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CORRELATION OF LEFT VENTRICULAR EJECTION FRACTION WITH PROTEIN ARGININE METHYLTRANSFERASE-1 MRNA EXPRESSION IN PATIENTS WITH DILATED CARDIOMYOPATHY: A CROSS-SECTIONAL STUDY

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Abstract

INTRODUCTION : In dilated cardiomyopathy (DCM) patients, elevated circulating asymmetric-dimethylarginine (ADMA) concentration is well-established, however the underlying mechanism(s) for excess ADMA is unclear. Reduced circulating nicotinamide-adenine-dinucleotide (NAD+) and sirtuin1 (sirt1) mRNA expression in peripheral blood of DCM patients have been reported; whether this down-regulation influences LVEF (left-ventricular-ejection-fraction) is uncertain.

OBJECTIVE : To investigate whether the expression of cardiac genes (protein arginine methyltransferase1, PRMT1 and sirt1) and circulating analytes (ADMA and NAD+) are altered in peripheral blood and if such changes are associated with LVEF in DCM.

METHODOLOGY : Peripheral blood cells (PBCs) and plasma were isolated from sixty DCM patients. Based on LVEF, patients were categorized as moderate- or severe-left ventricular dysfunction (LVD). Gene expression was determined by quantitative reverse-transcription-polymerase-chain-reaction. Quantification of analytes was assessed by reverse-phase high-performance-liquid-chromatography.

RESULTS : In patients with severe LVD, PRMT1 mRNA and circulating ADMA were higher, while sirt1 mRNA expression and circulating NAD+ were reduced, than moderate LVD. Positive correlation exists between PRMT1 expression- and ADMA-versus decreasing LVEF. Whereas reduced sirt1 expression and NAD+ were negatively correlated with decreasing LVEF.

CONCLUSION : As disease severity increases, mRNA expression of two cardiac genes and circulating concentrations of analytes were altered, possibly reflecting stress response effects. This study shows that PBCs-signatures of arginine methylation and cardio-protection can be defined for LVEF and that the peripheral blood mRNA expressions could predict LVD.

KEYWORDS : Dilated cardiomyopathy, Heart failure, Protein arginine methyltransferase1, Sirtuin1, Asymmetric dimethylarginine, Nicotinamide adenine dinucleotide

INTRODUCTION

Dilated cardiomyopathy (DCM) has variable clinical presentation, including impaired left ventricular ejection fraction (LVEF), which progresses to heart failure (HF)1. An underlying factor in DCM is cardiomyocyte dysfunction2. Nitric oxide (NO), a vasodilator produced by endothelial nitric oxide synthase (eNOS), regulates cardiomyocyte functions. In DCM, NO signaling is impaired because of the accumulation of eNOS inhibitor asymmetric dimethylarginine (ADMA)3. Plasma ADMA concentrations

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are elevated in DCM patients. ADMA up-regulated systemic oxidative stress (OS) by promoting superoxide formation. Excess OS is a characteristic feature in DCM and HF. Thus, excess ADMA contributes to pathogenesis of HF. ADMA is biosynthesized via arginine methylation reaction, catalyzed by the redox-sensitive enzyme protein arginine methyltransferase1 (PRMT1)4. The association between excess ADMA and DCM suggests that ADMA biosynthesis by PRMT1 is altered. However, whether PRMT1 expression becomes upregulated in DCM or if PRMT1 expression is associated with LVEF is unclear, which is the base of this study.

Sirtuin-1 (sirt1), a cardiomyopathy modifier-gene, is a nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylase enzyme, which is associated with several fundamental biological processes, including OS and cardiomyocyte survival. While sirt1-overexpression in cardiomyocytes renders cytoprotection, sirt1-deficiency leads to apoptosis, excess OS and inflammation5. In HF patients, NAD+ depletion and reduced sirt1 expression in peripheral blood cells (PBCs) and cardiomyocytes contribute to HF6. The roles of PBC sirt1 mRNA and circulating NAD+ in cardiac function are poorly characterized in DCM. Our question was, whether or not, PBC sirt1 mRNA and circulating NAD+ levels are altered; and whether such changes could influence LVEF in DCM. This study aimed to define the gene expression of selected cardiac genes in PBCs and their respective circulating biochemical analytes and relate them to LVEF in order to identify whether there is a link between the elements of i) arginine methylation (PRMT1 and ADMA) and ii) cardioprotection (sirt1 and NAD+), with cardiac function, in DCM patients. We evaluated i) the mRNA levels of PRMT1 and sirt1 in PBCs and ii) the circulating concentrations of ADMA and NAD+ to echocardiographic indices of LV function in DCM patients.

