Circulatinglevelsof inflammation-associatedmiR-155 and endothelial-enriched miR-126 in patients with end-stage renal disease on regular haemodialysis

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Abstract: Objective: The aim of this study is to assess the circulating levels of inflammation-associated miR-155 and endothelial-enriched miR-126 to determine their regulation in children with ESRD, expecting to be able to provide new insights into the development of ESRD. **Subjects & Methods:**In this study we investigated 50 cases which were divided into two groups as Patients group: include 30 ESRD children who are receiving maintenance hemodialysis and Control group: include 20 healthy children without any evidence of CKD or inflammatory disorders. **Results:** the expression of miR-155 and miR-126 were significantly reduced in ESRD patients. Also, but did not significantly differ between pre-HD and post-HD with slight increase in pre-HD (where miRNA126 level quantity mean ± SD pre-HD12.68 ± 0.58 and in post-HD 11.67 ± 1.86 compared to 15.33 ± 0.51 in control group, while miRNA155 level quantity mean ± SD in pre-HD13.77 ± 0.90 and in post-HD 13.19 ± 0.60 compared to 16.44 ± 0.02in control group). Nevertheless, further studies are required for the validation and extension of these results. miRNA126 correlated significantly and positively with sex (being higher in males), while no correlation between miRNA155 and sex. Circulating miR-155 and miR-126 levels correlated positively with eGFR, hemoglobin and CRP, while they correlated negatively with creatinine. Circulating miRNA155 correlated significantly and negatively with calcium, while circulating miRNA155 correlated significantly and negatively with phosphate. **Conclusion:** The expression of miR-155 and miR-126 were significantly reduced in ESRD patients, but did not significantly differ between pre-HD and post-HD. Our data suggested that miR-126 and miR-155 might be useful predictive tools in ESRD. The reduction of circulating miR-126 and miR-155 might be accompanied by a series of clinical symptoms.

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1. Introduction

In the guideline for handling pediatric patients with chronic renal failure it is recommended that when renal function is less than 15ml/min/1.73m^2, renal replacement therapy must be initiated, which includes peritoneal dialysis, hemodialysis and kidney transplant ***(1)*.**

MicroRNAs (miRNAs) are a class of small (~22 nucleotides) non-coding RNAs that regulate gene expression at the post-transcriptional level by either translational repression or messenger RNA degradation ***(2)***.

MiRNAs have been shown to play critical roles in various cellular processes, inflammation, and angiogenesis ***(3)*.** In particular, miR-126 and miR-155 have been reported to control vascular homeostasis, angiogenesis, and vascular repair. In addition, miR-155, an important multifunctional miRNA has been shown to regulate the endothelial inflammatory response and migration mediated by angiotensin α ***(4)*.**

Moreover, accumulating evidence points to an essential role of miRNAs in kidney disease, which is supported by the finding that the depletion of the miRNA-processing enzyme Dicer results in defects in renin cells (juxtaglomerular cells), angiogenesis, and renal morphological integrity and function ***(5)*.**

Specific miRNA expression profiles have been described for diabetic nephropathy, polycystic kidney disease and kidney allograft rejection ***(6)***. Cardiovascular diseases are the leading cause of morbidity and mortality in patients with end-stage renal disease (ESRD) ***(7)***, and this cardiovascular risk cannot be completely elucidated by traditional risk factors. Thus, more emphasis has been placed on non-traditional risk factors such as vascular calcification, inflammation, endothelial dysfunction and oxidative stress, and sympathetic overactivation***(8)*.**

A few specific miRNAs have been implicated in the functional regulation of endothelial cells (ECs), as well as inflammatory responses and peripheral angiogenic signaling ***(9)*.**

Thus, differentially expressed miRNAs are responsible for non-traditional risk factors of chronic kidney disease (CKD). Given that miR-126 and miR-

155 play important roles in EC functional regulation, endothelial dysfunction has been reported to be associated with ESRD. Therefore, we hypothesized that miR-126 and miR-155 may be involved in vascular damage-related pathophysiology in patients with ESRD. MiRNAs were previously considered to act as intracellular RNAs to regulate gene expression. However, increasing evidence suggested that miRNAs can be detected in the circulation in remarkably stable form that withstands repeated freezing/thawing cycles ***(10)*.**

