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Study Of Circulating Levels Of Mir-126 And Vascular Complication In End Stage Renal Disease Patients On Heamodialysis

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Abstract:

Vascular calcification is a major complication of End stage renal disease patient on hemodialysis. A cross sectional cohort study in Beni Suef university hospital for a group of patients with End stage renal disease on regular hemodialysis. In total, 70 participants were divided into the study (50 patients) and (20 control groups). group showed higher miR-126 levels compared with the control group. Carotid intimal medial thickness (CIMT) was positively correlated with miR- 126.

Keywords: miR-126 , Vascular complication , End stage renal disease.

1. Introduction:

MicroRNAs (miRNAs) are a class of small (~22 nucleotides) non-coding RNAs that regulate gene expression by either translational repression or messenger RNA degradation. MiRNAs have been shown to play critical roles in various cellular processes, inflammation, and angiogenesis. In particular, miR-126 have been reported to control vascular homeostasis, angiogenesis, and vascular repair. Has been recognized that specific miRNAs could serve as potential biomarkers of various diseases in the circulation. Therefore, they assayed the circulating levels of endothelial-enriched miR- 126 in patients with ESRD, expecting to be able to provide new insights into the development of ESRD and the manifestation of its vascular complications. Atherosclerotic diseases are now a major global public health problem and increased Carotid intimal medial thickness (CIMT) is a hallmark of atherosclerotic diseases (Schönhage et al,. 2008).

Study demonstrated that the rate of miRNA degradation is increased in plasma from patients with severe CKD. Therefore, one may speculate that the increased circulating miR-126 might be associated with the increased miRNA degradation rate in patients with ESRD. (Neal et al,. 2011).

Subclinical atherosclerosis patients had significantly higher miR-126. CIMT was shown to be positively correlated with miR-126. Importantly, after adjustments for age, body mass index, systolic blood pressure and CIMT was still closely correlated with miR-126. An important finding of the study is that miR-126 would be a good new biomarker for the identification of subclinical atherosclerosis. the data was going significantly and meaningfuly with another study that showed that the miR-126 is a better indicator of preclinical atherosclerosis. (Kaptoge et al ,. 2010)

It is widely acknowledged that atherosclerosis is a complex inflammatory process and, recently, circulating miRNAs have been recognized as novel biomarkers and potential therapeutic targets for CVD, including atherosclerosis. MiR-126, a key circulating cytokine, has been shown to play a multifaceted and vital role in atherosclerosis. they have shown that miR-126 levels are closely correlated with CIMT. MiR-126 has also been reported to be associated with endothelial cell dysfunction. (Mott et al,.2008) reported that miR-126 is an important endogenous regulator, which plays a role in endothelial cell apoptosis by inhibiting the anti-apoptotic gene, myeloid cell leukemia-1. Xiaoying and his team also showed that miR-126 mediates the deposition of collagen and fibrosis via IL-6 and tumor growth factor-β (Xiaoying et al ,. 2019).

MiR-126 has also been suggested to suppress the proliferation and migration of vascular smooth muscle cells, possibly through inhibition of myeloid cell leukemia-1 and matrix metalloproteinase-2, indicating that miR-126 may serve as a valuable therapeutic tool to treat CVD, such as atherosclerosis (Lee et al ,. 2015). Although miR-126 has a number of clear effects on different stages in the formation of atherosclerosis and are, individually, important markers for CVD. As indicated by the results, circulating levels of miR-126 may serve as underlying and ponderable biomarkers for detecting subclinical or clinical atherosclerosis.(Stein et al ,. 2008). the study demonstrated that miR-126 is positively correlated to increase in carotid intimal median thickness as a predictor for atherosclerosis and CVD.

