Micro RNA-192 Is Negatively Associated With Cardiovascular Events Among Wait-Listed Potential Kidney Transplant Recipients on Hemodialysis Over a 5-year Follow-up Period

Mona G. Hassan[a], Fadia Morise[a], Noha A. Osman[b], Lobna Abdel Salam[c], Hesham Yehia[d], Mohamed Hamdi[e], Noha M. El Husseiny[f], and M.M. NasrAllah[b,*]

[a]Department of Nephrology & Department of Internal Medicine, Kasr Alainy School of Medicine, Cairo University, Cairo, Egypt; [b]Department of Nephrology, Kasr Alainy School of Medicine, Cairo University, Cairo, Egypt; [c]Genome Unit, Kasr Alainy School of Medicine, Cairo University, Cairo, Egypt; [d]Department of Cardiology, Kasr Alainy School of Medicine, Cairo University, Cairo, Egypt; [e]Department of Critical Care, Kasr Alainy School of Medicine, Cairo University, Cairo, Egypt; and [f]Department of Internal Medicine, Kasr Alainy School of Medicine, Cairo University, Cairo, Egypt

ABSTRACT

Background. Patients with chronic renal disease are susceptible to accelerated vascular calcification and cardiovascular morbidity and mortality. Micro RNAs (miRNAs) have been linked to the pathogenesis of cardiovascular diseases in the general population.

Aim. This study was carried out to evaluate the link between miRNA 192 and vascular calcification, pre-existing as well as newly occurring major adverse cardiovascular events, and mortality among hemodialysis patients who are also considered to be potential kidney transplant recipients.

Methods. We screened 64 potential transplant recipients on hemodialysis at our university hospital. Pre-existing overt cardiovascular disease was recorded; new adverse cardiovascular events and all causes of death over an observational period of 5 years were prospectively followed. Vascular calcification was measured in the aorta using computerized tomography scans, and micro RNA 192 was measured.

Results. The final study population included 55 patients followed for 63 months. Micro RNA 192 was significantly lower in patients who had preexisting cardiovascular disease ($P = .015$) as well and in all patients who had experienced any event by the end of the observational period ($P = .012$). A multiregression analysis model including micro RNA, age, dialysis vintage, intradialytic hypotension, vascular calcification, diabetes, systolic blood pressure, and smoking found the only independently correlating factor to cardiovascular events in this model to be micro RNA ($\beta = -0.286$, $P = .05$).

Conclusions. MiRNA 192 levels are significantly lower among patients experiencing cardiovascular events while on hemodialysis awaiting kidney transplantation.

MICRO RNAs (miRNAs) are noncoding RNAs that have the ability to repress gene expression [1]. They have been linked to different conditions [2–4]. miRNAs are stable in plasma [5,6], and this makes them useful markers of disease [7,8] including cardiovascular disease and allograft kidney injury [9].

Patients with chronic kidney disease (CKD) are susceptible to accelerated vascular calcification [10,11] exposing them to higher morbidity and mortality [10,12,13] although the pathogenesis and predictors are still not fully elucidated [14–17]. Several markers and molecules with possible pathogenic roles have been reported to be involved in this including FGF–23 [18,19], fetuin A [20], esRAGE [21],

*Address correspondence to M.M. NasrAllah, Kasr AlAiny School of Medicine, Saray Street, Cairo, Egypt. Tel: +201001150099. E-mail: mmnasrallah@kasralainy.edu.eg

