



# Reduced SIRT1 expression correlates with enhanced oxidative stress in compensated and decompensated heart failure

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## ABSTRACT

Sirtuin-1 (SIRT1) is a longevity factor in mammals initiating the cell survival mechanisms, and preventing ischemic injury in heart. In the etiopathogenesis of heart failure (HF), impairment in cardiomyocyte survival is a notable factor. Oxidative stress comprises a critical impact on cardiomyocyte lifespan in HF. The aim of the present study was to investigate SIRT1 expression in patients with compensated (CHF) and decompensated HF (dHF), and its correlation with oxidative stress. SIRT1 expression in peripheral leukocytes was examined using quantitative RT-PCR in 163 HF patients and 84 controls. Serum total oxidant status (TOS) and total antioxidant status (TAS) were measured via colorimetric assays, and oxidative stress index (OSI) was calculated. Lipid parameters were also determined by routine laboratory methods. SIRT1 mRNA expression was significantly downregulated in HF with more robust decrease in dHF ( $p=0.002$ , control vs CHF;  $p<0.001$ , control vs dHF). Markedly increased oxidative stress defined as elevated TOS, OSI and low TAS levels were detected in HF patients comparing with the controls (TAS;  $p=0.010$ , control vs CHF,  $p=0.045$  control vs dHF, TOS;  $p=0.004$  control vs CHF;  $p<0.001$  control vs dHF, OSI;  $p<0.001$  for both comparisons, respectively). With SIRT1 expression levels, TAS, TOS, OSI, and high density lipoprotein levels in CHF and dHF were determined correlated. SIRT1 expression were significantly reduced in both HF subtypes, particularly in dHF. SIRT1 expression was correlated with the oxidant levels and antioxidant capacity. Data suggest that SIRT1 may have a significant contribution in regulation of oxidant/antioxidant balance in HF etiology and compensation status.

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## 1. Introduction

Sirtuin family comprising of a specific group of highly conserved, functional histone deacetylases has indisputable importance in many critical pathways in living organisms. Among the total of seven members in mammals, Sirtuin-1 (SIRT1; silent mating type information regulation two homolog-1) gene encodes the largest member of sirtuin family proteins in human, SIRT1 enzyme, sharing the closest homology with yeast silent information regulator 2 (Sir2) [1]. SIRT1 is a NAD<sup>+</sup>-dependent class III

histone/protein deacetylases, and is suggested to ensure the protection of chromatin structure [2]. Actual roles of human sirtuins have not yet been fully documented, but functions of their yeast homolog suggest their importance in cell biology. SIRT1 demonstrates crucial functions in wide-ranging cellular processes including cell survival, apoptotic pathways, oxidative stress and regulation of gene expression [2–4]. SIRT1 gene product mediates cellular metabolic functions, and serves as an important regulator of cellular response to stress conditions through mediating antioxidant mechanisms [5,6]. In heart, SIRT1 is a longevity factor protecting cells against apoptosis, and mediating survival of cardiac myocytes under *in vitro* stress conditions [3,4].

Heart failure (HF) is a major cardiovascular disease characterized with a progressive decline in cardiac functions. This condition

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is one of the most significant public health concern today causing remarkable mortality and morbidity worldwide. Main etiopathogenetic factors responsible for the development of HF are varying in a range between hemodynamic/ischemic factors to some genetic mutations [7]. It has been well documented that many of those underlining etiologic factors involve redox imbalance at their background, and oxidative stress increased in HF [8,9]. Oxidative stress is a well-established phenomenon participating in numerous diseases and complications in the organ systems, and the cardiovascular system is not an exception [10].

Based on the symptoms and physical examination, HF is divided into two main categories: compensated HF (CHF) and decompensated HF (dHF). In the literature, more evidence is required to better understand the nature of those two conditions to develop better therapeutic approaches. In patients with HF having multifactorial causes in the etiology, the role of SIRT1 gene has not been clearly documented to date. It is important to determine expression level of SIRT1 in patients with HF, and verify its possible correlation with oxidative stress. Thus, the current study aimed to investigate differences, if any, between CHF and dHF with a closer attention to the redox capacity and determine SIRT1 gene expression levels in patients with either CHF or dHF, and to show its correlation with oxidative stress.