2. Patients and Methods:

The study included fifty ESRD patients who had been receiving maintenance hemodialysis 3 times weekly for at least 3 months. Twenty healthy adults without any evidence of chronic

kidney disease (CKD) or inflammatory disorders were included in the control group. consent from study participants before study has been taken and also ethical committee approval from beni suef university and Internal medicine department

Inclusion Criteria:

End stage renal disease patients on regular haemodialysis diagnosed by:

1- ultrasound (small sized kidneys Grade 3-4 nephropathy)
2- Estimated Glomerular Filtration Rate (eGFR) less than 10 ml/min

Exclusion Criteria:

1- Patients with normal kidney function (Estimated Glomerular Filtration Rate eGFR 90 ml/min)
2- vascular diseases such as:
   Vasculitis (Behcet's Disease -Buerger's Disease -Eosinophilic Granulomatosis with Polyangiitis, formerly Churg-Strauss Syndrome - Cryoglobulinemia -Giant Cell Arteritis- Henoch-Schönlein Purpura -Microscopic Polyangiitis- Polyarteritis Nodosa -Rheumatoid Vasculitis - Takayasu's Arteritis- Granulomatosis with Polyangiitis)
3- Familial hyperchostorlemia patients

All participants were subjected to the following

- Full History taking.
- Clinical examination including standered anthropometric (height, weight, body mass index {BMI}, systolic and diastolic blood pressure).
- Routine laboratory investigations including: (complete blood count – sodium – potassium – calcium – phosphorus).

Special investigations

1- duplex of carotid arteries with measurement of carotid intimal medial thickness .
2- Measure of MiR-126 by PCR technique in serum and correlation of MiR-126 results by Duplex on intimal medial thickness of carotid artery. Blood samples was collected from End stage renal diease patients on regular heamodialysis in haemodialsysis unit of beni Suef University

Statistical methodology:

• Analysis of data was done by IBM computer using SPSS (statistical program for social science) as follows;

- Description of quantitative variables as mean, SD and range.

- Description of qualitative variables as number and percentage.

- Unpaired t-test was used to compare quantitative variables, in parametric data (SD <
50 % mean)

• P value > 0.05 insignificant
• P < 0.05 significant
• P < 0.01 highly significant [20].

3. Results:

The current study was conducted at Beni-Suef university hospital within six months from April to October 2018. This study included fifty patients with end stage renal failure who had been receiving maintenance hemodialysis 3 times weekly for at least 3 months. Twenty healthy adults without any evidence of chronic kidney disease (CKD) or inflammatory disorders were included in the control group. Study group: including 50 patients

- 20 patients with end stage renal disease due to hypertension
- 5 patients with end stage renal disease due to Diabetus Mellitus
- 10 patients with end stage renal disease due to Glomerulonephritis
- 15 patients with end stage renal disease due to impacted renal stones.

Table (1): comparison between two patients' groups as regards age:

Age	Groups						T-Test	
	Study Group			Control Group			t	P-value
Range	6	-	72	18	-	42	-1.150	0.254
Mean ±SD	36.36	±	21.62	30.47	±	8.30		

P-value >0.05 is non-significant

This table shows that mean of age in study group was 36.36 ±21.62 and in control group was 30.47 ± 8.30 years. Comparison between two patients' groups shows that means of age were nearly equal between both groups with no statistically significant difference (p>0.05).

Table (1): show no significant difference between both groups as regards basic characteristics (age, weight, and BMI).

Table (2): comparison between two patients' groups as regards sex.

Sex	Groups						Chi-Square	
	Study Group		Control Group		Total			
	N	%	N	%	N	%	X2	P-value
Male	30	60.00	15	75.00	45	64.00	1.115	0.291*
Female	20	40.00	5	25.00	25	36.00		
Total	50	100.00	20	100.00	70	100.00		

P-value 0.05 is non-significant

This table shows that males were more prevalent in both groups 60% and 75% in study and control groups respectively. No statistically significant difference found by comparison between two patients' groups as regards gender.

