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Original Article 1

Expression of miR-15a-5p, miR-17-5p, and miR-103-3p in hypertension-associated chronic kidney disease and hemodialysis patients

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Background
MicroRNAs (miRs), which can be released into the extracellular environment, are important modulators of how cells respond to various stimuli. Prior research over the past decade has focused on miRs as new clinical targets or diagnostic tools. Numerous studies on chronic kidney disease (CKD) have focused on miRs. One prevalent risk factor for CKD is hypertension (HTN), which damages targeted organs, including the kidneys, heart, and arteries.
Objective
Our goal was to investigate the expressions of miRs (17-5p, 15a-5p, and 103-3p) in prerenal failure and renal failure patients under hypertension conditions, as these could be biomarkers of kidney damage caused by hypertension.
Patients and methods
Quantitative real-time polymerase chain reaction (RT-PCR) was utilized to assess the expression levels of the above-mentioned miRs. A total of 110 studied cases that met the eligibility criteria of HTN-associated CKD were grouped into a pre-renal failure group (n = 50) and a renal failure group (n = 60), which included end-stage renal disease cases under hemodialysis beside 40 healthy controls.
Results and conclusion
Our research revealed that miR-103-3p was upregulated; however, miRs (15a-5p and 17-5p) were significantly downregulated in the studied HTN-CKD groups compared with control subjects. The degree of deregulation correlated with the severity of the disease suggests that these miRs may be involved in the pathophysiology of HTN-related CKD, which in turn encourages additional research into their possible clinical use in the prevention and management of CKD. Moreover, these miRs showed areas under the curve near 1 in receiver operating characteristics (ROC) analysis. Consequently, they might be used as markers for CKD.
Keywords: chronic kidney disease (CKD), hypertension (HTN), microRNAs (miRs), miR-103-3p, miR-15a-5p, miR-17-5p.

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Introduction

Chronic kidney disease (CKD) encompasses all levels of loss in kidney function, ranging from mild to severe chronic renal failure [1]. CKD is defined as a progressive decline in renal function that eventually results in kidney impairment or a glomerular filtration rate (GFR) of less than 60 ml/min per 1.73 m^2 for more than three months[2]. One of the most prevalent primary conditions that leads to CKD and eventually end-stage renal disease (ESRD) is hypertension (HTN)[3]. There is an epidemiological relationship between HTN and CKD. The prevalence of HTN has been reported to be greater than 85% in stage 3 and above 90% are in stage 4 and stage 5 CKD patients [4]. Most patients with mild to moderate CKD have no obvious clinical symptoms, so it is difficult to diagnose and as the disease progresses further it may develop into ESRD, which may be irreversible[5]. Albuminuria and GFR are the primary criteria used to diagnose and stage CKD; these tools, however, are mostly capable of diagnosing CKD after renal damage has occurred. They merely offer a limited amount of information regarding kidney disease activities and do not offer any etiological clues [6]; therefore, in order to

lower CKD-related mortality, earlier CKD diagnosis and therapy may be more appropriate. Thus, a deeper insight into the mechanisms behind the onset and progression of CKD is required. Recently, many microRNA (miRs) molecules have been discovered that play an essential role in the pathogenesis of many diseases. Investigating miRs as biomarkers for CKD has emerged as a fascinating area of research [7]. MiRs are defined as short non-coding RNAs that bind to the 3′ untranslated regions of their target mRNAs, causing translational repression and mRNA degradation. They are important modulators of gene expression [8],which play a very important and extensive role in a number of cellular regulatory processes, including differentiation, proliferation, development, and apoptosis [9]. They are also involved in the development and proper operation of the kidneys, including the maintenance of fluid, electrolyte, acid-base, and blood pressure [10]. In many biological fluids, these RNA species have longer half-lives and are more stable, such as urine, plasma, serum, and saliva [11]. Therefore, miRs dysregulation may result in cell dysfunction and development of CKD[12-15]. Previous investigations also showed changes in miR expression in the onset and progression of hypertensive nephropathy [16–20]. Prior study revealed that the miR-103 expressions were increased in the plasma of hypertension cases relative to that of normal individuals [18]. A previous study conducted in the United States of America revealed dysregulation of miR-15a-5P and miR-17-5p in hypertensive patients with CKD [21].The role of miRs (103-3p, 15a-5p, and 17-5p) in HTN associated with CKD needs more investigation in different populations. Hence, the objective of this study is to assess the levels of miR-15a-5p, miR-103-3p, and miR-17-5p in Egyptian CKD patients associated HTN and to evaluate the effect of these miRs levels in the progress of ESRD,which should assist shed light on how ESRD develops and establish a relationship with kidney function and HTN.

Subjects and methods

Study population

The EL-Edwa Hospital, Minia, Egypt, accepted the study protocol and each participant gave their informed consent. The subjects were assigned into three groups according to kidney function test and estimated glomerular filtration rate (eGFR) [22]. Group 1 (control group) consisted of forty healthy volunteers with an age range of 45 to 63 years; all of them had no relevant medical history and were not taking any medication on a regular basis, with an eGFR > 90 mL/min, serum creatinine up to 1.3 mg/dL, and normal blood pressure (BP). Group II (pre-renal failure group) included fifty hypertensive patients with chronic kidney disease (CKD), age range 43-62 years, untreated newly diagnosed hypertension, with 15 < eGFR < 60 mL/min, and serum creatinine ranged from 1.3 to 3 mg/dL. Group III (renal failure group): sixty patients (age range 47-65 years) with end-stage renal disease (ESRD) with a long history of chronic hypertension, eGFR < 15 mL/min, and creatinine > 4.0 mg/dL. All had received hemodialysis therapy for at least 6 months, three times a week for four hours per session. Hypertension was defined according to the European Society of Hypertension clinical practice guidelines [23]. Patients with co-morbid conditions including liver disease, autoimmune diseases, or malignancies, as well as patients with renal disease due to pathologies other than hypertension, excessive smoking, and relevant medications (anti-hypertensive drugs), were excluded from the study. Treatments with corticosteroids, cytotoxic medicines, or non-steroidal anti-inflammatory drugs at the time of the study were additional exclusion factors.

