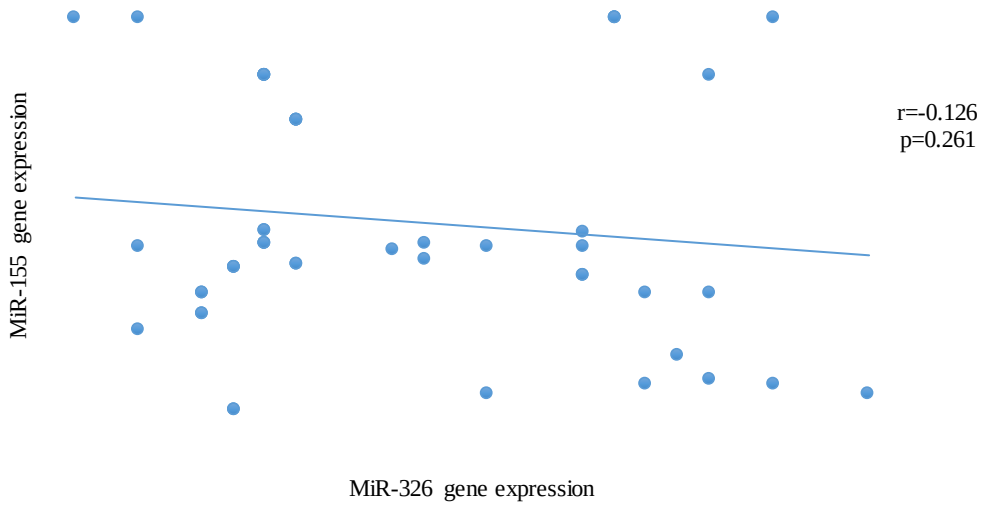

Fig. 1. The correlation scatter miR-155 and miR-326 levels in patients with EH

Correlations between immunological parameters in patients with EH

Correlation analysis demonstrated that the immunological parameters were significantly correlated, with IL-17A and IL-23 showing a positive correlation among patients with essential hypertension (r=0.618, p<0.001, Fig. 2).

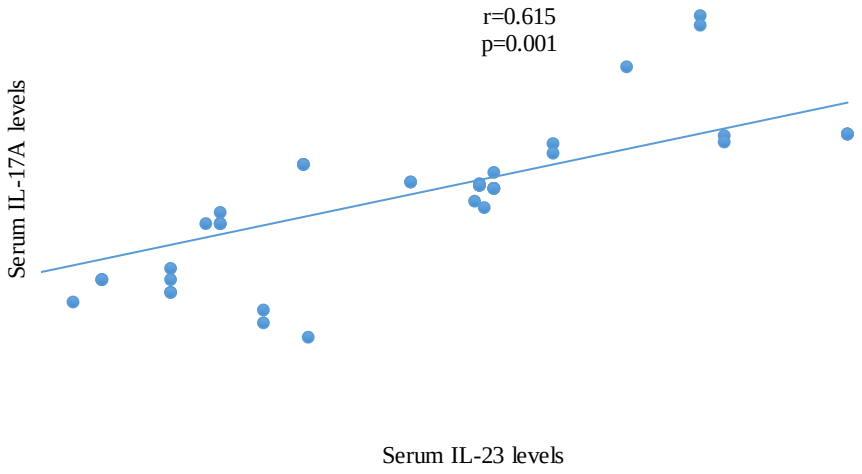

Fig. 2. The correlation scatter of IL-17A and IL-23 levels in patients with EH

Correlation between miRNAs expression parameters and other parameters in patients with EH

Among patients with EH, miR-155 expression demonstrated significant positive correlations with age (r=0.383, P=0.006) and BMI (r=0.502, P=0.001). Expression of miR-326 was significantly negatively correlated with BMI (r=−0.323, P=0.042) but not with age (r=−0.113, P=0.435). Neither miR-155 nor miR-326 expression correlated significantly with serum IL-17A or IL-23 concentrations (for all P>0.05).

Table 6. Correlation between miRNAs gene expression parameters and cytokine axis and other parameters in patients with EH*

Parameters	**miRNAs gene expression parameters**			
	miR-155 gene expression		**miR-326 gene expression**	
	r	p	r	p
IL-17A	0.181	0.209	-0.210	0.194
IL-23	0.171	0.234	-0.103	0.528
Age	0.383	0.006*	-0.113	0.435
BMI	0.502	0.001*	-0.323	0.042*

* r – correlation coefficient

In silico target prediction analysis identified SOCS1 as a potential target of miR-155 and Ets-1 as a potential target of miR-326. Both genes have been implicated in cytokine signaling and regulation of Th17-cell differentiation.