## 2. Materials and methods

### 2.1. Subjects

One hundred and sixty-three patients with HF and 84 sex- and age-matched healthy controls enrolled to the study were divided into 3 categories as CHF, dHF and control groups. The patients were chosen among the ones applied to the cardiology clinics at Harran University Hospital, Ahmet Inan Education and Research Hospital with cardiac failure symptoms, diagnosed with acute dHF and hospitalized at coronary intensive care unit or diagnosed with CHF with no hospitalization requirement within the last month. The study was planned and performed according to the local and international ethical criteria, and was approved by the Institutional Review Board of Harran University School of Medicine on January 13<sup>th</sup>, 2014. Informed consent was obtained from all patients and controls who participated to the study. The first group consisted of 84 patients with CHF with ejection fraction (EF) <40%, who came to the cardiology clinics for routine monitorization, with no acute coronary event or not required hospitalization for decompensation of failing heart within the last month. The second group, diagnosed with dHF, consisted of 79 patients having EF <40%, and hospitalized at coronary intensive care unit. The last group was control formed by sex- and age-matched 84 healthy volunteers with EF ≥ 50%. Exclusion criteria included a hospitalization within the last month due to acute coronary syndromes or decompensation for the patients with HF, having a neurodegenerative disease with decreased SIRT1 expression; having EF lower than 50% despite no diagnosis with HF and a critical infection history within 1 month.

### 2.2. Blood samples, serum and leukocyte isolation

Nine ml blood was drawn by venipuncture at the antecubital vein from the patients and controls, and collected into EDTA tubes (5 ml) and standard blood collection tubes (4 ml) separately for leukocyte isolation and analysis of oxidative stress markers. Peripheral blood leukocytes were isolated by density gradient centrifugation within the first 2 h of collection and stored at −80 °C until analysis. For serum isolation, following 1 h of incubation at room temperature, blood samples were centrifuged at 700 g for

15 min, and sera samples were collected and stored at −80 °C until analysis.

### 2.3. Real-Time quantitative PCR (RT-qPCR)

RNA was obtained from blood samples of patients and controls. Isolation of RNA from blood was performed using QIAamp RNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). Total RNA quality and quantity were determined using Epoch Micro-Volume Spectrophotometer System (BioTek, Winooski, United States). cDNA synthesis from total RNA was performed using RT2 First Strand Kit (Qiagen).

Commercially available, ready-to-use primers for SIRT1 (catalog number: PPH02188A-200) and housekeeping gene  $\beta$ -actin (ACTB; catalog number: PPH00073G-200) were purchased (SABiosciences, Qiagen, Germany). cDNA was amplified using RT<sup>2</sup> SYBR Green ROX FAST Mastermix (Qiagen GmbH, Hilden, Germany) with primer pairs for SIRT1 and  $\beta$ -actin (SABiosciences). qPCR was performed using a Rotor Gene 6000 Real-Time PCR Machine (Qiagen GmbH, Hilden, Germany). The amplification conditions were as follows: a 15 min preincubation at 95 °C followed by 40 cycles of 15 s at 95 °C and one minute at 60 °C. SIRT1 expression levels were normalized to the corresponding  $\beta$ -actin gene expression levels, and calculated relative to the mean level in those acquired from the healthy controls by the comparative  $2^{-\Delta\Delta CT}$  method [11].

### 2.4. Measurement of TAS, TOS and calculation of OSI

Serum TAS and TOS levels were determined using commercially available kits (Rel Assay Diagnostics; Mega Tip, Gaziantep, Turkey), and Oxidative Stress Index (OSI) were calculated. Serum TAS and TOS levels were determined colorimetrically using Spectramax M5 Microplate Reader (MV05047, Molecular Devices in Sunnyvale, CA) with measurement methods developed by Erel. Serum TAS levels were presented as  $\mu$ mol Trolox equiv./Liter [12,13]. Serum TOS assay was calibrated with hydrogen peroxide ( $H_2O_2$ ) and the results were expressed in  $\mu$ mol  $H_2O_2$  equivalent/l ( $\mu$ mol  $H_2O_2$  equiv./Liter) [14]. As a reliable index of oxidative stress in the body, OSI was calculated as described in literature, and presented in arbitrary units [15].