It was discovered that miRNAs circulate within membrane-bound vesicles that are resistant to RNase digestion ***(11)***. One study additionally demonstrated that circulating Argonaute 2 complexes, carrying a number of miRNAs independent of vesicles, are one mechanism responsible for the stability of circulating miRNAs***(12)*.**

2. Subjects and Methods

This study was carried out on 50 cases which were divided into two groups; Patients group: include 30 ESRD children who are receiving maintenance hemodialysis in the Pediatric dialysis unit, Benha University Hospitals. This group was further categorized into two subgroups: Pre-hemodialysis group: include the patients group before receiving hemodialysis, Post-hemodialysis group: include the patients group after receiving hemodialysis. Control group: 20 healthy children without any evidence of CKD or inflammatory disorders. All cases were subjected to the following: Full history taking, Full clinical examination.

Sample collection and preparation as follows:In the pre-hemodialysis (pre-HD) patients, whole blood samples (3 mL) were collected immediately pre-HD from fistula needles before machine connection or any heparin exposure. In the post-hemodialysis (post-HD) patients, samples were also collected from fistula needles. Blood samples were collected for each case in a tube containing EDTA and processed for the isolation of plasma within 4 hrs of collection. The blood was centrifuged at 2000 g for 10 min at room temperature. Plasma was then transferred to RNase-free tubes for storage at -80°C until further processing. then Laboratory Investigation were done as the following: (a) Molecular laboratory investigations: Quantification of miR-126and miR-155 Expression in Plasma as follows:**1-** RNA extraction: MicroRNA was extracted using RNeasy Protect Animal Blood Kit, Qiagen following the manufacturer instructions.**2-**MicroRNA Reverse Transcription: Reverse transcription of the extracted microRNA was done using TaqMan® MicroRNA Reverse Transcription Kit, Applied Biosystems following the manufacturer instructions. Real time quantification RT- PCR (RT-qPCR) using Cyber green: Real time PCR were performed using SYBR green (DreamTaqTM Green PCR Master Mix (2x), supplied by Fermentas, life science – Germany). Applied Bio-systems 7900HT real-time PCR machine were used (Applied Biosystems, USA). Cel-lin-4 were used as positive internal control.(b)Other routine laboratory measurements include: estimated glomerular filtration rate (eGFR), hemoglobin, calcium, phosphate, albumin and C-reactive protein (CRP). Data were collected, statistically analyzed and tabulated. Statistical analysis was performed using mean ± SD, Chi square ($\chi 2$), Student-t-test (t), Pearson correlation (r) to calculate the probability (*P*). *P*>0.05 was not significant (NS), <0.05, significant (S), <0.01, highly significant (HS), <0.001 was considered very highly significant (VH).

3. Results

Table (1): Comparison between the two studied groups according to miRNA 126 level quantity.

	Patient (n = 30)		**Control (n = 20)**
	Before	**After**	
miRNA 126			
Min. – Max.	11.37- 13.42	9.84 – 14.82	14.71 – 15.75
Mean ± SD	12.68 ± 0.58	11.67 ± 1.86	15.33 ± 0.51
Median	12.61	11.04	15.71
t_1 (p)	2.457* (0.020*)		
t_2 (p)	16.599* (<0.001*)	10.196* (<0.001*)	

4. Discussion

Recently, miRNA control has also been found to have a critical regulatory role in epithelial mesenchymal transition (EMT), which may play an important role in the progressive kidney damage in CKD ***(13)***.

MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate gene expression at the post-transcriptional level by either translational repression or messenger RNA degradation ***(14)***.

MiRNAs have been shown to play critical roles in various cellular processes, inflammation, and angiogenesis. In particular, miR-126 and miR-155 have

been reported to control vascular homeostasis, angiogenesis, and vascular repair. In addition, miR-155, an important multifunctional miRNA, has been shown to regulate the endothelial inflammatory response and migration mediated by angiotensin-α ***(4)***.