0041-1345/19
https://doi.org/10.1016/j.transproceed.2019.02.044

Table 1. Characteristics of the Study Population and Subgroups

	All Patients	Patient With Cardiovascular Events	Patient Without Cardiovascular Events	*P* value*
Number	55	17 (30.9%)	38 (69.1%)	
Age (years)	37.5 (18–67)	47 (26–67)	35 (18–59)	.01
DBP (mm Hg)	90 (60–106.8)	90 (60–100)	90 (60–106.8)	.57
SBP (mm Hg)	136.7 (96.7–186.7)	140 (96.7–173.3)	136.7 (100–2)	.96
KT/V	1.4 (0.17–2.3)	1.3 (0.2–2.3)	1.5 (0.5–2.2)	.24
Calcium (mg/dL)	8.3 (5.8–10.6)	8.9 (7.1–9.6)	8.2 (5.8–10.6)	.48
Phosphorus (mg/dL)	4.5 (1.7–9.5)	3.9 (2–7.7)	4.7 (1.7–9.5)	.52
PTH pg/mL	323.1 (23.7–14)	288 (28–1369)	367 (23.7–1271)	.84
Hemoglobin (g/dL)	9.8 (7.1–1)	10.5 (7.7–13.2)	9.6 (7.1–14)	.94
Albumin (mg/dL)	4.2 (3.5–4.9)	4.3 (3.8–4.7)	4.2 (3.5–4.9)	.070
Dialysis vintage (months)	6 (9–288)	60 (9–288)	60 (12–192)	.5
BMI	23.5 (13.2–40.47)	25.5 (19.5–40.4)	23.1 (13.2–33.4)	.09
Uric acid (mg/dL)	7.2 (3–10.3)	7.1 (3.4–8.6)	7.2 (3–10.3)	.49
Cholesterol (mg/dL)	149.8 (74–226.6)	151 (74–226.6)	147.5 (96–218.5)	.98
Triglyceride (mg/dL)	138.6 (52.6–8)	130 (52.6–398)	139 (72–815)	.21
Alkaline phosphatase (IU/L)	204 (204.5–1753)	203 (77–668)	206 (43–1753	.86
Vascular calcification (n, % +ve)	23 (41.8%)	10 (58.8%)	13 (34.2%)	.09
Parathyroidectomy (n, %)	1 (1.8%)	1 (5.88%)	none	.31
Calcium carbonate (n, %)	54 (98.2%)	16 (94.1%)	38 (100%)	.31
Alfacalcidol (n, %)	47 (85.5%)	14 (82.4%)	33 (86.8%)	.69
Erythropoietin (n, %)	24 (43.6%)	6 (35.3)	18 (47.4%)	.56
Original kidney disease (n)				
Hypertension	24	8	16	
Glomerulonephritis	11	3	8	
Diabetes	4	2	2	
Urological	5	1	4	
Polycystic kidney disease	4	1	3	
Unknown	7	2	5	
Sex (male: n, %)	26 (47.3%)	10 (58.8%)	16 (42.1%)	.38
Smoking (n, %)	18 (32.7%)	7 (41.2%)	11 (29%)	.54

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; EPO, erythropoietin; PTH, parathyroid hormone; SBP, systolic blood pressure.
*Results for Mann-Whitney test for group with cardiovascular events and group without cardiovascular events. Quantitative data are summarized as median (minimum and maximum in parenthesis); qualitative data are summarized as number and (percentage in parenthesis).

inflammation, oxidative stresses [17], matrix GLA protein [14,15,22], sclerostin [23], and osteocalcin [15]. However, despite the ubiquity of candidate markers and pathogenic molecules, the full picture is far from being complete [14–17]. miRNAs may be a new piece in the puzzle, particularly miRNA 192 levels, which were found to be altered among non-CKD patients with myocardial infarction and heart failure [24]. It is also highly expressed in the kidneys [25,26] and in the glomeruli of diabetic mice [27]. This study evaluates the link between miRNA 192 and hard clinical endpoints, namely: vascular calcification, major adverse cardiovascular events, and mortality over a 5-year period of prospective follow-up among potential kidney recipients while on regular hemodialysis.

SUBJECT AND METHODS

We included 64 subjects from our university hospital dialysis unit. Patients on oral anticoagulation therapy were excluded. The study population were CKD stage 5 receiving 4-hour hemodialysis sessions, 3 times per week, on low flux polysulphone dialyzers and dialysate calcium 1.5 mmol/L for at least 6 months. The study protocol received approval from the ethical committee at our university hospital.

Pre-existing overt cardiovascular events that occurred after the start of hemodialysis treatment but before commencement of the study were recorded. New cardiovascular events were followed. The relevant episodes recorded were myocardial infarction, heart failure, acute coronary syndrome, ischemic cerebrovascular disease, and/or symptomatic peripheral vascular disease. All-cause mortality was also recorded. Vascular calcification was measured in the aorta by CT scans of the abdominal aorta using a previously described and validated sensitive score [10] and reported qualitatively as positive or negative.

We measured micro RNA-192 for all included subjects. Total RNA was extracted from EDTA anticoagulated blood using a High Pure Total RNA preparation kit (Roche Applied Science, Germany) followed by cDNA synthesis for each extracted sample by Transcription First Strand cDNA synthesis kit (Roche Applied Science, Germany).