### 2.5. Statistical analyses

Data was analyzed with SPSS 21 statistics software package (IBM software, Pointe Claire, Quebec, Canada). Distribution patterns of the groups were analyzed using the one-sample Kolmogorov–Smirnov test. Parametric variables between the groups were compared using one way ANOVA. Multiple comparisons were analyzed using Bonferroni post hoc test. Sex distribution, the distribution of individuals with DM, HT and smoking status in groups were evaluated using Chi-Square test. Covariance between SIRT1 and interested variables was tested applying Pearson Correlation Coefficient. The results were presented as means  $\pm$  SD if not stated otherwise. Differences were considered significant for  $P < 0.05$ .

## 3. Results

A total of 84 patients diagnosed with CHF, 79 patients with dHF and 84 age- and sex-matched controls were included to the study. The mean ( $\pm$  SD) ages, sex, waist circumference and calculated body mass index (BMI) values, along with lipid profiles and fasting glucose levels are presented in Table 1.

Triglyceride levels were significantly elevated in the control

**Table 1**  
Demographic and clinical characteristics of the study groups.

	Control n=84	CHF n=84	dHF n=79	P value	ANOVA
Age (years)	58.3 ± 6.8	61.1 ± 13.6	61.9 ± 11.7	0.090	NS
Male/Female (n) <sup>a</sup>	39/45	51/33	39/40	0.148	
BMI (kg/m <sup>2</sup> )	26.9 ± 2.2	27.1 ± 4.0	27.4 ± 4.3	0.686	NS
Waist C. (cm)	97.6 ± 5.8	99.7 ± 11.5	99.2 ± 13.8	0.428	NS
Triglyceride (mg/dl)	149.8 ± 75.0	145.4 ± 67.5	122.2 ± 65.5	0.036 <sup>a</sup>	
T. Cholesterol (mg/dl)	191.4 ± 37.3	175.7 ± 38.8	153.5 ± 33.9	< 0.001 <sup>b</sup>	
LDL (mg/dl)	120.7 ± 32.5	113.7 ± 31.3	95.1 ± 27.5	< 0.001 <sup>c</sup>	
HDL (mg/dl)	40.7 ± 9.7	38.5 ± 9.3	36.5 ± 11.9	0.025 <sup>b</sup>	
Hypertension <sup>a</sup> , n (%)	13(15.5%)	24(28.6%)	21(26.6%)	0.099	
DM <sup>a</sup> , n (%)	8(9.5%)	14(16.7%)	17(21.5%)	0.107	
Smoking <sup>a</sup> n (%)	12(14.3%)	15(17.9%)	18(22.8%)	0.371	

<sup>a</sup> Chi-square test. n, number of individuals; The results are shown as mean ± SD. BMI, body mass index; Waist C., waist circumference; T. Cholesterol, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; DM, diabetes mellitus; CHF, compensated heart failure; dHF, decompensated heart failure.

<sup>a</sup>  $p=0.047$  (Control vs dHF).

<sup>b</sup>  $p<0.001$  (Control vs dHF);  $p=0.021$  (control vs CHF);  $p=0.001$  (CHF vs dHF).

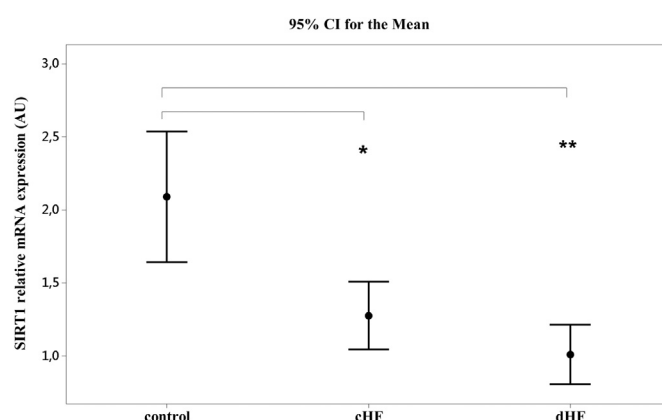
<sup>c</sup>  $p<0.001$  (Control vs dHF);  $p=0.001$  (CHF vs dHF).