Sample collection and measurements

Samples of blood were taken under fasting conditions from every participant in the morning. Blood was collected in vacutainer EDTA for plasma isolation plus serum tubes for blood biochemistry determined by routine methods. At room temperature, the blood samples were separated at 3000 rpm for ten minutes. The plasma was gathered and kept at -80 °C until analysis. Serum creatinine level, age, and sex were utilized to assess the eGFR. Individuals were diagnosed with CKD if their eGFR was less than 60 mL/min/1.73 m^2.

Molecular analysis

The quantitative real-time PCR (qPCR) method was used to evaluate the chosen miRs. We selected three miRs (miR-103-3p, miR-15a-5p and miR-17-5p) for investigation according to involvement in HTN-CKD-related pathways as reported in pertinent literature. Total RNA was extracted from the plasma after the samples were thawed at room temperature using the miRNeasy Plasma Advanced Kit (Qiagen Inc., Germantown, Maryland, USA) according to the manufacturer's instructions. The extracted RNA concentrations were measured by reading the absorbance at 260 nm using Thermo Fisher Scientific Inc., Walthman, MA, USA NanoDrop 2000c spectrophotometer [24]. MiRs were reverse-transcribed to complementary DNA using cDNA Synthesis kit (Applied Biosystems, Foster, California, USA). MiRs expressions were determined via specific primers illustrated in Table 1 [25-27] and the cDNA product was used as a template for the detection of miR expression by using PCR master mix (SYBR green) and utilizing an applied biosystems, USA, real-time PCR System 2700. A conserved gene U6 served as the internal standard. Data are shown as fold changes ($2^{-\Delta\Delta Ct}$) [28].

Table 1 Primers for reverse transcriptase polymerase chain reaction (RT-PCR).

MiRNAs	Primer sequences	References
MiRNA-15a-5p	Forward:5'-ACACTCCAGCTGGGTAGCAG CACATAATGGTTTGT-3' Reverse:5'-CTCAACTGG TGTCGTGGAGTCGGCAATTCAGTTGAGCACAAAC-3'	[25]
MiRNA-17-5p	Forward:5'-TGCGCCAAAGTGCTTACAGTGCA-3' Reverse:5'- CCAGTGCAGGGTCCGAGGTATT-3'	[26]
MiRNA-103-3p	Forward:5'-ACACTCCAGCTGGGAGCAGCATTGTAC-3' Reverse:5'-TGGTGTCGTGGAGTCG-3'	[27]

Statistical analysis

All measured variables' distributions were checked for normality. One-way analysis of variance and the student's test were used for continuous values with a normal distribution, and the variables were displayed as mean ± standard deviation (SD). Pearson's correlation analysis was performed to assess correlation coefficients (r). Furthermore, the specificity and sensitivity of the regression models that included the related miRs were evaluated using receiver operating characteristics (ROC). SPSS version 27.0 (SPSSInc, Chicago, IL, USA) was used for all tests. A *p*-value of less than 0.05 was regarded as significant.

Results

The clinical and demographical features of the studied subjects are summarized in Table 2. The participant's ages ranged from 43 to 65 years. There was no significant difference in age (P = 0.123) or sex (P = 0.67) between control, prerenal failure (PRF), and renal failure (RF) groups. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly elevated in CKD patients than controls. Likewise, serum creatinine and urea were the highest in RF cases (for creatinine: RF cases = 8.30 ± 2.57 mg/dL; PRF cases = 2.01 ± 0.42 mg/dL; controls = 0.80 ± 0.12 mg/dL, p< 0.001) (for urea: RF cases = 144.2 ± 32.99 mg/dL; PRF cases = 77.66 ± 26.85 mg/dL; controls = 23.63± 3.53 mg/dL, p< 0.001). As expected, the mean eGFR was significantly lower in the RF group (7.53±2.52 ml/min/1.73 m^2) than PRF cases (38.14±10.78 ml/min/1.73 m^2) and control groups (105.78±16.93 ml/min/1.73 m^2) (p< 0.001). Similarly, significant differences were observed between the studied groups with respect to hemoglobin (Hb) (P = 0.01), and the level of electrolytes was relatively similar across the groups. As shown in Tables 3 & 4 and Figures 1 & 2, the plasma of hypertensive patientsin the study groups contains higher levels of miR-103-3p than those found in healthy individuals; its expression in PRF and RF cases increased 1.397-fold and 2.831-fold, respectively, compared to controls (p< 0.001). Alternatively, miR-15a-5p and miR-17-5p are characterized by lower expression levels in CKD cases. There was a 0.722-fold and 1.412-fold decrease in the expression of miR-17 -5p in PRF & RF cases, respectively (p<0.001). We also reported a 0.974-fold lower level of miR-15a-5p expression in the RF group (p < 0.001) than the PRF group (log2 fold change = -0.482, p< 0.001). Correlations between the studied plasma miRs and clinical parameters in the studied groups were evaluated and represented in Table 5 and Figures 3 & 4. Correlation analysis showed that miR-103-3p was positively linked to SBP, serum creatinine, and urea levels and a corresponding negative correlation with eGFR in PRF&RF groups. In contrast, levels of miR-15a-5P and miR-17-5p were negatively related to SBP, serum creatinine, and urea levels, and a significant positive correlation was obtained with eGFR. No other significant relationship was found among the studied miRs and other clinical parameters. The diagnostic utility of the chosen miRs for the CKD and control groups was then assessed using receiver operating characteristic (ROC) curve analyses. Regarding the PRF group, the area under curves (AUCs) which ranged from 0.783 to 0.898 was displayed in Figure 5 and Table 6. The diagnostic effect of the combined panel of the three miRs was then assessed using the frequency table and ROC curves. The 3-miR panel's ROC curve showed a high degree of diagnostic accuracy. (AUC, 0.978; 95% CI, 0.952–1.00; p< 0.001), which was superior to that of most of the single miRs. Concerning the RF group, as can be observed in Figure 6 and Table 7, the AUCs ranged from 0.893 to 0.995, and the ROC curve for the 3-miR panel had the highest AUC (AUC, 1.00; 95% CI, 1.00–1.00; p<0.001). These findings imply that these miRs are powerful diagnostic indicators for CKD patients with hypertension.