ROC curve analysis was conducted to evaluate the preliminary discriminatory performance of both cytokines and miRNA in patients with essential hypertension compared with controls, as shown in Table 7 and Table 8. IL-17A, at a cutoff value of >50.37 pg/mL yielded an AUC of 0.974 (95% CI: 0.950–0.998), achieving a sensitivity of 96.0% and a specificity of 92.0%. IL-23 at a cutoff of >92.38 pg/mL yielded an AUC of 0.946 (95% CI: 0.908–0.985), with corresponding sensitivity and specificity values of 92.0% and 94.0%, respectively.

Table 7. Accuracy of IL-17A and IL-23 by ROC curve analysis*

Biomarker	Cutoff	AUC (95% CI)	Sensitivity %	Specificity %	PPV %	NPV %
IL-17A	>50.37 pg/mL	0.974 (0.950–0.998)	96.0	92.0	92.3	95.8
IL-23	>92.38 pg/mL	0.946 (0.908–0.985)	92.0	94.0	93.9	92.2

* CI – confidence interval, AUC – area under the curve, NPV – negative predictive value, PPV – positive predictive value

At a cutoff of >2.24-fold expression, miR-155 yielded an AUC of 0.988 (95% CI: 0.973–1.000), with sensitivity of 96.0% and specificity of 98.0%. miR-326 at a cutoff of <0.22-fold expression yielded an AUC of 0.927 (95% CI: 0.848–1.000), with 90.0% specificity and 92.0% sensitivity. MiR-155 achieved the highest AUC across all four biomarkers. These findings require external validation before any clinical application.

Table 8. Accuracy of miR-155 and miR-326 by ROC curve analysis*

Biomarker	Cutoff	AUC (95% CI)	Sensitivity %	Specificity %	PPV %	NPV %
miR-155	>2.24 fold	0.988 (0.973–1.000)	96.0	98.0	98.0	96.1
miR-326	<0.22 fold	0.927 (0.848–1.000)	92.0	90.0	90.2	91.8

* AUC – area under the curve CI – confidence interval, PPV – positive predictive value, NPV – negative predictive value

To account for potential confounding by age and BMI, multivariable binary logistic regression models were applied to examine whether the relationships of miR-155, miR-326, IL-17A, and IL-23 with essential

hypertension remained statistically significant. The results demonstrated that all four biomarkers remained significantly associated with hypertension.

Table 9. Multivariable logistic regression model of related factors in patients with EH

Model	Variable	*B*	*SE*	χ^2Wald	*df*	p	OR	95% CI for OR
miR-155	Age	0.057	0.101	0.325	1	0.569	1.059	0.869–1.291
	BMI	-0.511	0.255	4.000	1	0.045	0.600	0.364–0.990
	miR-155	-6.310	2.654	5.652	1	0.017	0.002	0.000–0.330
miR-326	Age	-0.005	0.030	0.027	1	0.870	0.995	0.939–1.055
	BMI	-0.069	0.044	2.490	1	0.115	0.933	0.856–1.017
	miR-326	2.412	0.612	15.542	1	0.000	11.153	3.363–36.993
IL-17	Age	-0.005	0.059	0.008	1	0.931	0.995	0.885–1.118
	BMI	-0.192	0.090	4.574	1	0.032	0.825	0.692–0.984
	IL17	-0.358	0.077	21.569	1	0.000	0.699	0.601–0.813
IL-23	Age	0.003	0.055	0.002	1	0.963	1.003	0.900–1.117
	BMI	-0.187	0.071	6.910	1	0.009	0.830	0.722–0.954
	IL23	-0.230	0.057	16.090	1	0.000	0.795	0.710–0.889

Discussion

This case-control study provides clinical evidence for the dysregulation of circulating miR-155 and miR-326, as well as the IL-17/IL-23 inflammatory axis in patients with essential hypertension. An experimental study demonstrated that IL-17A is not a secondary marker, but part of pathogenesis of hypertension.[20] Wang et al. demonstrated significantly elevated circulating Th17 cells and IL-23 levels in patients with hypertension, supporting the involvement of Th17-mediated inflammation in hypertension.[15] Ahmed et al., demonstrated significant activation of the IL-23/Th17 in Egyptian patients with grade 2 hypertension, supporting the role of Th17-mediated inflammation in hypertension.[12] Furthermore, Yao et al. reported elevated serum IL-17 concentrations even among individuals with prehypertension, suggesting that activation of the IL-17 pathway may occur early during blood pressure dysregulation and persist throughout disease progression.[21]