<sup>d</sup>  $p=0.02$  (Control vs dHF).

group than those in dHF ( $p=0.047$ ). Total cholesterol levels were significantly increased in the control group comparing with both CHF ( $p=0.021$ ) and dHF ( $p<0.001$ ). The same parameter was significantly elevated in patients with dHF in compared to those with CHF ( $p=0.001$ ). Low density lipoprotein (LDL) levels were significantly higher in both control and CHF groups in compared to dHF group ( $p\leq 0.001$  for both comparisons). For total cholesterol and LDL, all measurements were within the normal ranges in respect to the laboratory cut-off values. HDL levels were significantly lower in the patients with dHF than those in the control individuals ( $p=0.02$ ). There was no statistically significant difference in waist circumference and BMI among the groups.

The mean serum TAS levels in the control, CHF and dHF groups were  $1.17 \pm 0.19$ ,  $1.09 \pm 0.14$  and  $1.10 \pm 0.21$  mmol Trolox Equiv. Liter<sup>-1</sup>, respectively. Antioxidant levels were significantly decreased in the patients with HF comparing with the controls, particularly in the compensated group ( $p=0.007$ ). The mean TOS levels in the control, CHF and dHF are  $13.96 \pm 1.83$ ,  $15.56 \pm 3.55$ ,  $15.96 \pm 3.87$   $\mu\text{mol H}_2\text{O}_2$  Equiv. Liter<sup>-1</sup>, respectively ( $p<0.001$ ). TOS levels were significantly elevated in the HF groups in compared to those in the control group. The mean OSI level calculated based on TAS and TOS levels, a representation of general redox status in the body, was significantly elevated in both the patients with compensated and decompensated HF ( $p<0.001$ ) (Table 2).

The expression levels of SIRT1 mRNA were evaluated in peripheral leukocytes harvested from the patients and controls, and the results were expressed in arbitrary units (AU). The mean SIRT1 mRNA levels were 2.1 (95% CI 1.64–2.52), 1.3 (95% CI 1.07–1.55)



**Fig. 1.** Relative SIRT1 mRNA expression levels in the study groups. SIRT1 expression is reduced in patients with both compensated and decompensated heart failure. One way ANOVA, 95% confidence interval plots are displayed for the mean SIRT1 expression levels in arbitrary units (AU). \*  $p=0.002$  control vs compensated HF; \*\*  $p<0.001$  control vs decompensated HF.

and 1.0 (95% CI 0.81–1.20) AU in the control, CHF and dHF groups, respectively (Fig. 1). SIRT1 expression was significantly attenuated in HF with a particular decrease in the dHF group without reaching the significance between the HF subgroups ( $p=0.002$  control vs CHF;  $p<0.001$  control vs dHF).

Pearson's product moment correlation coefficient was used to assess a possible covariance between the SIRT1 expression levels and measured oxidative stress along with the recorded lipid parameters. For compensated HF, SIRT1 expression was negatively correlated with OSI ( $r=-0.348$ ,  $p=0.001$ ) and TOS ( $r=-0.218$ ,  $p<0.046$ ), and positively correlated with TAS ( $r=0.333$ ,  $p=0.002$ ). Additionally, between HDL cholesterol levels and SIRT1, a positive correlation ( $r=0.459$ ,  $p<0.001$ ) was determined within the compensated HF group.

On the other hand, a similar pattern to above mentioned group in the correlation study was shown in the dHF group. SIRT1 expression levels have shown a negative correlation with OSI and TOS, a positive correlation with TAS and HDL levels within this group ( $r=-0.560$ ,  $p<0.001$ ;  $r=-0.342$ ,  $p=0.002$ , and  $r=0.546$ ,  $p<0.001$ ;  $r=0.486$ ,  $p<0.001$ , respectively).