Table 2 Demographic, clinic and laboratory characteristics among the studied groups.

Variables	**Controls(n=40)**	**PRF group (n=50)**	**RF group (n=60)**	***P* value**
age (years)	50.15± 3.93	52.04 ± 4.17	55.52± 4.61	0.123
Sex (male/female)	30 /10	38 /12	47 /13	0.67
SBP(mm Hg)	116.91±7.11	142.26±15.34	140.83±16.16	0.01
DBP(mm Hg)	78.11±3.77	91.66±7.56	90.93±8.31	0.01
Creatinine (mg / dL)	0.80±0.12	2.01±0.42	8.30±2.57	<0.001
Urea (mg / dL)	23.63±3.53	77.66±26.85	144.2±32.99	<0.001
eGFR (ml /min / 1.73 m^2)	105.78±16.93	38.14±10.78	7.53±2.52	<0.001
Ca^{++}(mmol/L)	1.18±0.05	1.17±0.07	1.15±0.07	0.06
Na^{+}(mmol/L)	141.54±4.12	140.84±7.01	136.37±4.53	0.12
k^{+}(mmol/L)	4.23±0.41	4.27±0.54	5.12±0.83	0.08
Hb(g/dL)	13.01±0.79	12.55±1.18	9.05±1.97	0.01

Data represented as mean ± standard deviation (SD); PRF: Prerenal failure; RF: Renal failure; SBP: systolic blood pressure; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; Hb: hemoglobin.

Table 3 The relative expression for studied miRs of the population recruited.

Variables	**Controls(n=40)**		**PRF group(n=50)**		**RF group(n=60)**		*P* value
	M ± SD	**95 % CI**	**M ± SD**	**95 % CI**	**M ± SD**	**95 % CI**	
miR-103-3p	1.23±0.75	0.99-1.46	3.22±0.28	2.86-3.59	8.72±0.26	7.87-9.56	<0.01
miR-15a-5p	1.13±0.26	1.05-1.21	0.81±0.33	0.72-0.90	0.58±0.19	0.53-0.62	<0.01
miR-17-5p	1.27±0.44	1.13-1.41	0.77±0.34	0.67-0.87	0.48±0.21	0.42-0.53	<0.01

M ± SD: mean ± standard deviation; 95% CI: 95%; confidence interval; PRF: Prerenal failure; RF: Renal failure.

Table 4 The fold change for the expression levels of prerenal and renal failure groups.

Variables	**PRF group (n=50)**			**RF group (n=60)**		
	log2 FC	**expression change**	***P* value**	**log2 FC**	**expression change**	***P* value**
miR- 15a-5p	-0.482	down	<0.001	-0.974	Down	<0.001
miR- 17-5p	-0.722	down	<0.001	-1.412	Down	<0.001
miR- 103-3p	1.397	up	<0.001	2.8314	Up	<0.001

FC; fold change; PRF: Prerenal failure; RF: Renal failure.

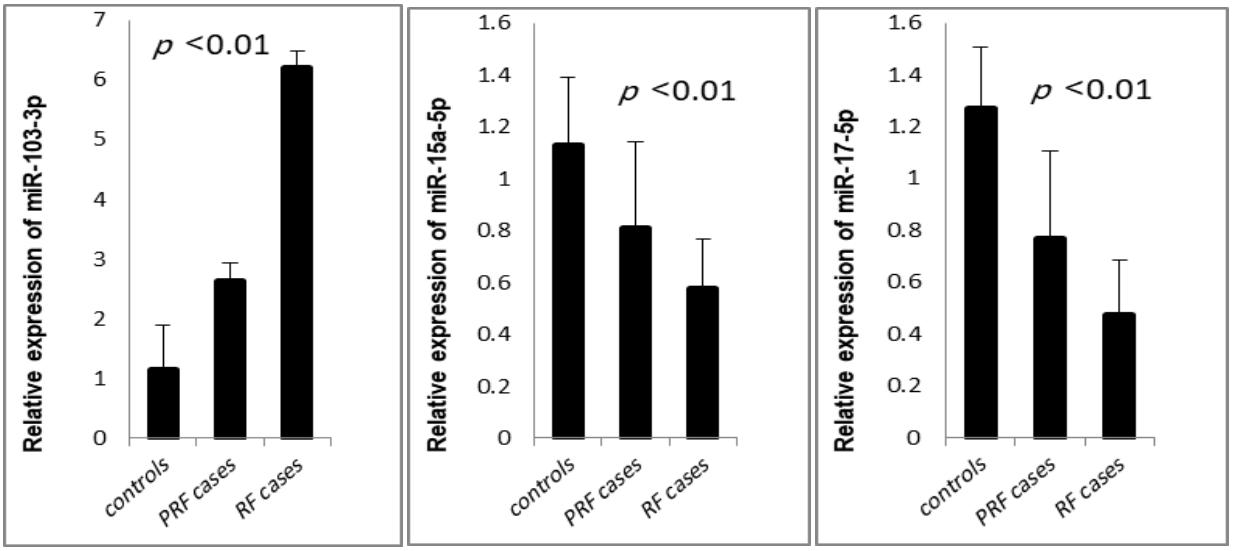

Fig. 1. Relative expression of evaluatedmicroRNAs (miRs) in the studied groups: prerenal failure (PRF) andrenal failure (RF).