METHODS

Sixty DCM patients had been enrolled from the out-patient facility in the Department of Cardiology in SRM Medical College Hospital and Research Center, Kattankulathur. This study is a hospital-based cross-sectional study, therefore convenience sampling was chosen. All patients had an echocardiographic diagnosis of DCM. This study is approved by the Institutional Ethics Committee at SRMIST, Kattankulathur, and all patients provided informed consent to participate in the study. The present study conforms to the Declaration of Helsinki for using human tissue samples or subjects. Table 1 shows the baseline data.

Echocardiography: At enrolment, echocardiographic indices were assessed by using the Epiq &c Philips ultrasound system. LVEF was calculated using the biplane Simpson's method. Based on the LVEF, patients were categorized as Group A (moderate LVD; LVEF 31 – 45%) and Group B (severe LVD; LVEF < 30%). Table 1 shows the echocardiographic parameters.

Analysis of ADMA and NAD+: Plasma and blood cells were isolated using peripheral blood samples. Following ultra-filtration of plasma samples, through 3-kDa molecular weight cut-off filters, samples were utilized for the simultaneous quantification of ADMA and NAD+ using dual wavelengths in reverse-phase high-performance-liquid-chromatography, as described.[7]

RNA isolation and qRT-PCR: Total RNA was extracted from blood cells using the TRIzol reagent (Sigma) method. The concentration and purity of RNA were determined by using the NanoDrop 2000c spectrophotometer (ThermoFisher Scientific, USA). Using PrimeScript RT reagent kit (TaKaRa bio-Inc) cDNA was synthesized. Quantitative reverse-transcription-polymerase-chain-reaction (qRT-PCR) was carried out in a Light-Cycler-480 (Roche applied science), using the TB-Green-Premix-Ex-Taq-II (Ta-Ka-Ra bio-Inc). By using the 2-ΔΔCt method, relative gene expression was calculated; data shows the normalized fold change in gene expression against GAPDH.

Primer sequences:

PRMT1:

Forward 5'-TTGACTCCTATGCCCACT-3'

Reverse 5'-CCACATCCAGCACCACC-3'.

Sirt1:

Forward 5'- TCAGCTCTGGGATGACCTT-3'

Reverse 5'- ACCATCAAGCCGCCTACTAATCTG-3'.

GAPDH:

Forward 5'- TTCAGCTCTGGGATGACCTT-3'

Reverse 5' - CTCATGACCACAGTCCATGC- 3'.

Statistical analysis: Data represent mean(s) and standard-deviation for continuous-variables or per-centages for categorical-variables. Differences between the groups (A and B) were tested using student-t-test for continuous-variables and chi-square test was used for categorical variables. Correlations between continuous variables are evaluated using the Spear-man cor-relation co-efficient. Significant changes in gene-expression bet-ween the groups are evaluated by a two-tailed Mann–Whitney test. All tests were two-sided, and analysis is performed with the SPSS statistical package (Windows version 16.0). Multivariate linear regression models are obtained to establish whether the independent

variables, mRNA expressions or circulating concentrations of biochemical analytes, in the presence of other risk factors, can predict the disease severity of DCM. P-value less than 0.05 is considered stat-istically significant.

RESULTS

In DCM patients, mRNA expression levels of the two cardiac genes, PRMT1 and sirt1 in PBCs, as well as the circulating concentrations of biochemical analytes ADMA and NAD+, which are associated with the selected genes, are assessed to LVEF.