Table (3): comparison between two patients' groups as regards Hypertension:

Hypertension	Groups						Chi-Square	
	Study Group		Control Group		Total			
	N	%	N	%	N	%	X2	P-value
Positive	20	40.00	0	00.00	20	28.5	10.702	0.001*
Negative	30	60.00	20	100.00	50	71.5		
Total	50	100.00	20	100.00	70	100.00		

P-value <0.05 is significant

The table shows that the number of hypertensive patients in study group was 20 (40%) and totally negative in control group. There was statistically significant difference between both groups as regards hypertension (p-value<0.05).

Table (4): comparison between two patients' groups as regards Diabetes Mellitus:

Diabetes Mellitus	Groups						Chi-Square	
	Study Group		Control Group		Total			
	N	%	N	%	N	%	X2	P-value
Positive	5	10.00	0	00.00	5	7.2	2.048	0.152*
Negative	45	90.00	20	100.00	65	92.8		
Total	50	100.00	20	100.00	70	100.00		

P-value >0.05 is non-significant

The table shows that the number of diabetic patients in study group was 5 (10%) and totally negative in control group. There was no statistically significant difference between both groups as regards diabetes (p-value>0.05)

Table (5): comparison between two patients' groups as regards miR126:

miR126	Groups						T-Test	
	Study Group			Control Group			t	P-value
Range	2.2	-	2.9	2.20	-	2.32	-5.416	0.000*
Mean ±SD	2.492	±	0.194	2.24	±	0.043		

P-value <0.05 is significant

This table shows that mean of miR126 in study group was 2.2492 ± 0.194 and control group was 2.24 ± 0.043. Comparison between two patients' groups shows that means of miR126 were not equal with statistically significant difference (p<0.05).

Table (6): comparison between two patients' groups as regards Carotid intimal medial thickness:

Carotid intimal medial thickness	Groups						T-Test	
	Study Group			Control Group			t	P-value
Range	0.30	-	0.60	0.23	-	0.33	-7.555	0.000*
Mean ±SD	0.434	±	0.102	0.252	±	0.030		

P-value <0.05 is significant

This table shows that mean of Carotid intimal medial thickness in study group was 0.434 ± 0.102 and control group was 0.252 ± 0.030. Comparison between two patients' groups shows that means of Carotid intimal medial thickness were not equal with statistically significant difference (p<0.05).

Table (7): Correlation between years of dialysis and miR126 in study groups

Years of Dialysis	miR126
R	0.806**
p-value	0.000

Correlation is significant at the 0.01 level

The table showed that there was strong positive correlation between years of dialysis and miR126 (r=0.806) at statistically significant level (p-value<0.05).