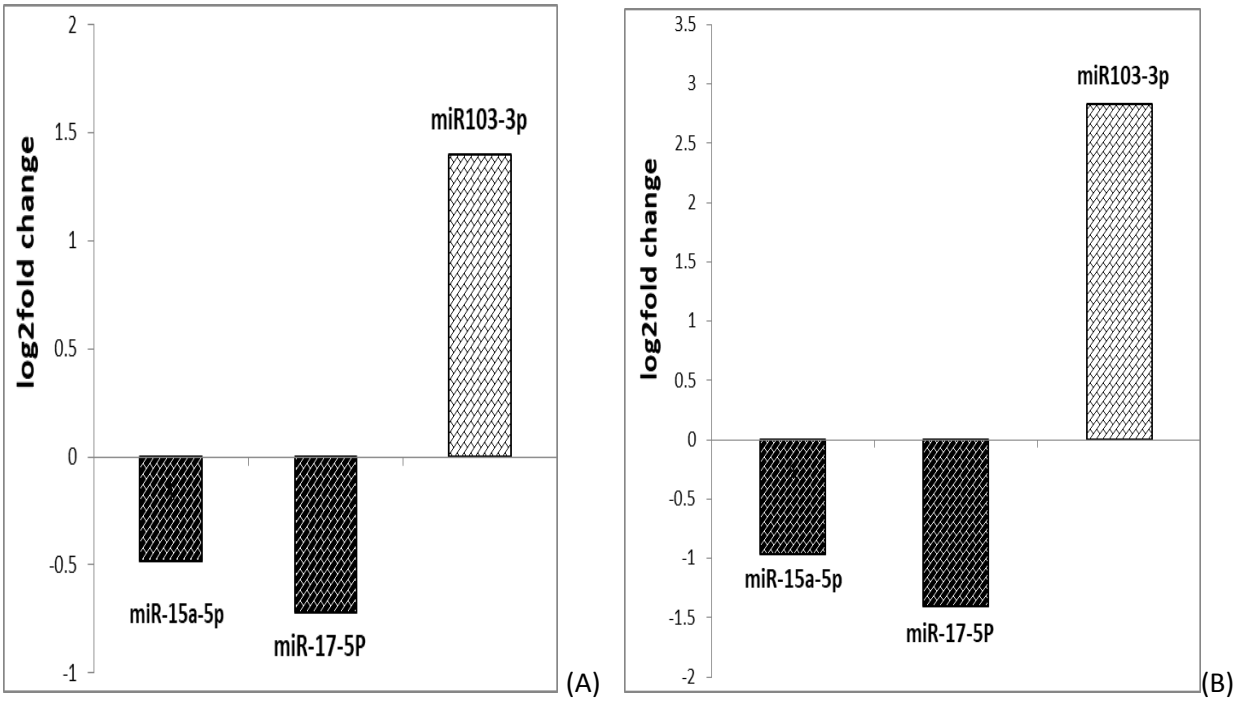

Fig. 2. The fold change for the relative expressions inthe studied groups; (A) for prerenal failure (PRF) cases and (B) for renal failure (RF) cases.

Table 5. Correlation analysis between the expression of the three studied plasma miRs and clinical parameters in the studied groups using Pearson's correlation analysis.

	Pre renal failure group n= 50						**Renal failure group n= 60**					
variables	**miR-15a-5p**		**miR-17-5p**		**miR-103-3p**		**miR-15a-5p**		**miR-17-5p**		**miR-103-3p**	
	r	***p*-value**	***r***	***p*-value**	***r***	***p*-value**	***r***	***p*-value**	***r***	***p*-value**	***r***	***p*-value**
Age	**-0.174**	**0.534**	**-0.238**	**0.311**	**0.23**	**0.153**	**-0.138**	**0.198**	**-0.109**	**0.334**	**0.089**	**0.498**
SBP	-0.602	<0.001	-0.611	<0.001	0.505	0.001	-0.539	<0.001	-0.57	<0.001	0.501	0.001
DBP	-0.205	0.152	-0.102	0.211	0.304	0.06	-0.102	0.09	-0.136	0.074	0.208	0.07
Hb	0.328	0.069	0.249	0.121	-0.125	0.09	0.037	0.778	0.046	0.727	-0.112	0.395
Creatinine	-0.593	< 0.001	-0.695	<0.001	0.714	<0.001	-0.84	<0.001	-0.868	<0.001	0.526	0.005
Urea	-0.464	0.001	-0.626	<0.001	0.588	<0.001	-0.763	<0.001	-0.819	<0.001	0.643	0.030
eGFR	0.443	0.001	0.551	<0.001	-0.681	<0.001	0.808	<0.001	0.778	<0.001	-0.632	<0.001
Na^+	-0.156	0.113	-0.199	0.811	0.272	0.09	-0.107	0.958	-0.135	0.304	0.172	0.586
K^+	-0.245	0.128	-0.218	0.177	0.084	0.606	-0.169	0.196	-0.122	0.869	0.151	0.701
Ca^{++}	0.212	0.08	0.268	0.095	-0.29	0.069	0.102	0.437	0.061	0.644	-0.146	0.727

r: correlation coefficient;eGFR: estimated glomerular filtration rate SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 6. Receiver operating characteristic (ROC)curve analysis of plasma miRs assessed in prerenal failure patients

Variable(s)	**AUC**	**SE**	***P* value**	**95% CI**	
				Lower Bound	**Upper Bound**
miR-17-5p	0.783	0.051	< 0.001	0.683	0.882
miR-15a-5p	0.842	0.041	< 0.001	0.763	0.922
miR-103-3p	0.898	0.033	< 0.001	0.832	0.963
miR-Panel	0.978	0.013	< 0.001	0.952	1.004

ROC: receiver operating characteristic; AUC: area under curve; SE: standard error; CI: confidence interval.