#### 4. Discussion

SIRT1 is a nuclear protein possessing many key roles in a range of critical cellular pathways including cellular senescence,

**Table 2**  
Oxidative stress parameters of the three groups defined as TAS, TOS and OSI.

	Control n=84	CHF n=84	dHF n=79	p value
TAS (mmol trolox equiv. l <sup>-1</sup> )	1.17 ± 0.19	1.09 ± 0.14	1.10 ± 0.21	0.007 <sup>a</sup>
TOS ( $\mu\text{mol H}_2\text{O}_2$ equiv. l <sup>-1</sup> )	13.96 ± 1.83	15.56 ± 3.55	15.96 ± 3.87	< 0.001 <sup>c</sup>
OSI [arbitrary units (AU)]	1.22 ± 0.26	1.45 ± 0.38	1.50 ± 0.47	< 0.001 <sup>d</sup>

TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index. CHF, compensated heart failure; dHF, decompensated heart failure. The results are shown as mean ± SD.

<sup>a</sup>  $p=0.010$  control vs CHF;  $p=0.045$  control vs dHF.

<sup>c</sup>  $p=0.004$  control vs CHF;  $p<0.001$  control vs dHF.

<sup>d</sup>  $p<0.001$  control vs CHF, and control vs dHF.

apoptosis, response to oxidative stress and gene expression control [3,16]. Studies investigating the functions of SIRT1 in cardiac system are increasing, but key functions of SIRT1 expression in heart have not been clearly shown as of today. It has been reported that SIRT1 increases tolerance of heart against ischemia and oxidative stress enhancing endothelial nitric oxide synthase (eNOS) [17]. SIRT1 also functions preventing cardiac hypertrophy and showing beneficial effects on lipid metabolism via enhancing the activation of peroxisome proliferator-activated receptor alpha [18]. A study with transgenic mice has revealed that SIRT1 expression decreased markedly in ischemic/reperfusion model of heart. It was also shown in the same transgenic model that moderately increased expression of SIRT1 in heart prevented programmed cell death and aging-associated alterations [3]. In the same study, SIRT1 expression of cardiac myocytes has been found upregulated at the end of 4-week period of pressure load.

On the other hand, SIRT1 expression have been shown down-regulated in human left ventricle samples harvested from patients with HF undergoing cardiac transplantation, even though the sample size was quite low [4]. In patients with severe HF subsequent to dilated cardiomyopathy, cardiac SIRT1 expression has been determined significantly downregulated along with enhanced oxidative stress parameters in the harvested tissue samples [19]. In a recent study involving 48 male participants including patients with either acute coronary syndrome or stable coronary artery disease and the controls, SIRT1 mRNA expression has been determined significantly reduced in peripheral monocytes in the patients [20]. Those reports indicate that SIRT1 expression in HF is of interest and yet to be clearly documented to guide in clinical approach. The current study was conducted on a total of 163 patients with either compensated or decompensated HF, and SIRT1 transcript expression has been found significantly decreased in harvested leukocytes, especially in patients with dHF even though the difference did not reach the significance. Lower levels of SIRT1 expression in the cells could be related to increased susceptibility to cellular oxidative stress based on the literature.

It is now a known fact supported through a number of clinical and bench studies that oxidative stress increase in HF, not surprisingly, especially in ischemic type of cardiac failure. Enhanced myocardial oxidative stress, diminished antioxidant levels and disrupted redox balance have been reported in a rat model of mild and severe heart failure developed subsequent to myocardial infarction, and those changes have shown to be ameliorated at some degree via supplementation of an antioxidant, vitamin E [21,22]. Similarly, oxidative stress parameters have been found significantly increased in an animal model of congestive heart failure [23]. In patients with chronic ischemic heart failure, Ellidag et al. reported significantly increased oxidative stress index, and attenuated total antioxidant status [24]. Markedly increased oxidative stress along with inflammation has been documented in a clinical study on patients with severe decompensated congestive HF [25]. In another study conducted on patients with congestive heart failure at varying degrees, elevated oxidant stress indicated as increased lipid peroxidation, and decreased antioxidant reserve were recorded [26].