Moreover, accumulating evidence points to an essential role of miRNAs in kidney disease, which is supported by the finding that the depletion of the miRNA-processing enzyme Dicer results in defects in renin cells (Juxta-glomerular cells), angiogenesis and renal morphological integrity and function ***(15)***.

Specific miRNA expression profiles have been described for diabetic nephropathy, polycystic kidney disease and kidney allograft rejection ***(14)***.

Table (2): Comparison between the two studied groups according to miRNA 155 level quantity

	Patient (n = 30)		**Control (n = 20)**
	Before	**After**	
miRNA 155			
Min. – Max.	12.12 – 14.52	12.30 – 14.30	16.43 – 16.47
Mean ± SD	13.77 ± 0.90	13.19 ± 0.60	16.44 ± 0.02
Median	14.18	13.14	16.44
t_1 (*p*)	4.036* (<0.001*)		
t_2 (*p*)	16.327* (<0.001*)	29.781* (<0.001*)	

Table (3): Correlation between miRNA 126 level quantity and miRNA 155 level quantity with different parameters in patients group

	miRNA 126 level		**miRNA 155 level**	
	R	***p***	**r**	***p***
Age	0.446*	0.013	0.094	0.621
Ca	-0.252	0.180	-0.476*	0.008
Phosphate	-0.603*	<0.001	-0.199	0.291
Albumin	0.280	0.134	-0.092	0.628
creatinine	-0.576*	0.001	-0.437*	0.016
CRP	0.604*	<0.001	0.660*	<0.001
Hb	0.612*	<0.001	0.398*	0.029
GFR	0.606*	<0.001	0.335	0.070

This study conducted on 30 patients with End Stage Renal Disease (ESRD) on regular hemodialysis, their ages ranged between 9-17 years with a mean age of 14.7 years. Fifty percent of our cases were males and fifty percent were females with a male to female ratio of 1:1. These results disagree with Wang and his colleagues who found in their study group that the mean age was 52.17 years and males (73%) were more common than females (27%) with a male to female ratio of 2.7:1 ***(14)***.

Also, Hu and coworker found a mean age of 46.5 years in their patients and more affection of males (61.7%) of their cases with a male to female ratio 1.6:1 which is in contradict with our results ***(16)***.

Our results showed that phosphate levels increased in patients of our study. These results disagreed with Wang and his colleagues who found a negative correlation between phosphate levels in their patients with increase expression of miRNA155 ***(14)***.

Also, ***Neal et al, (17)*** found in their study an inverse correlation between the phosphate levels in the sera of their study and the levels of expression of miRNA155.

Our results showed no change in the calcium level in patients of our study. This agrees with ***Wang et al, (14)*** who found no specific correlation between calcium level in sera of their patients and increase expression of miRNA155.

In agreement with our results, Neal and his colleagues (2011) found in patients of their study that there were no association was seen between calcium levels and levels of circulating miRNAs.

Our results showed a decreased level of albumin in the sera of our patients with increased expression of miRNA155 and 126.

This was disagree with results of Wang and his colleagues who found no specific relation between expression of miRNA126 and 155 with the level of albumin in the sera of their patients ***(14)***.

Increased creatinine levels found in patients of our study in correlation with increased expression of miRNA155 and miRNA126.

Our results were in agreement with Szeto and his colleagues who stated from their study that the expression of some miRNA targets in the urinary

sediment correlated with proteinuria, renal function, and the degree of tubulo-interstitial fibrosis ***(13)***.

Our results disagree with results of ***Neal et al, (17)*** who found in their studied patients that there was no association was observed between urinary miRNA level and kidney function.

Our results documented increase in the CRP in patients of our study with increased expression of miRNA155 and miRNA126. Our results were in agreement with ***Hu et al, (16)*** who found a positive correlation between the level of serum CRP and the increased expression of miRNA155.

The results disagree with ***Wang et al, (14)*** who found negative correlation between expression of miRNA155 and miRNA126 and level of CRP in patients of their study. Glomerular filtration rate was markedly decreased in patients of our study group with increased expression of miRNA155 and miRNA126. This result was in agreement with ***Wang et al, (14)*** who found positive correlation between expression of miRNA155 and miRNA126 and GFR in patients of their study.