Table 7 Receiver operating characteristic(ROC) curve analysis of plasma miRs estimated in renal failure group.

Variable(s)	**AUC**	**SE**	***P* value**	**95% CI**	
				Lower Bound	**Upper Bound**
miR-17-5p	0.971	0.015	< 0.001	0.942	1.001
miR-15a-5p	0.893	0.033	< 0.001	0.828	0.958
miR-103-3p	0.995	0.004	< 0.001	0.987	1.003
miR-Panel	1.00	0.00	< 0.001	1.00	1.00

ROC: Receiver operating characteristic; AUC: area under curve; SE: standard error; CI: confidence interval.

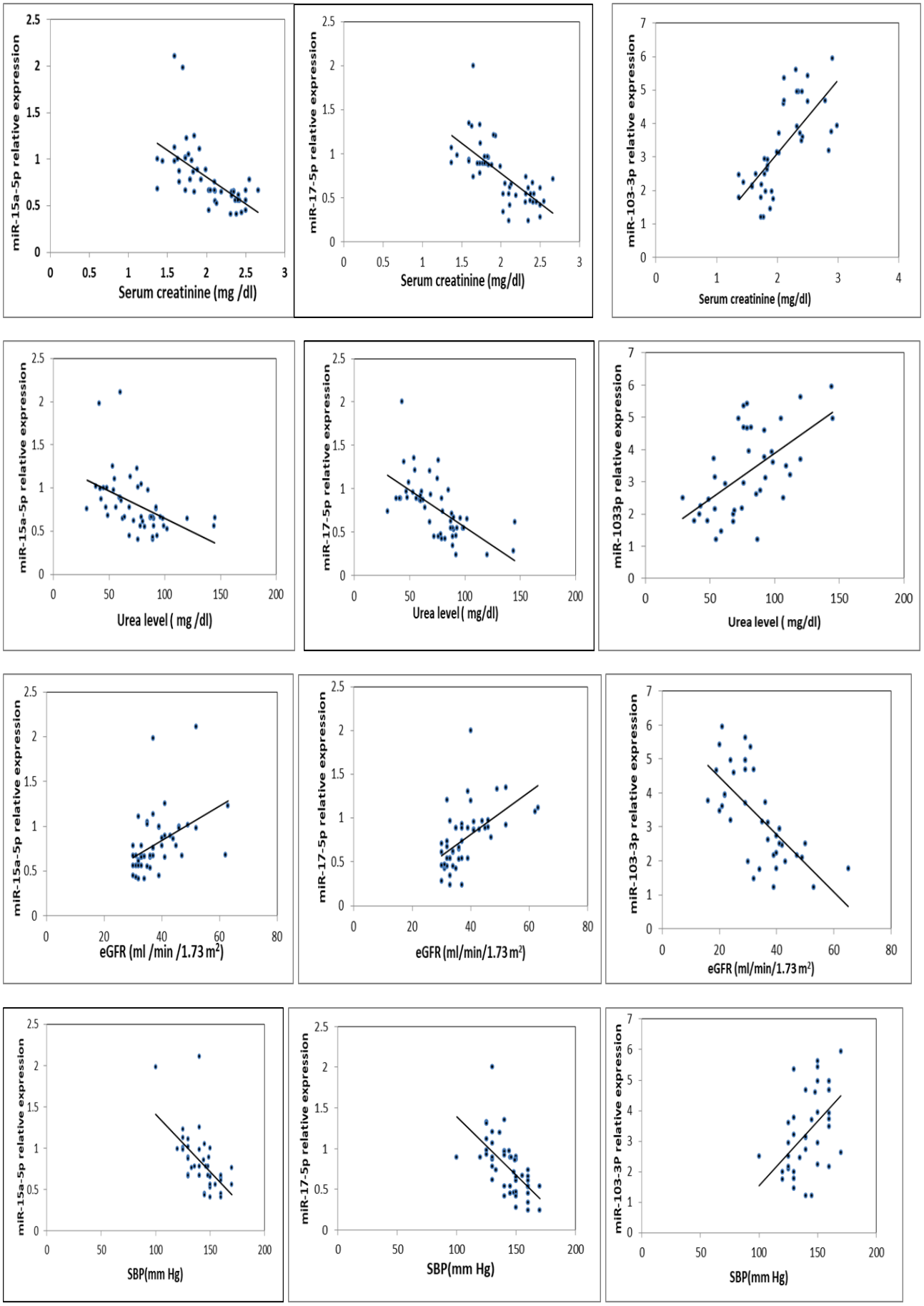

Fig. 3. Significant correlations between three measured plasma microRNAs (miRs) and clinical parameters in prerenal failure (PRF) cases.