SPSS version 22 (IBM SPSS Statistics for Windows, version 22.0, IBM Corp., Armonk, NY) was used for data analysis. Data were summarized as percentage, median, maximum, and minimum. Based on the sample distribution and the relationship between different samples, comparisons were performed using Mann-Whitney U test. Pearson's χ^2 test was used for categorical data and Fischer extract test for cells with expected counts of < 6. Stepwise multiple regression analysis was performed to determine the independence of statistical

Table 2. Multiregression Analysis of Factors Associated With Cardiovascular Events

Factor	β	95% CI	*P* value
micro RNA-192	−0.286	(0.00–0.00)	.05
Age	0.274	(−0.003 to 0.023)	.13
Vascular calcification	0.122	(−0.203 to 0.432)	.47
Diabetes	0.052	(0.458–0.642)	.74
Systolic blood pressure	−0.047	(−0.008 to 0.006)	.76
Smoking	0.032	(−0.254 to 0.317)	.83

Abbreviation: CI, confidence interval.

associations of the studied parameters to cardiovascular events. $P \leq .05$ was considered statistically significant.

RESULTS

We screened 64 patients on hemodialysis at the dialysis center in Kasr Alainy University Hospital. Nine were excluded (2 did not meet the inclusion criteria, and 7 declined consent). The final population included 55 patients followed for 63 months (mean observation 46.8 [3–63 months]) (Table 1).

Cardiovascular Events and Mortality

Evidence of preexisting cardiovascular disease was present in 8 patients and abdominal aortic calcification in 23 patients. We recorded 19 new cardiovascular events in 17 patients (15 acute coronary syndrome, 2 acute peripheral vascular disease, and 2 ischemic stroke). The characteristics of those patients are shown in Table 1. We also recorded 5 cases of mortality during the study period.

A multiregression analysis model was performed to study the factors correlating to the cardiovascular events in all patients (preexisting and new). This model included micro RNA, age, vascular calcification, diabetes, systolic blood pressure, and smoking. The only factor independently correlating to events was micro RNA-192(Table 2).

MiRNA−192

Micro RNA−192 count was lower in patients with preexisting cardiovascular disease (median 49; min 5, max 11,370) compared to those without (median 392; min 0, max 98,000) ($P = .015$). It was also significantly lower among patients with any documented cardiovascular event (before and during the observation period) (median 99; min 0, max 9980) compared to those with no events (median 440; min 5–98,000) ($P = .012$). Micro RNA count was numerically lower among patients who expired during the study (median 143; min 0, max 11,370) compared to those who survived (median 308; min 15, max 98,000) ($P = .4$) and lower among those with vascular calcification (median 112; min 0, max 72,900) compared to those without (median 308; min 5, max 98,000) ($P = .47$).

DISCUSSION

This study was carried out to evaluate the link between mi RNA 192 and cardiovascular outcomes in hemodialysis patients awaiting kidney transplantation over 5 years of prospective follow-up. We found a significantly lower micro RNA−192 level in patients with pre-existing cardiovascular disease as well as in all patients with documented cardiovascular events (before and during the observation period).

The low level of miRNA-192 in patients with cardiovascular events was demonstrated in previous studies where the circulating level of micro RNA-192 was associated with coronary heart disease [24]. These findings might be related to transforming growth factor β, which may reduce micro RNA-192 in CKD [28]. Transforming growth factor β is elevated in CKD and is involved in many cardiac disorders [29–31]. Nonetheless, low levels of micro RNA in CKD patients may be due to the accumulation of RNA causing degradation of circulating micro RNA [32,33]. Recently it was demonstrated that many circulating micro RNA are bound to protein [34] and lipoprotein [35] complexes rather than being within vesicles rendering them subject to degradation.

CKD is a risk factor for cardiovascular disease [36,37]. Hyperphosphatemia may induce medial calcification [38] by inducing osteochondrogenic switch of vascular smooth muscle cells (VSMCs) [11], and involvement of micro RNA has been evidenced in this phosphate-induced phenotypic switching [39].

Previous studies documented the expression of micro RNA in atherosclerosis obliterans and in destabilized human plaques - [39-41]. Cipollone et al [42] suggested that the unstable phenotypes of the atherosclerotic plaques and their ability to rupture induced by micro RNA might be related to its involvement in hydroxyapatite crystals formation. However, in our study, we did not find a significant difference in micro RNA levels between patients with vascular calcification and those without. This might be explained first by the fact that we measured circulatory and not tissue expressed micro RNA, which may be very different [40-41].