Our results were in agreement with ***Hu et al, (16)*** who found a negative correlation between the level of GFR and the increased expression of miRNA155. Another study documented from their studies decline of GFR and the increased expression of many miRNA***(13)***.

Our study revealed no specific difference between the hemoglobin level and the expression of miRNA155 or 126. This was in agreement with ***Neal et al, (17)*** who found a significant inverse correlation between hemoglobin levels and miR-155 indicating the presence of lower hemoglobin levels in patients with CKD. Another study was in agreement with this results positive correlation between expression of miRNA155 and miRNA126 and hemoglobin level in patients of their study ***(14)***.

Our results revealed significantly decreased level of miRNA126 in patients of our study. ***Wang et al, (14)*** found in their study a significant reduction in miRNA126 in ESRD patients.

Our results were in agreement with ***Le Meuth et al, (18)*** who established in their study that miR-126 decreased during the later stages of CKD in all pathological conditions.

There was slight change in the level of miRNA126 before and after hemodialysis. These results were agreed with results of ***Wang et al (14)*** who found in their study that there was no significant change in the level of miRNA126 before and after hemodialysis.

Our results revealed significantly decreased level of miRNA155 in patients of our study. In agreement with these results, ***Wang et al, (14)*** found in their study a significant reduction in miRNA155 in ESRD patients.

Our results were in contradict with ***Hu et al, (16)*** who stated that miR-155 has been detected in end-stage renal failure patients and there is no clarification of the mechanism of miR-155 in promoting kidney disease progression.

There was slight change in the level of miRNA155 before and after hemodialysis. In agreement with such results; ***Wang et al, (14)*** found in their study that there was no significant change in the level of miRNA155 before and after hemodialysis.

The slight or no change in the level of miRNA126 and miRNA155 before and after hemodialysis in ESRD patients suggesting might be associated with the responsiveness to hemodialysis, or heparin anticoagulation may potentially interfere with the RT-qPCR-based assay ***(14)***.

Neal et al, (17) demonstrated that the rate of miRNA degradation is increased in plasma from patients with severe CKD. Therefore, one may speculate that the reduced circulating miR-126 and miR-155 might be associated with the increased miRNA degradation rate in patients with ESRD.

Our results showed significant correlation between miRNA126 and age, CRP, Hb and GFR, while correlates negatively with phosphate and creatinine and not correlated with calcium. These results were in agreement with ***Wang et al, (14)*** in that expression of miRNA126 positively correlated with GFR and Hb level, negatively correlated with phosphate and with no correlation to calcium level. But disagree with them because they found negative correlation between CRP and expression of miRNA126.

We document that miRNA155 correlated positively with CRP and Hb, but correlates negatively with calcium and creatinine while showed no correlation with age, phosphate, albumin and GFR. These results were in agreement with ***Hu et al, (16)*** who found positive correlation between miRNA155 and CRP levels but disagree with them because they found negative correlation between miRNA155 and GFR. These results were in agreement with ***Wang et al, (14)*** in that expression of miRNA155 positively correlated with GFR and Hb level, negatively correlated with phosphate with no correlation to calcium level and albumin. Also, the results agreed with ***Neal et al, (17)*** who found negative correlation with phosphate level but disagree with them because they found negative correlation between miRNA155 and hemoglobin.

Conclusion

From the above, it is concluded that the expression of miR-155 and miR-126 were significantly reduced in ESRD patients, but did not

significantly differ between pre-HD and post-HD with slight increase in pre-HD Nevertheless, further studies are required for the validation and extension of these results. miR-155 and miR-126 can be considered as candidate peripheral markers that may enable the prediction of ESRD progression. miR-126 and miR-155 might be useful predictive tools in ESRD, however, these results should be validated in a large clinical population, in which comparisons with standard risk factors could be made. The circulating miR-155 and miR-126 levels correlated positively with eGFR, hemoglobin and CRP, while they correlated negatively with creatinine. This finding suggested that the reduction of circulating miR-126 and miR-155 might be accompanied by a series of clinical symptoms.

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