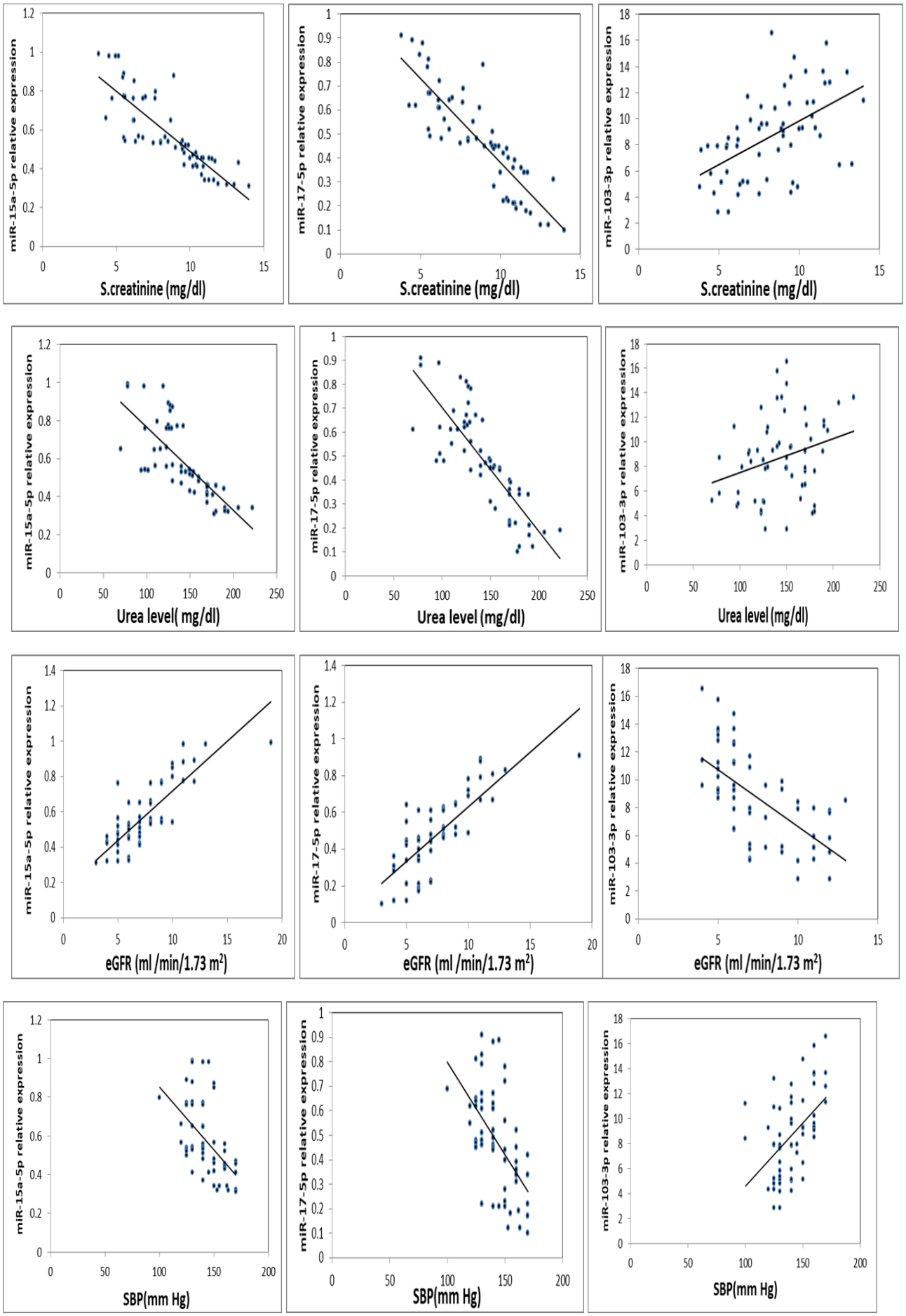

Fig. 4. Significant correlations between the plasma microRNAs (miRs) and clinical parameters in renal failure (RF) cases.