Table 1: Demographic and echocardiographic characteristics of enrolled patients

Parameters	Group A LVEF: 31 – 45 % (n=30)	Group B LVEF: 25 – 30 % (n=30)	p Value
Gender, Male (%)$	66% (20)	60% (18)	NS
Age (years)*	59.05±8.54	53.5±6.28	NS
Height (cm)*	161.9±8.46	163.5±5.25	NS
Weight (Kg)*	67.1±14.68	63.4±6.64	NS
Heart Rate (bpm)*	76.3±8.75	76.4±11.16	NS
Systolic BP (mmHg)*	120. 5±14.68	127±17.67	NS
Diastolic BP (mmHg)*	77±9.79	78±11.35	NS
Hypertension	33%	40%	NS
Diabetic	43%	50%	NS
CAG – Normal (%)$	70%	70%	NS
CAG – SVD (%)$	20%	30%	NS
CAG – DVD (%)$	10%	0%	NS
Beta Blockers$	60%	45%	NS
Diuretics$	80%	55%	NS
Antiplatelet$	90%	85%	NS
Statins$	80%	95%	NS
LVEF (%) – Simpson	32.77±4.57	27.6±1.58	< 0.0001
LV mass (g)	228.13±41.72	231.2±22.58	NS
EDV (mL)	152±37.7	140.1±5.43	NS
ESV (mL)	101±21.71	104.8±11.49	NS
SV (L/min)	48.5±20	35.5±10.19	0.0092
CO (L/min)	3.09±1.09	2.919±0.72	NS
LVIDD (cm)	5.62±0.27	5.73±0.18	NS
LVIDS (cm)	4.67±0.27	4.77±0.27	NS
E (cm/s)	0.69±0.18	0.82±0.1	0.0021
A (cm/s)	0.58±0.26	0.33±0.08	< 0.0001
E/A	1.6±1.25	2.585±0.85	0.0011
e' Septal (cm/s)	0.05±0.01	0.047±0.01	NS
E/e' Septal	13.6±4.21	17.55±2.34	< 0.0001
e' Lateral (cm/s)	0.07±0.01	0.065±0.01	NS
E/e' Lateral	10.51±2.97	12.8±1.87	0.001
GLS (%)	-9.32±5.06	-7.623±1.77	NS

Table 1. Demographic and echocardiographic characteristics of enrolled patients. Data are presented as mean ± standard deviation. A – atrial systole, BP – blood pressure, CAG – coronary angiogram, CO – cardiac output, DVD – double vessel disease, E – early rapid filling in diastole, E/e' - ratio of transmitral blood flow velocity to tissue doppler velocity, e' – early diastolic filling velocity, EDV – end diastolic volume, ESV – end systolic volume, GLS – global longitudinal strain, LV mass – left ventricular mass, LVEF – left ventricular ejection fraction, LVIDD – left ventricular internal dimension in diastole, LVIDS – left ventricular internal dimension in systole, NS – non significant, SV – stroke volume, SVD – single vessel disease.

Demographic and clinical characteristics of patients, along with baseline echocardiographic parameters, are shown in Table.1. In the echocardiographic parameters, statistically significant differences between the groups were observed for LVEF, stroke volume (SV), E wave, A wave, E/A, E/e' septal and E/e' lateral ($p<0.05$).

In severe LVD, PRMT1 and ADMA are up-regulated, however, sirt1 and NAD+ are down-regulated, than in moderate LVD (Figure 1, A-D). A significant negative correlation exists between ADMA concentration and PRMT1 mRNA expression versus LVEF (Figures 2A and 2C). A significant positive correlation exists between NAD+ concentration versus LVEF and sirt1 mRNA expression versus LVEF (Figures 2B and 3D). These observations suggest that elements associated with arginine methylation (PRMT1 and ADMA) are up-regulated, however, cardioprotective elements (sirt1 and NAD+) are down-regulated, as LVEF decreases.

Multivariate linear regression analysis models are derived to investigate the relationships between gene expression, biochemical analytes, and LVEF (Table 2). Before or after adjusting for demographic factors and cardiovascular models, all the models showed that LVEF was significantly associated with PRMT1 gene expression and circulating analytes (ADMA, sirt1, and NAD+). Therefore, changes in the gene expression of PRMT1 and sirt1 and circulating analytes could be associated with LVEF.