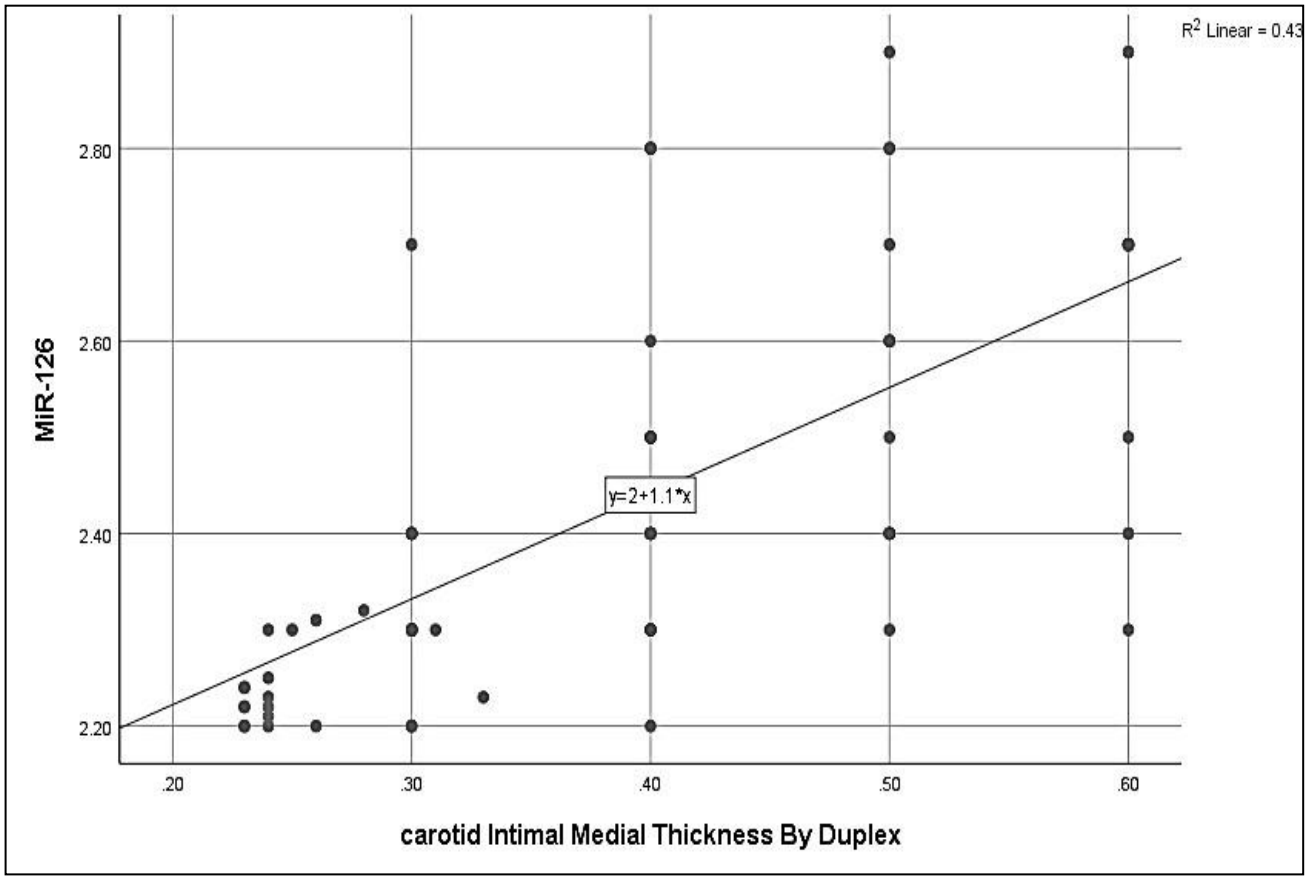
Table (8): Correlation between years of dialysis and Carotid intimal medial thickness in study group:

Years Of Dialysis	**Carotid intimal medial thickness**	
	R	0.677**
	p-value	0.000

Correlation is significant at the 0.01 level

The table showed that there was strong positive correlation between years of dialysis and carotid intimal medial thickness (r=0.677) at statistically significant level (p-value< 0.05)

Figure (1): Correlation between MIR126 and Carotid intimal medial thickness in study group



4. Discussion:

Circulating Zlevels of inflammation- associated miRNAs were significantly dysregulated in patients with ESRD receiving maintenance hemodialysis compared to healthy controls; moreover, the levels of these miRNAs tended to be significantly increased in patients with ESRD. (Zernecke et al,. 2009).

It is important to realize that endothelial dysregulation starts in the early stage of CKD and is a common event identified in both chronic and acute renal failure and in ESRD. In addition, studies have suggested that inflammation exists in patients with ESRD and might be aggravated by hemodialysis. Similarly, studies have demonstrated that miRNA can regulate vascular inflammation, endothelial homeostasis, and angiogenesis. In particular, miR-126 has been shown to be involved in vascular dysfunction and inflammation. MiR- 126 is highly enriched in ECs and endothelial apoptotic bodies and governs the maintenance of vascular integrity and angiogenesis (Fish et al,. 2008).

