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Research Article

Alteration of metabolic genes in peripheral blood isolated from patients with acute myocardial infarction

Eman F. Wasfey[a], Rania S. Abd El-Razik[a], Nadia M. Hamdy[a], Wael M. El-Kilany[b], Hala O. El-Mesallamy[a*]

[a]*Department of Biochemistry, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt*

[b]*Department of Cardiology, Faculty of Medicine, Ain Shams University, Cairo, Egypt*

ABSTRACT

Accumulating evidence suggests that molecular alterations of peripheral leucocytes are possible diagnostic markers of acute myocardial infarction (AMI). Changed lipid/glucose metabolism is a prominent feature of the pathogenesis of AMI. Silent mating type information regulation 2 homolog 1 (*SIRT1*) and peroxisome proliferator-activated receptor gamma coactivator factor-α (*PGC-1α*) regulate mitochondrial function and energy metabolism. Thus, the gene expression of *SIRT1* and *PGC-1α* in peripheral leucocytes isolated from AMI patients and their association with dyslipidemia have been evaluated. Fifty-five male subjects were divided into 40 patients with AMI and 15 healthy control subjects. Peripheral blood samples were obtained on the first day of AMI. Lipid profile parameters were assessed spectrophotometrically. Relative *SIRT1* and *PGC-1α* expression were measured by real-time PCR. Compared with the control group, *SIRT1* and *PGC-1α* expression were significantly decreased in the AMI group. *SIRT1* expression was significantly negatively correlated with the age of the participants. SIRT1 expression was significantly positively correlated with *PGC-1α* expression. Both *SIRT1* and *PGC-1α* expression were negatively correlated with markers of dyslipidemia. In conclusion, *SIRT1* and *PGC-1α* expression are reduced in the acute phase of AMI, which addresses their possible role as potential biomarkers for AMI.

Keywords: *AMI; gene expression; PGC-1 alpha; SIRT1; mitochondrial dysfunction*

***Correspondence** | Prof. Dr. Hala O. El-Mesallamy, Department of Biochemistry, Faculty of Pharmacy, Ain Shams University, African union organization street Abassia, Cairo 11566, Egypt. Email: hala_elmosalamy@hotmail.com

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1. INTRODUCTION

Ischemic heart disease (IHD) is a major cause of death and disability worldwide. Acute myocardial infarction (AMI) is responsible to the major percent of IHD deaths due to its accompanied fatal complications[1]. Recently, gene expression studies are gaining attention in revealing potential molecular biomarkers for AMI detection[2,3]. Peripheral leucocytes were found to exhibit an alteration in their gene expression profile in patients with AMI[4].

Disrupted lipid/glucose metabolism is associated with the oxidative stress and inflammatory response encountered in the pathophysiology of atherosclerosis and plaque instability[5]. Moreover, AMI is associated with

augmented fatty acid (FA) oxidation and increased myocardial oxygen consumption resulting in complications such as arrhythmias or reduced cardiac contractility[6].

Silent information regulator factor 2-related enzyme 1 (*SIRT1*) is NAD^+ dependent deacetylase, which can deacetylate both histone and non-histone proteins to regulate multiple cellular functions including energy metabolism, autophagy, apoptosis, and cellular senescence[7]. SIRT1 controls the cellular energy metabolism through the deacetylation and activation of the master metabolic regulator, the peroxisome proliferator-activated receptor gamma coactivator factor-α (*PGC-1α*)[8]. *PGC-1α* upregulates the expression of several genes of the tricarboxylic acid cycle and mitochondrial FA oxidation pathway[9]. Additionally, the *SIRT1/PGC-1α* pathway plays a role in regulating the activity of antioxidant enzymes[10].

Previous reports described the role of *SIRT1/PGC-1α* in protecting against myocardial ischemia in which activation of *SIRT1* and/or *PGC-1α* was associated with smaller infarct size and better post-AMI outcome[11-13]. However, this study was conducted to address the possible diagnostic role of *SIRT-1* and *PGC-1α* expression in peripheral leucocytes isolated from AMI patients and the association between these markers and the dyslipidemic profile observed in AMI patients.