Figure 1. Comparison of concentrations of circulating analytes (ADMA (A) and NAD+ (B)) and gene expression levels (PRMT1 (C) and Sirt1 (D)) in peripheral blood of dilated cardiomyopathy patients according to left ventricular ejection fraction (LVEF). Group A - moderate LV dysfunction with LVEF 31 – 45%; Group B – severe LV dysfunction with LVEF < 30%; AU – arbitrary units; ADMA – asymmetric dimethylarginine; NAD+ – nicotinamide adenine dinucleotide; PRMT1 – protein arginine methyltransferase1; Sirt1 – sirtuin1. Data are presented as mean ± SD. *P<0.05.

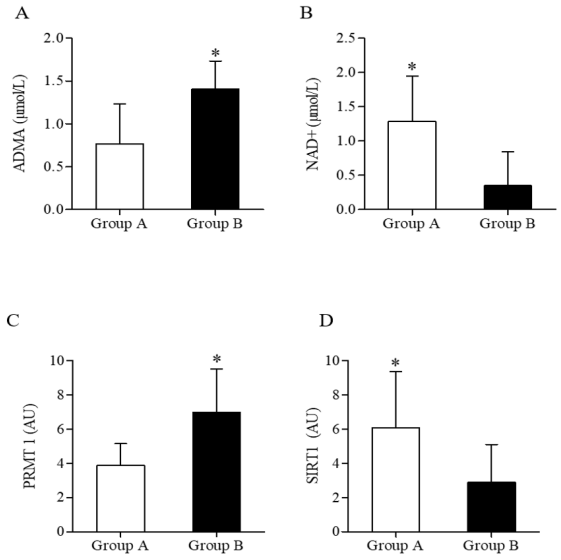
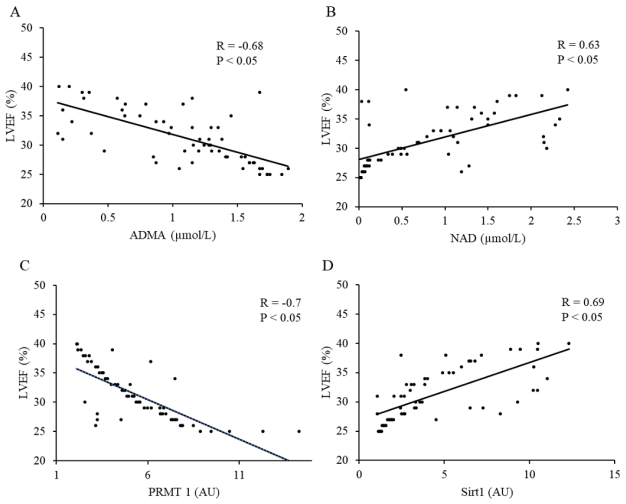


Figure 2. The associations between percentage of the left ventricular ejection fraction and circulating analytes (ADMA (A) and NAD+ (B)) and peripheral blood mRNA expression (PRMT1 (C) and Sirt1 (D)) in dilated cardiomyopathy patients (n=60, Spearman correlation analysis). AU – arbitrary units; ADMA – asymmetric dimethylarginine; NAD+ – nicotinamide adenine dinucleotide; PRMT1 – protein arginine methyltransferase1; Sirt1 – sirtuin1.



INDEX	MODEL 1			MODEL 2			MODEL 3		
	β	95% CI	P-value	β	95% CI	P-value	β	95% CI	P-value
ADMA	-0.07	-0.09, -0.05	1.19E-09	-0.07	-0.09, -0.05	4.54E-10	0.07	-0.09, -0.05	1.12E-09
PRMT 1	-0.42	-0.51, -0.32	3.55E-12	-0.41	-0.51, -0.31	1.24E-11	-0.40	-0.49, -0.30	1.53E-11
NAD+	0.10	0.06, 0.13	6.48E-08	0.10	0.07, 0.13	5.41E-08	0.10	0.07, 0.13	5.68E-08
Sirt1	0.48	0.35, 0.62	6.98E-10	0.10	0.07, 0.13	5.41E-08	0.48	0.34, 0.61	2.09E-09

Table 2. Multivariate Regression Analysis. ADMA – asymmetric dimethylarginine, NAD+ – nicotinamide adenine dinucleotide+, PRMT1 – protein arginine methyltransferase1, Sirt1 – sirtuin1. Model 1: no covariate; Model 2: includes age and gender as covariates; and Model 3: includes age, gender and traditional cardiovascular risk factors (type-2 diabetes, arterial hypertension) as covariates.