Studies have revealed that miRNAs previously identified in specific cells can also be detected in the circulation. The study have shown that circulating levels of total and specific miRNAs were increased in patients with severe chronic renal failure, with important implications for the use of circulating miRNAs as biomarkers in patients with renal impairment and for the pathogenesis of uremia. In another study, they measured the expression of miR-126, which were significantly increased in ESRD patients. the data suggested that miR- 126 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.(Amabile et al,. 2012).

Circulating miRNAs are packed within membrane-bound vesicles. These vesicles are byproducts released from cell apoptosis or activation, and are involved in cell-cell communication. However, the increased concentration of circulating miR-126 detected in patients with ESRD was surprising because one would expect that ECs and the inflammatory cell activation that occurs in ESRD contribute to the release of microparticles and remnants of apoptotic cells, thus increasing the expressed levels of circulating miRNAs. However, another study demonstrated that the rate of miRNA degradation is increased in plasma from patients with severe CKD. (Neal et al,. 2011).

Moreover, in one study, they observed that the circulating levels of miR-126 was also significantly increased in ESRD patients, further studies are required for the validation and extension of these results. they also found that circulating miR-126 levels correlated positively with eGFR and hemoglobin but were inversely correlated with phosphate levels. This finding suggested that the reduction of circulating miR- 126 and might be accompanied by a series of clinical symptoms. Although preliminary, the results showed that the two miRNAs can be considered as candidate peripheral markers that may enable the prediction of ESRD progression. (Neal et al,. 2011).

Additionally, accumulating evidence suggests that apoptotic bodies and microparticles can be transferred to other cell types, and that high circulating levels might result from increased delivery of miR-126 to recipient cell, and then act as physiologically functional molecules to exert gene silencing through the same mechanism as cellular miRNAs, suggesting the potentially important roles of circulating miRNAs in the development of CKD. Therefore, it is at least theoretically probable that circulating miR-126 is involved in the development of ESRD. (Prokop et al,. 2009).

Another study specifically addressed the levels and regulation of inflammation- associated and endothelial-derived miRNAs. Although altered levels of miR-126 in patients with ESRD patients, miR-126 deserve special consideration as novel biomarkers for risk estimation and classification. Meanwhile, they need to validate these data in large clinical populations. Furthermore, the effect of drug treatment on circulating miR-126 levels remains to be studied. However, the findings of the current study do allow us to propose that circulating miR-126 levels may be of clinical importance in ESRD.(Zernecke et al,. 2009).

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It is widely acknowledged that atherosclerosis is a complex inflammatory process and, recently, circulating miRNAs have been recognized as novel biomarkers and potential therapeutic targets for CVD, including atherosclerosis. MiR-126, a key circulating cytokine, has been shown to play a multifaceted and vital role in atherosclerosis. they have shown that miR-126 levels are closely correlated with CIMT. MiR-126 has also been

reported to be associated with endothelial cell dysfunction. (Mott et al,.2008) reported that miR-126 is an important endogenous regulator, which plays a role in endothelial cell apoptosis by inhibiting the anti-apoptotic gene, myeloid cell leukemia-1. Xiaoying and his team also showed that miR-126 mediates the deposition of collagen and fibrosis via IL-6 and tumor growth factor-β (Xiaoying et al ,. 2019).

MiR-126 has also been suggested to suppress the proliferation and migration of vascular smooth muscle cells, possibly through inhibition of myeloid cell leukemia-1 and matrix metalloproteinase-2, indicating that miR-126 may serve as a valuable therapeutic tool to treat CVD, such as atherosclerosis (Lee et al ,. 2015).

Our study demonstrated that miR-126 is positively correlated to increase in carotid intimal median thickness as a predictor for atherosclerosis in patients with end stage renal disease on regular hemodialysis , Moreover (Neal et al,. 2011) observed that the circulating levels of miR-126 was also significantly increased in ESRD patients.

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