These clinical observations are further supported by recent large-scale evidence highlighting the central role of immune and inflammatory pathways, particularly the IL-23/IL-17/Th17 axis, in the development and progression of hypertension. A systematic review and meta-analysis of circulating cytokines in hypertension underscored the contribution of immune mediators to development of hypertension, while contemporary reviews have emphasized the central role of inflammation disease progression.[22,23]

The positive correlation between IL-17A and IL-23 levels identified in our study (R^2=0.3816) reinforces potential the interdependent nature of these cytokines and is consistent with prior reports demonstrating a synergistic relationship within the axis in patients with essential hypertension.[15]

The exploratory discriminatory performance of IL-17A (AUC=0.974) and IL-23 (AUC=0.946) in distinguishing patients with essential hypertension from healthy controls underscores their potential as inflammatory biomarkers, although requiring further validation.

The concurrent upregulation of miR-155 and elevation of IL-17A in patients with essential hypertension may be associated with inflammatory marker. This finding is partly agreement with previous clinical studies suggesting a role for miR-155 in hypertension-associated inflammation. Huang et al. reported an association between increased circulating gene expression as well as miR-155 with elevated inflammatory markers in patients with white coat hypertension, supporting the involvement of miR-155 in early hypertensive inflammatory responses.[3] While Kara et al. did not observe differences in miR-155 levels among patients newly diagnosed hypertension, resistant hypertension, and normotensive controls.[24] These discrepancies may reflect differences in patient populations, disease phenotypes and treatment status. Collectively, the available evidence suggests that the role of miR-155 may be dependent on and potentially influenced by both inflammatory and clinical characteristics of the studied population.

Conversely, the significant downregulation of miR-326 in patients with essential hypertension, occurring together with elevated IL-17A and IL-23 levels, presents a complex regulatory scenario. Similar alterations in circulating miRNA profiles have been reported in patients with essential hypertension. A study reported significant upregulation of miR-4516 and downregulation of miR-145 in patients with hypertension, supporting the concept that miRNA dysregulation may associated with the pathophysiology of hypertension.[25]

Previous studies in autoimmune diseases, particularly multiple sclerosis, have reported increased miR-326 expression associated with enhanced Th17 differentiation through suppression of Ets-1.[14] In contrast, the present study demonstrated a marked reduction in circulating miR-326 levels in patients with essential hypertension. Furthermore, the relationship between miR-326 and IL-17A does not appear to be straightforward; a study in celiac disease reported increased IL-17A levels despite downregulation of miR-326, suggesting that increased IL-17A production may occur independently of miR-326 expression under certain inflammatory conditions.[26] This apparent discrepancy suggests that the biological role of miR-326 may be disease-specific and associated with differences in the immune microenvironment, tissue origin, or

metabolic factors. Furthermore, Th17 differentiation is controlled by a complex regulatory network involving multiple microRNAs and signaling pathways.[27] This discrepancy may indicate that the regulatory role of miR-326 is highly dependent and differs between autoimmune diseases and cardiovascular inflammatory conditions such as essential hypertension.

It can be hypothesized that IL-23-driven inflammatory mechanisms may become dominant in sustaining Th17 activity, potentially overriding or compensating for miR-326 regulatory role. However, this hypothesis was not directly investigated in the present study and requires experimental validation.

The significant positive correlations between miR-155 expression, age and BMI observed in this study are clinically relevant. Advancing age is associated with heightened oxidative stress and low-grade inflammation, while adipose tissue is a recognized source of circulating miR-155 that engages macrophage polarization and sustains pro-inflammatory signaling.[28]

The downregulation of miR-326 expression and its negative correlation with BMI indicate that metabolic factors, particularly obesity-related immune dysregulation, may modulate miR-326 expression in a dependent manner. As knowing miRNA secretion from the growing adipose tissue which is one of the sources of circulating miRNAs and vary significantly as obesity develops.[29,30]

There is no correlation between miRNA and the measured cytokine, despite their concurrent dysregulation in patients with essential hypertension. Therefore, the present findings do not provide direct evidence of a mechanistic interaction between these biomarkers. There is no evidence of a molecular cascade in this study. Further studies are required to clarify whether selected biomarkers interact mechanistically or reflect independent aspects of hypertensive inflammation.