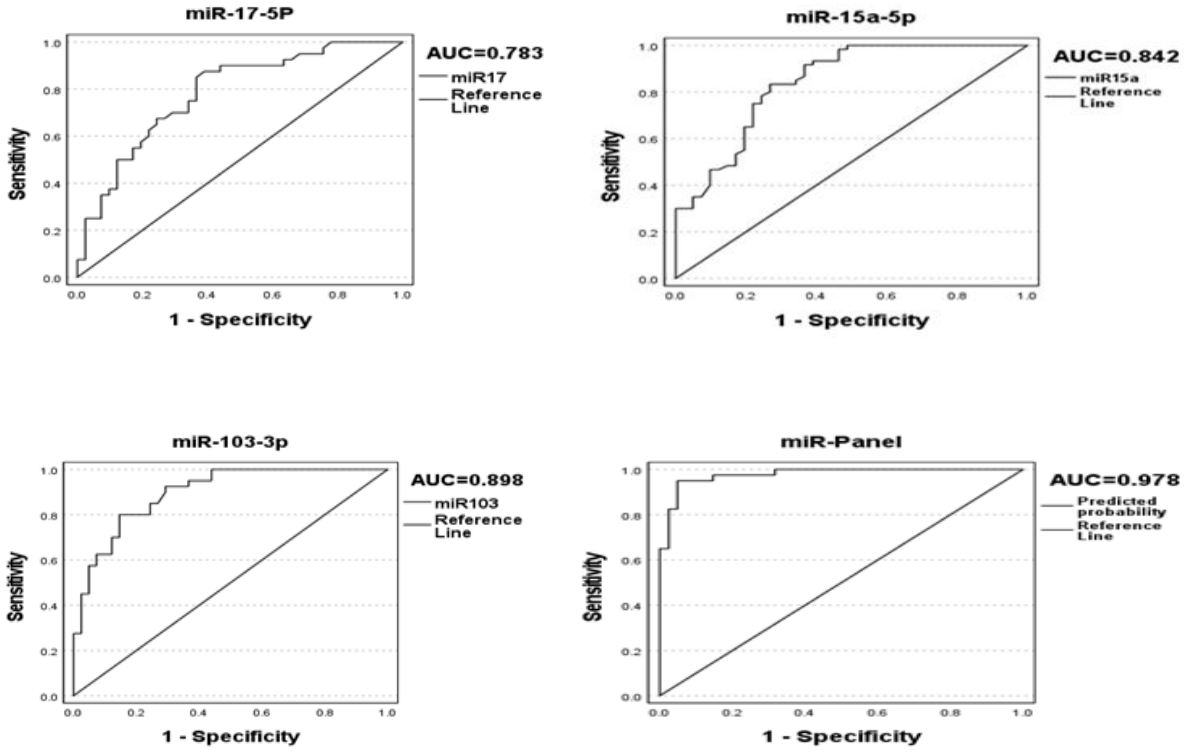

Fig. 5. Receiver operating curve (ROC) of the three microRNAs (miRs) investigated in patients with prerenal failure (PRF) compared to controls.

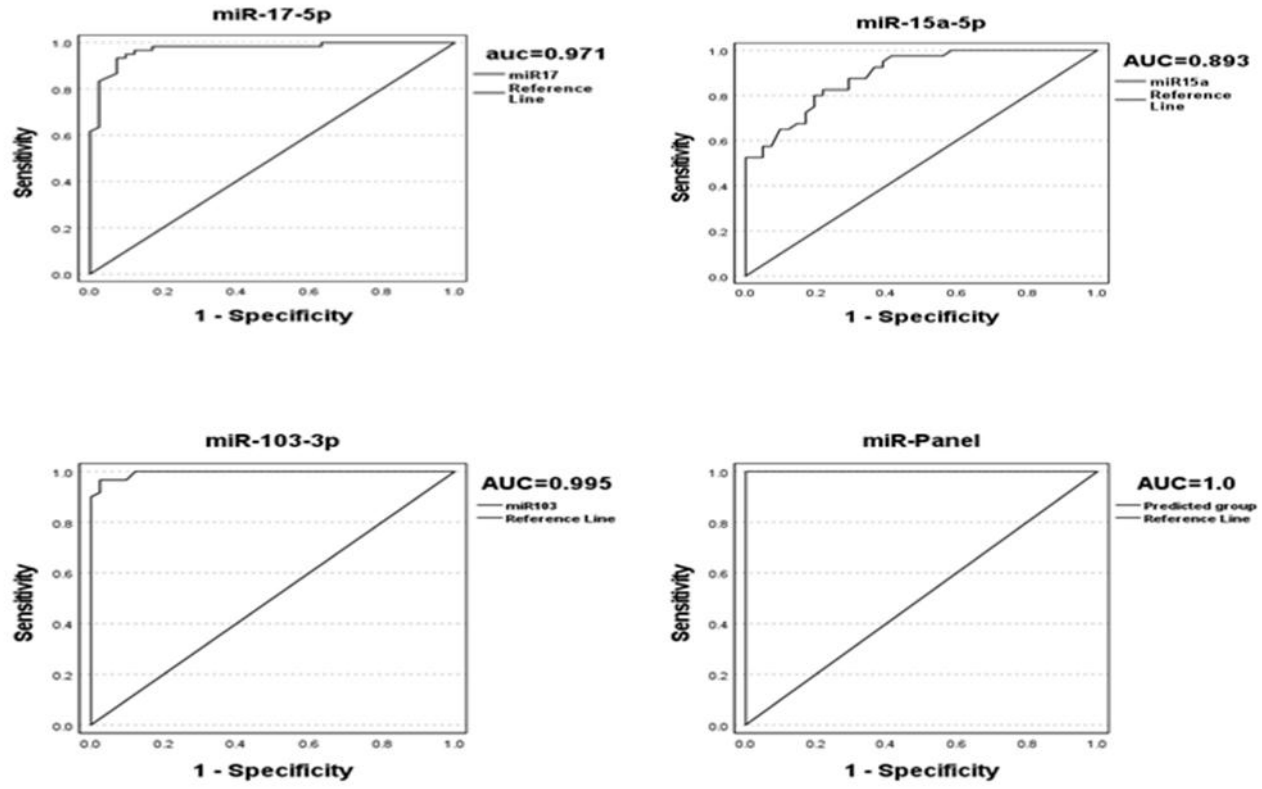

Fig. 6. Receiver operating characteristic (ROC) curve analysis of microRNAs (miRs) in renal failure (RF) cases versus controls.