DISCUSSION

The current study shows the differences in the expression of cardiac genes (PRMT1 and sirt1) in PBCs and circulating concentrations of biochemical analytes (ADMA and NAD+) appear to be associated with LV function in DCM. Our study provides data for the first time, that suggests, in DCM patients, when LVEF decreases i) PRMT1 mRNA expression is upregulated in blood cells and that it could be an underlying factor for the excess circulating ADMA concentration; ii) PBC sirt1 mRNA expression and circulating NAD+ concentration are down-regulated; iii) a correlation exists between the transcript levels of genes (PRMT1 and sirt1) and LVD. These observations imply that changes in PBC mRNA expression and concentration of circulating analytes indicate the disease severity in terms of LV function and that it is possible that peripheral blood (PBCs and plasma) could be an alternative sample for the gene expression analysis (of PRMT1 and sirt1) and to monitor the disease progression in DCM; and iii) Multivariate regression analysis revealed that the increase in PRMT1 mRNA expression, decrease in sirt1, elevated ADMA concentration and decrease in NAD+ concentration are independent predictive factors for the decrease in LVEF, suggesting that the elements of arginine methylation (PRMT1 and ADMA) and cardioprotective factors (sirt1 and NAD+) have an antagonistic effect on the LV function. Therefore, it is possible that the components of arginine methylation and cardio-protection could predict LVD.

PRMT1 – ADMA – LVEF: As for the molecular mechanism that leads to excess ADMA, our data indicates that PRMT1 gene expression is upregulated in severe LVD than in moderate LVD. Studies have shown that i) ADMA accumulation is an indicator of excess systemic OS; OS is upregulated in failing hearts[8] and iii) excess OS triggers PRMT1 expression and thus ADMA accumulation.[9] Based on this evidence and our observations, excess systemic OS may lead to increased PRMT1 mRNA, which results in excess circulating ADMA. On the link between ADMA and LVEF, ADMA alters the LV structure and function, and remodeling processes. ADMA is one of the biomarkers of myocardial injury and remodeling in HF and ADMA is an independent risk factor for several pathological conditions that contribute to the development of HF, including hypertension and coronary artery disease.[10] Hence, it is possible that in DCM upregulated PRMT1 expression regulates LVEF via excess ADMA biosynthesis.

Sirt1 – NAD+ – LVEF: Reduced myocardial sirt1 expression[11] and NAD+ concentration12 contribute to the pathogenesis of DCM6.[13] Our observation shows that sirt1 expression and plasma NAD+ concentration are impaired, wherein severe LVD (group B) had significantly lower sirt1 and NAD+ than the moderate LVD (group A). This finding indicates that reduced sirt1 mRNA in blood cells and, or diminished circulating NAD+ concentration, may reflect LV systolic dysfunction.

Physiologically, sirt1 and NAD+ elicit cardioprotection by detoxifying OS and regulating energy production. Under excess OS, as in DCM and HF, surplus free radicals attenuate NAD+ and sirt1. These effects cumulatively result in cardiac remodelling and impaired cardiac performance.[14] Thus, in this study, it is possible that reduced sirt1 mRNA and, or NAD+ concentration could mediate the changes in redox imbalance, which affects the LVEF and results in LVD in DCM.

CONCLUSION

In DCM patients, LVEF deterioration appears to be associated with two events: alterations in the i) expression of genes associated with arginine methylation and cardioprotection in PBCs, and ii) circulating concentrations of ADMA and NAD+.

LIMITATIONS

1. A Major limitation is the small sample size.
2. Instead of assessing the direct enzyme activities of PRMT1 and sirt1, indirect activities were determined.
3. We could not determine the cause of the relationship between altered mRNA expressions and deteriorating LVEF, as this investigation was cross-sectional.
4. We could not establish the role of the DCM milieu in the levels of mRNA and analytes because healthy control subjects were not recruited in this study.

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