The main drawback of this study is the relatively small number of patients studied. However, it was a prospective study in which we evaluated and followed up the patients for 5 years to detect solid outcomes of cardiovascular events and mortality. Moreover, computed tomography scanning of the abdominal aorta, a sensitive tool [10], was done to evaluate aortic calcification.

REFERENCES

[1] Espinoza-Lewis RA, Wang DZ. Micro RNAs in heart development. Curr Top Dev Biol 2012;100:279–317.
[2] Ulitsky I, Bartel DP. LincRNAs: genomics, evolution, and mechanisms. Cell 2013;154:26–46.
[3] Morris KV, Mattick JS. The rise of regulatory RNA. Nat Rev Genet 2014;15:423–37.
[4] Ikeda S, Pu WT. Expression and function of MicroRNAs in heart disease. Curr Drug Targets 2010;11:1–13.
[5] Valadi H, Ekstrom K, Bossios A, et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nature Cell Biol 2007;9:654–9.

[6] Ratajczak J, Wysoczynski M, Hayek F, et al. Membrane-derived microvesicles (MV): important and under-appreciated mediators of cell-to-cell communication. Leukemia 2006;20: 1487–95.

[7] Chen X, Ba Y, Ma L, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other disease. Cell Res 2008;18:997–1006.

[8] Mitchell PS, Parkin RK, Kroh EM, et al. Circulating micro-RNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci (USA) 2008;105:10513–8.

[9] Mas VR, Dumur CI, Scian MJ, Gehrau RC, Maluf DG. MicroRNAs as biomarkers in solid organ transplantation. Am J Transplant 2013;13:11–9.

[10] Nasr Allah MM, Nassef A, Elshaboni TH, Morise F, Osman NA, Sharaf El Din UA. Comparing different calcification scores to detect outcomes in chronic kidney disease patients with vascular calcification. Int J Cardiol 2016;220:884–9.

[11] Aikawa E, Aikawa M, Libby P, et al. Arterial and aortic valve calcification abolished by elastolytic cathepsin S deficiency in chronic renal disease. Circulation 2009;119:1785–94.

[12] Hjortnaes J, Bouten CV, Van Herwerden LA, Grundeman PF, Kluin J. Translating autologous heart valve tissue engineering from bench to bed. Tissue Eng Part B Rev 2009;15: 307–17.

[13] Rajamannan NM, Evans FJ, Aikawa E, et al. Calcific aortic valve disease: not simply a degenerative process: a review and agenda for research from the National Heart and Lung and Blood Institute Aortic Stenosis Working Group. Executive summary: Calcific aortic valve disease–2011 update. Circulation 2011;124: 1783–91.

[14] AP S, Tintut Y, LL D. Regulatory mechanisms in vascular calcification. Nat Rev Cardiol 2010;7:528–36.

[15] Schlieper G, Schurgers L, Brandenburg V, Reutelingsperger C, Floege J. Vascular calcification in chronic kidney disease: an update. Nephrol Dial Transplant 2016;31:31–9.

[16] Floege J, Ketteler M. Vascular calcification in patients with end-stage renal disease. Nephrol Dial Transplant 2004;19:V59–66.

[17] Nasrallah MM, El-Shehaby AR, Osman NA, et al. The association between fibroblast growth factor–23 and vascular calcification is mitigated by inflammation markers. Nephron Extra 2013;3: 106–12.

[18] Nasr Allah MM, El-Shehaby AR, Salem MM, Osman Na, Salem M, Sharef El Din U. Fibroblast growth factor–23 (FGF–23) is independently correlated to aortic calcification in haemodialysis patients. Nephrol Dial Transplant 2010;25:2679–85.

[19] Jean G, Bresson E, Terrat J. Peripheral vascular calcification in long-hemodialysis patients: associated factors and survival consequences. Nephrol Dial Transplant 2009;24:948–55.

[20] Ketteler M. Fetuin-A and extraosseous calcification in uremia. Curr Opin Nephrol Hypertens 2005;14:337–42.

