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Macrophage migration inhibitory factor is associated with vascular dysfunction in patients with end-stage renal disease

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ABSTRACT

Background: Patients with end-stage renal disease (ESRD) show a high prevalence of cardiovascular disease with arterial stiffness, atherosclerosis and endothelial dysfunction, leading to increased morbidity and mortality. The cytokine macrophage migration inhibitory factor (MIF) exhibits proinflammatory and proatherogenic functions and has recently emerged as a major regulator of atherogenesis. Studies examining the relationship between circulating MIF levels and vascular dysfunction in this high-risk population do not exist.
Methods: In patients with ESRD ($n = 39$) and healthy controls ($n = 16$) we assessed endothelial function by flow-mediated dilation of the brachial artery and arterial stiffness (augmentation pressure, augmentation index and pulse pressure) using applanation tonometry. High-sensitive Troponin and subendocardial viability ratio were determined to assess myocardial injury.
Results: Patients with ESRD had impaired endothelial function and higher plasma MIF levels. MIF levels negatively correlated with endothelial function ($r = -0.345$, $P = 0.031$) and positively with arterial stiffness indices in patients with ESRD (pulse pressure $r = -0.374$, $P = 0.019$ and augmentation pressure $r = -0.423$, $P = 0.025$). In multivariate regression models besides age, gender, weight, and heart rate, MIF was an independent predictor for arterial stiffness. Impact on myocardial end-organ damage was reflected by correlation with high-sensitive Troponin I ($r = 0.43$, $P = 0.009$).
Conclusion: Our findings show that high MIF plasma levels are associated with diminished endothelial function and arterial stiffness and are correlated with myocardial injury. Further studies are necessary to investigate whether modulation of MIF might have an impact on atherosclerotic disease in this high-risk population.

1. Introduction

Patients with end-stage renal disease (ESRD) suffer from increased morbidity and mortality due to higher incidences of cardiovascular events [1]. The major contributors to the pathogenesis of cardiovascular disease (CVD) and preceding vascular dysfunction in ESRD are vascular calcifications and concomitant arterial stiffness [2,3]. These are highly prevalent, progressive and imply reduced arterial elasticity. Measures of arterial stiffness include pulse pressure (PP) and central augmentation index (AIx), which have shown an independent predictive value of all-cause mortality in ESRD patients [4,5]. Increased stiffness affects timing and magnitude of central pulse wave reflections, hence determining ventricular load and coronary blood flow [6].

Although atherosclerosis, characterized by the presence of plaques and arterial occlusions, is the most frequent underlying cause of CVD, many vascular complications happen in the absence of clinically detectable disease. Endothelial dysfunction is regarded as one of the earliest phenomena of atherosclerotic CVD indicating inappropriate regulation of the vascular tone and was identified as prognostically relevant in studies investigating patients with cardiovascular risk factors [7]. Mechanistically, the endothelial dysfunction is perpetuated through increased oxidative stress and low-grade inflammation, the latter being concurrent, interrelated and in particular ascribed to patients with ESRD [8].

Vascular stiffness, atherogenic alterations and endothelial dysfunction lead to structural and functional changes at the level of conduit arteries and the microcirculation in ESRD with detrimental impact on the myocardium and coronary perfusion [9,10]. Classical therapeutic approaches to fight CVD in this collective appear insufficient. Therefore, early diagnosis and timely fashioned intervention against the effect of alterations in both conduit vessels and microcirculation are of increasing importance. It has recently been shown that levels of macrophage inhibitory cytokine correlate to increased mortality in ESRD [11].

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The cytokine macrophage migration inhibitory factor (MIF) is an evolutionarily conserved ubiquitously expressed protein and a modulator of the innate immune response [12]. Due to its pro-inflammatory functions, MIF has recently emerged as a major mediator of atherosclerosis and might be implicated in modulation of disease progression [13,14]. While MIF is in spotlight of experimental atherosclerosis research, clinical studies investigating the relationship with CVD are limited. Recently, it was shown that patients with acute myocardial infarction display high amounts of circulating MIF and the latter showed a correlation with vulnerable plaques, suggesting a role of MIF in risk assessment for patients suffering from acute coronary syndromes [15,16].