SIRT1 is a longevity factor with eminent roles in cellular senescence. SIRT1 attenuates oxidative stress and prevent cells against oxidative damage and eventual apoptosis via several mechanisms including the activation of eNOS and Poly (ADP-ribose) polymerase alpha (PARP  $\alpha$ ) in heart. To be noted here, a robust overexpression of PARP $\alpha$  and SIRT1 itself also enhance apoptosis and negatively affect normal functioning of heart [3,4]. In a hamster model of chronic HF, nuclear SIRT1 expression induced by resveratrol, a SIRT1 activator, increased Mn-SOD levels in cardiomyocytes and potentiated resistance against oxidant load, reduced oxidative stress and suppressed cell death [27]. Additionally,

patients with cardiovascular disease carrying certain SIRT1 gene polymorphisms have been found to be associated with increased TOS and OSI levels suggesting the protective effect of SIRT1 against oxidative stress in heart [28]. SIRT1, along with SIRT3, are known to enhance anti oxidative mechanisms in heart to increase scavenger capacity against redox imbalance, and modulate cardiomyocyte longevity through a number of mechanisms (reviewed in [27]).

In the present study, our results were in concordance with the literature confirming that redox balance is disturbed in the patients with HF, without a significant difference between the subgroups. We have shown significantly enhanced oxidative stress indicated by elevated TOS and OSI levels and markedly decreased TAS level in both CHF and dHF groups suggesting an unbalanced redox status in HF independent of the compensation condition. Interestingly, we reported here that there was no significant difference in TAS and TOS levels between compensated and decompensated groups although SIRT1 expression levels seemed to be more severely reduced in patients with dHF. It may be a masked condition achieved by rush antioxidant therapies along with tight diet modifications applied to the patients with dHF during their stay at the hospital as a supplemental support to the main cardiac therapies. Unfortunately, there was no available information on the pharmacological treatments given to the patients along with the main cardiac therapies.

Studies on SIRT1 and serum lipid profile are relatively new in literature. It was described in a recent study involving randomly selected Japanese volunteers that a SIRT1 allele carrying a set of distinct single nucleotide polymorphisms was found associated with low LDL and enhanced HDL levels suggesting a link between SIRT1 expression and serum lipid profile in the same population [29]. In a study conducted on Ashkenazi Jews, some SIRT1 polymorphisms were found associated with HDL and LDL sizes but not with longevity [30]. Interestingly, in an *in vitro* study with peripheral monocytes harvested from patients with coronary artery disease, Breitenstein et al. [20] reported that HDL taken from healthy subjects induced SIRT1 expression in monocytes more prominently than HDL taken from patients with cardiac disease suggesting a signal mediating interaction between HDL and SIRT1, and disruption of this healthy interaction in patients with cardiac problems. Based on the output of the present study, serum HDL levels were significantly reduced in the patients with dHF in compared with the controls. Even though not reaching at the significance, HDL levels were lower in dHF group than those in CHF group. HDL levels also showed a positive correlation with SIRT1 expression levels in both CHF and dHF groups. A similar results reported by Breitenstein et al. between HDL levels and SIRT1 expression in patients with coronary artery disease [20]. Although it needs more studies to delineate its function, HDL may be an amelioration factor in the disease course in HF.

Increased LDL and total cholesterol levels detected in control and CHF groups comparing with the dHF group revealed a complicated outcome, since the data may imply some specific medical care given to those patients during their follow-up period, although we did not record any dyslipidemia treatment. Regarding with serum triglyceride levels, patients with CHF have had higher levels than those in dHF and control groups. To be noted here, lipid measurements recorded in the patients and controls during the study were within the normal ranges according to the clinical data and laboratory cut-off values. More likely explanation to those results would be attributed to the tight diet modifications and daily life changes suggested to our study patients due to their health conditions.

In conclusion, we have reported here that the expression levels of SIRT1 transcript in peripheral blood leukocytes significantly decreased in patients with compensated and decompensated HF,



particularly in decompensated group although the difference did not reach the significance. Redox balanced was disturbed in HF, and evaluated SIRT1 mRNA levels correlated negatively with OSI and TOS, and positively with serum TAS and HDL levels in both patient groups with CHF and dHF. Reduced expression of SIRT1 in peripheral leukocytes may be associated with the compensation status of HF since slightly further decrease of SIRT1 expression was determined in dHF as compared to CHF and controls.

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