Discussion

It has been reported that miRNAs (miRs) are crucial molecular modulators. One miR molecule is able to control up to 200 distinct mRNA targets. Therefore, these non-coding RNAs play an important role in the regulation of many physiological and pathological cellular processes [29]. Recently, some studies have reported dysregulated patterns of miRs in chronic kidney disease (CKD) in patients with hypertension (HTN); these miRs could be employed as possible biomarkers and may have a role in the pathophysiology of CKD[30-32]. Findings of this study are that three plasma miRs (miR-15a-5p, miR-103-3p, and miR-17-5p) were significantly desregulated in hypertensive mild CKD individuals and patients with end-stage renal disease (ESRD). Our data displayed that miR-17-5p was significantly downregulated among CKD cases. This circulating miR belongs to a cluster of miRs located in intron 3 of C13orf25 at chromosome 13 [33]. This cluster plays a distinct role in the differentiation and maturation of immune cells [33]. MiR-15a-5p was also significantly downregulated in the studied cases; in contrast, miR-103-3p showed a significant elevation in cases compared to healthy individuals. The identified miR-15a-5p and miR-103-3p belong to the miR-15/107 group, which is widely expressed in many different mammalian tissues [34]. The biological functions of miRs from this group are involved in cell metabolism and division, angiogenesis, and stress response, assuming that deregulation of these miRs is a hallmark of numerous disease states [35-37]. We found in our study that expression levels of miR-15a-5p and miR-17-5p significantly decreased in ESRD cases than mild CKD individuals. We analyzed the link between eGFR and the expressions of miRs (15a-5p and 17-5p), and we observed that the expression levels of both miRs (15a-5p and 17-5p) decreased as kidney function deteriorated among the studied groups [38]. The decreased level of miRs may be due to their degradation by circulating RNase [39].In the present study, a negative correlation between SBP and these two miRs was detected in the studied groups. In the same line with our study, previous investigations have shown that expression of these miRs is critical for immune cell function and demonstrated that the expression of miR-17 plays an important role in the production of T cell survival [40,41], while the expression of miR-15 can enhance the induction of regulatory T cells[42].Likewise, the components of the immune system have a direct impact on the release of inflammatory cytokines and reactive oxygen species in hypertensive conditions [43–47], which are the main contributors to the deterioration of renal function, which leads to progressive kidney injury [48–50]. The reduction in expression of miRs (15a-5p and 17-5p) in HTN-CKD cases induced immune dysfunction that is well established among end-stage renal disease patients [51]. Thus, downregulation of these miRs in the context of hypertension may lead to the onset of CKD and have consequences for the development of CKD or its complications[21].Moreover, another study consistent with our finding illustrated that plasma miR-15b levels in hemodialysis patients were 2-fold lower compared to non-CKD, and the author referred to the lower level of miR-15b as being linked to the development of ESRD by modifying phosphate metabolism-related genes[52]. Also, there have been reports of associations between acute kidney injury (AKI) in human and animal models and the levels of miR-17 and miR-15 b within kidney tissues [53].On contrast, we found in the current study upregulation of miR-103-3p in mild HTN-CKD and ESRD patients; this upregulation was comparable to a study by Lu et al. that showed that, in comparison to healthy controls, hypertensive mice infused with angiotensin II hormone and patients with hypertensive nephropathy had overexpression of miR-103a3p in both serum and urine[18]. Our data also showed a positive correlation of miR-103-3p with SBP. According to our results, some previous studies reported that angiotensin-II levels are elevated in hypertension conditions; this hormone has pro-inflammatory and vasoconstrictive effects on post-glomerular arteries, resulting in kidney fibrosis and glomerular damage [54,55] and this renal injury is characterized by increased renal inflammation, glomerular fibrosis, extracellular matrix deposition, and glomerular protein filtration [56,57]. Lu and colleagues conducted additional studies to investigate the function of miR-103a-3p in angiotensin II-induced renal damage. They found that anti-miR-103a-3p knockdown decreased the effects of angiotensin II on the kidney, while overexpression caused kidney inflammation, kidney fibrosis, and albuminuria in animal models [18]. A target of miR-103a-3p, sucrose non-fermentable-related serine/threonine-protein kinase (SNRK), is expressed less when miR-103a-3p is present. SNKR has the ability to reduce inflammation. Mice with renal damage caused by angiotensin II also had lower SNRK levels; therefore,in contradiction to our finding, Lu et al. discovered that under hypertensive conditions, the levels of angiotensin-II and miR-103 were elevated and the expression of SNRK was decreased compared with healthy kidneys, and these conditions accelerated the progression of CKD [18]. Finally, depending on the estimated cutoff values of the area under the curve (AUC) of the receiver operating characteristic

(ROC) curve, all three miRs had an acceptable ability to predict CKD but miR-103-3p was excellent diagnostic biomarkers for mild HTN-CKD and ESRD cases, which showed a high predictive value however, the prediction model containing three tested miRs offered an additional advantage in predicting CKD in two studied groups. Our results were in consistent with studies by Garmaa et al that referred to the fact that miRs can be used as effective diagnostic markers for CKD and the use of a panel of miRs allowed more accurate diagnosis than relying on a single miR [58] .

In addition, as all samples in the study were obtained from clinical patients, we suspect that these miRNAs may be more relevant than those that have been identified in animal models, and may therefore be better able to elucidate the putative function of the miRNAs assessed in chronic kidney disease. However, the limitations of the study include the small sample size and the need to further validate the findings in a large patient cohort.

Conclusion

The current study demonstrated that miRNA-103-3p was up-regulated while miRNA-15a-5p and miRNA-17-5p were down-regulated in studied hypertension (HTN) associatedchronic kidney disease (CKD) cases. The dysregulation of the mentioned miRNAs was positively associated with prevalent chronic kidney disease in hypertensive individuals and associated with the development of end-stage renal disease (ESRD). Furthermore, we showed that each miRNA expression level was useful for predicting chronic kidney disease (CKD) under hypertension conditions. Thus, these miRNAs could develop into useful non-invasive biomarkers for hypertension (HTN) - chronic kidney disease (CKD); however, more thorough and extensive research is still required to determine the precise connection between miRNAs and hypertension relatedchronic kidney disease.

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Authors' contributions

Concepts and Design: A.A., N.S.K., M.D.E.A., and W.G.; Definition of intellectual content: M.G.; literature search, clinical studies, and data acquisition: H.N., W.G., and D.A.; data analysis and statistical analysis: H.N.; manuscript preparation: H.N. and W.G.; manuscript editing and review: N.S.K., M.D.E.A., A.A., and W.G.; Guarantor: A.A. and N.S.K.

Conflicts of interest

The authors declare there are no conflicts of interest.

Ethical considerations

This study was performed in line with the principles of the declaration of Helsinki. The study clearance was applied from the Institutional Review Board of the Faculty of Medicine, Minia University (approval No. 213-2022).

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