The association of plasma MIF levels with vascular stiffness and endothelial dysfunction in clinical settings has not been evaluated yet. We here aimed to explore the relationship of circulating MIF levels in vascular dysfunction in this high-risk population in a cross-sectional clinical approach.

2. Methods

2.1. Study population

Study procedures were in accordance with the Declaration of Helsinki and the Institutional Ethics Committee of the Heinrich-Heine University approved the study protocol (Clinicaltrials.gov: NCT01412320). $N = 39$ patients with ESRD were undergoing maintenance hemodialysis and $n = 16$ healthy controls gave written informed consent and were included in this cross-sectional study. Due to known circadian rhythm of MIF examinations were conducted and blood samples were drawn following a 15-min rest before 1:00 pm and before initiation of maintenance hemodialysis [17]. Brachial blood pressure (BP) was measured in duplicate in the non-fistula arm by cuff and mercury sphygmomanometer after the participant had rested in a seated position for 10 min and the average of the 2 measurements was recorded. Blood was drawn for clinical routine and the Institute of Clinical Chemistry and Laboratory Diagnostics, University Hospital Duesseldorf performed all analyses unless noted otherwise.

2.2. Endothelial function

Flow-mediated vasodilation (FMD) of the brachial artery was measured by a noninvasive technique to assess endothelial function as previously described [18–21]. Briefly, after a 15-min equilibration period in a temperature-controlled room, with the use of a 12-MHz linear-array transducer and the Vivid I system (GE Healthcare), the brachial artery diameter and Doppler-flow velocity were acquired proximal of the antecubital fossa before and immediately at 20, 40, 60, and 80 s after cuff deflation of 5-min forearm arterial occlusion at 250 mmHg of pressure with a 12.5-cm-wide cuff. End-diastolic frames were obtained and analyzed with an automated analysis system (Brachial Analyzer, Medical Imaging Applications, Iowa City, IO) by investigators blinded to the subjects. Nitroglycerin-mediated vasodilation (NMD) i.e., endothelium-independent vasodilation was measured at 4 min after 400 μg sublingual nitroglycerin [22]. FMD and NMD were determined as the maximal percent diameter change of the arterial diameter measurement relative to the baseline measurement.

2.3. MIF plasma-levels

MIF was determined as described previously [23]. Heparinized full blood was centrifuged at 800 g for 10 min (4 °C). The resulting plasma aliquots were snap frozen in liquid nitrogen and stored at −80 °C until further analysis. MIF levels were measured by quantitative sandwich enzyme-linked immunosorbent assay (ELISA) (Quantikine, R&D Systems, Minneapolis, USA) according to the manufacturer's protocols.

2.4. Vascular stiffness and pulse wave analysis

Arterial pulse pressure was determined by sphygmomanometry as the difference of systolic to diastolic blood pressure. The indirect measures of arterial stiffness and central hemodynamics were obtained with the subject in a supine position by using the SphygmoCor system (AtCor Medical, Sydney, Australia). Radial arterial pressure waveforms were obtained by applanation tonometry and central arterial waveforms generated using a validated inbuilt transfer function. Applanation tonometry has been validated to yield precise assessments of intraarterial pressures by comparison with simultaneous invasive pressure recordings [24]. The system provided a corresponding central aortic pulse waveform from which central SBP (cSBP), central DBP, central pulse pressure (cPP), subendocardial viability ratio (SEVR), augmentation pressure (AP) and normalized augmentation index (AIX@75 bpm) were identified (Fig. 2). Augmentation index is defined as the proportion of cPP to AP due to overlap between the forward and reflected pressure wave, and hence is a measure of peripheral wave reflection. SEVR is calculated as quotient of systolic to diastolic integral of central pulse waves.

PWV was calculated from sequential recordings of electrocardiogram referenced carotid and femoral pressure waveforms obtained by using tonometry with the SphygmoCor device and transducer. Wave transit time is determined by the software using the distance between carotid and femoral sites estimated from the distance between each artery location and the sternal notch and the R-wave of a simultaneously recorded electrocardiogram as reference frame [25].