[21] Nasrallah MM, El-Shehaby AR, Osman NA, Salem MM, Nassef A, El Din UA. Endogenous soluble receptor of advanced glycation end-products (esRAGE) is negatively associated with vascular calcification in non-diabetic hemodialysis patients. Int Urol Nephrol 2012;44:1193–9.

[22] Osman NA, El-Abd N, Nasr Allah MM. VKORC1 gene (vitamin K epoxide reductase) polymorphisms are associated with cardiovascular disease in chronic kidney disease patients on hemodialysis. Saudi J Kidney Dis Transpl 2016;27:908.

[23] Evenepoel MP, Goffin E, Meijers B, et al. Sclerostin serum levels and vascular calcification progression in prevalent renal transplant recipients. J Clin Endocrinol Metab 2015;100:4669–76.

[24] Matsumoto S, Sakata Y, Suna S, et al. Circulating p53-responsive microRNAs are predictive indicators of heart failure after acute myocardial infarction. Circ Res 2013;113:322–6.

[25] Tian Z, Greene AS, Pietrusz JL, Matus IR, Liang M. MicroRNA-target pairs in the rat kidney identified by microRNA microarray, proteomic, and bioinformatic analysis. Genome Res 2008;18:404–11.

[26] Sun Y, Koo S, White N, et al. Development of a micro-array to detect human and mouse microRNAs and characterization of expression in human organs. Nucleic Acids Res 2004;32:e188.

[27] Kato M, Zhang J, Wang M, et al. MicroRNA–192 in diabetic kidney glomeruli and its function in TGF- beta-induced collagen expression via inhibition of E-box repressors. Proc Natl Acad Sci USA 2007;104:3432–7.

[28] Krupa A, Jenkins R, Luo DD, Lewis A, Phillips A, Fraser D. Loss of MicroRNA–192 promotes fibrogenesis in diabetic nephropathy. JASN 2010;21:438–47.

[29] Edgley AJ, Krum, Kelly DJ. Targeting fibrosis for the treatment of heart failure: a role for transforming growth factor -β. Cardiovasc Ther 2012;30:e30–40.

[30] Schneiders D, Heger J, Best P, Piper H, Micheal, Taimor G. SMAD proteins are involved in apoptosis induction in ventricular cardiomyocytes. Cadiovasc Res 2005;67:87–96.

[31] Huntgeburth M, Tiemann K, Shahverdyan R, et al. Transforming growth factor -β oppositely regulates the hypertrophic and contractile response to β- adrenergic stimulation in the heart. PLoS One 2011;6:e26628.

[32] Neal CS, Michael MZ, Pimlott LK, Yong TY, Li JY, Gleadle JM. Circulating micro- RNA expression is reduced in chronic kidney disease. Nephrol Dialysis 2011;26:3794–802.

[33] Weickmann JL, Olson EM, Glitz DG. Immunological assay of pancreatic ribonuclease in serum as an indicator of pancreatic cancer. Cancer Res 1984;44:1682–7.

[34] Arroyo JD, Chevillet JR, Kroh EM, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci USA 2011;108:5003–8.

[35] Vickers KC, Palmisano BT, Shoucri BM, et al. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol 2011;13:423–33.

[36] Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis 1998;32:S112–9.

[37] Culleton BF, Larson MG, Wilson PW, et al. Cardiovascular disease and mortality in a community-based cohort with mild renal insufficiency. Kidney Int 1999;56:2214–9.

[38] Proudfoot D, Shanahan CM. Biology of calcification in vascular cells: intima versus media. Herz 2001;26:245–51.

[39] Rangrez AY, Massy ZA, Metzinger-Le Meuth V, Metzinger L. miR–143 and miR–145: molecular keys to switch the phenotype of vascular smooth muscle cells. Circ Cardiovasc Genet 2011;4:197–205.

[40] Li T, Cao H, Zhuang J, et al. Identification of miR–130a, miR–27b and miR–210 as serum biomarkers for atherosclerosis obliterans. Clin Chim Acta 2011;412:66–70.

[41] Raitoharju E, Lyytikainen LP, Levula M, et al. miR–21, miR–210, miR–34a, and miR–146a/b are up-regulated in human atherosclerotic plaques in the Tampere Vascular Study. Atherosclerosis 2011;219:211–7.

[42] Cipollone F, Felicioni L, Sarzani R, et al. A unique microRNA signature associated with plaque instability in humans. Stroke 2011;42:2556–63.