The multivariable logistic regression analysis provided additional support for the observed associations. when adjustment for age and BMI, miR-155, miR-326, IL-17A, and IL-23 remained significantly associated with essential hypertension, suggesting that these associations may not fully explain the differences in age or BMI between the study groups. Although, obesity is recognized as a low-grade chronic inflammatory condition may contribute to the inflammatory milieu associated with hypertension, capable of promoting Th17-cell activation and improving the production of pro-inflammatory cytokines, including IL-17.[31–33]

The simultaneous evaluation of circulating miRNAs and inflammatory cytokines offers a much more thorough insight into the nature of the inflammatory landscape in hypertension than the measurement of isolated markers. In silico target prediction was performed using publicly available databases (miRDB and TargetScan). The analysis identified SOCS1 as a potential target of miR-155 and Ets-1 as a potential target of both miR-155 and miR-326. The findings of present study generate hypotheses regarding possible regulatory pathways but do not provide experimental evidence.

Despite the high AUC value found through ROC analysis, the results should be interpreted cautiously not conclusive for diagnosis because of the small number limits the generalizability of the results, and the

observed exploratory discriminatory performance cannot be considered definitive evidence of diagnostic utility, although further studies on a larger scale are necessary.

Obesity and treatment status should also be considered when interpreting the present findings and may independently influence circulating cytokine concentrations and miRNA expression profiles.[34] The high prevalence of untreated disease (68.0%) in our cohort and detailed information regarding antihypertensive medication classes and treatment duration was unavailable, likely contributed to a persistently activated inflammatory milieu, further amplifying these cytokine levels. Likewise, antihypertensive medications have been reported to modulate inflammatory pathways and immune responses.[35] Therefore, the observed biomarker alterations may have been partially influenced by these factors, and future investigations with larger study populations and multivariable analyses are needed to further clarify their independent effects.

Study limitations

Several limitations in the present study. The sample size may insufficient to detect possible correlations between the expressions of miRNA and inflammatory cytokines levels. The single-center design generalizability to populations with different demographic and clinical characteristics. No formal adjustment for multiple comparisons was applied. In addition, multiple statistical comparisons across several biomarkers and correlation analyses may increase the risk of type I error. Findings of this study should be interpreted cautiously until confirmed in larger, independent investigation. Therefore, these findings suggest potential discriminatory value; however, validation in larger independent cohorts is required. It would be premature to consider diagnostic efficacy as per ROC analysis as definitive. Moreover, without any target gene validation for the specific miRNA, the study cannot prove its mechanism directly for example, SOCS1 is the target of miR-155. Future longitudinal and functional studies are needed to assess gene expression together with cytokine levels and miRNA profiling.

The use of a single housekeeping gene (GAPDH) for normalization, which may not fully comply with MIQE recommendations; however, its stable expression across study groups was confirmed by comparable Ct values. The multivariable regression models were adjusted only for age and BMI. Additional factors, including antihypertensive treatment characteristics and family history of hypertension, were not involved in the regression models and residual confounding cannot be completely excluded. Therefore, these factors should be considered in future studies.

Conclusion

Patients with essential hypertension showed significant upregulation of miR-155, IL-17A, and IL-23, along with downregulation of miR-326 compared with normotensive control. Although these biomarkers were significantly dysregulated, no direct correlation was identified between miRNA expression and cytokine concentrations. The present finding suggests that these biomarkers may perform distinct aspects of the

hypertension-associated inflammation rather than a confirmed molecular pathway. The findings should be regarded preliminary, require validation in larger, independent, preferably prospective cohorts before their potential clinical relevance can be established.

Acknowledgments

The authors extend their sincere gratitude to everyone who supported this research. We thank the College of Medicine, University of Al-Qadisiyah, and administrative team at Al-Diwaniyah Teaching Hospital for their unconditional support in carrying out this research.

Declarations

Funding

No external funding in this research.

Author contributions

Conceptualization, F.J.A. and M.M.K.; Methodology, F.J.A. and M.M.K.; Software, M.M.K.; Validation, F.J.A. and M.M.K.; Formal Analysis, M.M.K.; Investigation, F.J.A. and M.M.K.; Resources, F.J.A.; Data Curation, F.J.A. and M.M.K.; Writing – Original Draft Preparation, F.J.A. and M.M.K.; Writing – Review & Editing, M.M.K. and F.J.A.; Visualization, F.J.A.; Supervision, M.M.K.; Project Administration, M.M.K..

Conflicts of interest

The authors have no competing interests to declare in relation to this study.

Data availability

Data and material analyzed during the current study can be available by contacting the corresponding author upon reasonable requests.

Ethics approval

The research followed the ethical guidelines of the College of Medicine at the University of Al-Qadisiyah, in according with the Declaration of Helsinki (Approval Number 41, Date 20/10/2025).

Use of AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used AI to improve the language, grammar.

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