Table 1
Basic clinical and biochemical characteristics.

		ESRD	Healthy	*P*
n		39	16	
Age (y)		65 ± 12	64 ± 5	0.5
Male gender (n)		31	10	
Height (cm)		173 ± 10	173 ± 10	0.7
Weight (kg)		87 ± 18	78 ± 16	0.1
Systolic blood pressure (mmHg)		136 ± 20	138 ± 16	0.7
Heart rate (bpm)		70 ± 14	63 ± 8	0.02
Renal diagnosis (n)	Hypertensive/large vessel	13		
	Diabetic nephropathy	9		
	Glomerulonephritis	7		
	Polycystic kidney disease	4		
	Other/miscellaneous	6		
Dialysis vintage (months)		37 ± 27		
Cardiovascular disease (n)		15		
Risk factors (n)	Hypertension	35		
	Diabetes	13		
	Current smoker	11		
	Hypercholesterolemia	2		
Medication (n)	ASS	21		
	Statin	19		
	AT blocker	10		
	ACE-I	9		
	b blocker	22		
	Ca channel blocker	113		
	Diuretics	30		
	Steroids	2		
Chemistry Panel	Sodium (mmol/l)	140 ± 2.6	141 ± 1.5	0.07
	Potassium (mmol/l)	4.8 ± 0.8	4.0 ± 0.2	<0.001
	Calcium (mmol/l)	2.3 ± 0.2	2.2 ± 0.1	0.8
	C-reactive protein (mg/dl)	0.7 ± 1.4	0.36 ± 0.2	0.09
	Total protein (g/dl)	7.1 ± 0.5	7.1 ± 0.2	0.9
	Cystatin C (mg/dl)	5.5 ± 1.2	0.8 ± 0.1	<0.001
	Creatinine (mg/dl)	7.6 ± 2.5	0.8 ± 0.2	<0.001
	GFR (MDRD)	8.6 ± 4.8	94.7 ± 22.5	<0.001
	Urea nitrogen (mg/dl)	114 ± 35	34 ± 8	<0.001
	Total cholesterol (mg)	186 ± 45	217 ± 36	0.02
	HS-troponin (ng/l)	59 ± 36	6.8 ± 2.8	<0.001
	Creatin-kinase (mg/dl)	87 ± 51	126 ± 97	0.05
Blood Count	Hemoglobin (g/dl)	11.5 ± 1.2	14.2 ± 1.1	<0.001
	Hematocrit (%)	36 ± 3	42 ± 3	<0.001
	Platelets (/ll)	211 ± 57	237 ± 45	0.1
	Leucocytes (104/ll)	7.3 ± 2.2	5.9 ± 1.3	0.03

2.5. Statistical methods

Results are expressed as mean ± standard deviation (SD) unless stated otherwise. All data were checked for normality distribution using Kolmogorov–Smirnov test and no departures were noted. Differences between groups were compared using unpaired Student's two-tailed *t*-test. Correlations between individual parameters were calculated using univariate analyses. Results are expressed as Pearson's *r* and corresponding *p* values. Multivariate regression analyses were calculated using method "enter". Variables for the linear regression models were chosen based on simple correlation analyses and those variables known or thought to be associated with arterial stiffness, from published observations. Due to direct calculation of AP, AIX and PP derived from peripheral blood pressure systolic, diastolic and mean blood pressure were excluded from entering analysis. *R* square indicates change for each parameter. *P* values of less than 0.05 were regarded statistically significant. All statistical tests were conducted using SPSS 21.0 (IBM) and Prism 5.0 (GraphPad) for Mac OS.

3. Results

3.1. Subject characteristics

Thirty-nine patients undergoing maintenance dialysis and 16 healthy controls were included in this cross-sectional study. The etiology of ESRD was hypertensive renal disease (13 cases), diabetic renal disease (9 cases), chronic glomerulonephritis (7 cases), polycystic kidney disease