2. SUBJECTS AND METHODS

2.1. Subjects

A total of 55 men were enrolled in the study, 40 were AMI patients and 15 were healthy control subjects. The blood was collected on the first day of the infarction. Patients were recruited from the Intensive Care Unit, Cardiology Department, Ain Shams University Educational Hospitals, Cairo, Egypt. Exclusion criteria included inflammatory diseases, autoimmune disorders, malignancies, hematological diseases, skeletal muscle diseases, hepatic or renal diseases, acute or chronic infections, or administration of immunosuppressive drugs.

The study was conducted in accordance with the regulations and recommendations of the Declaration of Helsinki and was approved by the Faculty of Pharmacy, Ain Shams University Ethical committee (Ph.D.: no.22). An informed consent was obtained from all participants.

2.2. Sample preparation

Fasting peripheral blood samples were obtained from all participants. Samples were divided into 2 aliquots; the first aliquot was collected on plain vacutainer for lipids profile analysis. The second aliquot was collected on EDTA for immediate total RNA purification from the human whole blood.

2.3. Lipid profile analysis

Lipids profile parameters were measured colorimetrically by enzymatic methods[14-18] according to kits provided by Salucea, Netherlands using (Schimatzu 1650 UV/Visible, Japan).

2.4. RNA isolation and real-time quantitative PCR

Total RNA was extracted and purified from human whole blood using a QIAamp RNA blood mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol and stored at -80 °C until use. The mRNA expression levels of *PGC-1α, SIRT1* and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) as an internal control were measured by quantitative one-step QuantiFast Probe QRT-PCR Kit (Qiagen, Hilden, Germany) using Stratagene MX 3000P thermal cycler (Stratagene, USA) according to the manufacturer's protocol. The analysis was performed using the MxPro-Mx 3000P software

(Stratagene, USA). The expression levels in an unknown sample were normalized and analyzed by the $2^{-\Delta\Delta Ct}$ methods, where ΔΔCt = (Ct target gene − Ct GAPDH) sample − (Ct target gene − Ct GAPDH) calibrator.

2.5. Statistical Analysis

The IBM statistical package for social sciences (SPSS) statistics (V.23, IBM Corp., USA, 2015) and GraphPad Prism 7 (La Jolla, CA, USA) were used for data analysis. Data were presented as mean±SEM. Comparisons between groups were done using Students t-test. Spearman correlation test was used for correlation analysis. $p<0.05$ was considered statistically significant.

3. RESULTS

3.1. Clinical characteristics

Demographic and clinical characteristics of all participants are shown in **Table (1)**. Total cholesterol (TC), triacylglycerol (TAG) and low-density lipoprotein-cholesterol (LDL-C) were significantly higher in the AMI group when compared with the control group (all p= 0.000). On the other hand, the high-density lipoprotein cholesterol (HDL-C) was significantly lower in AMI patients in comparison with the control group (p= 0.008).

3.2. *SIRT1* expression

As shown in **Fig. 1**, *SIRT1* relative expression showed a significant decrease in the AMI group (0.49±0.03) compared with the control group (1.07±0.12) at p<0.0001.

3.3. *PGC-1α* expression

As depicted in **Fig. 2**, *PGC-1α* relative expression was significantly lower in the AMI group (0.76±0.7) compared with the control group (1.34±0.12) at p<0.001.

3.4. Correlation

As demonstrated in **Table 2**, *SIRT-1* expression was significantly negatively correlated with the age of the participants (*r*= -0.276, p=0.04). In addition, *SIRT-1* showed significant negative correlations with TC and LDL-C (*r*= -0.498, p= 0.000; *r*= -0.461, p= 0.000, respectively), but not with TAG or HDL-C (*r*= -0.258, p= 0.057; *r*= 0.100, p= 0.467, respectively).

PGC-1α expression was significantly negatively correlated with TAG, TC, and LDL-C (*r*= -0.369, p= 0.006; *r*= -0.327, p= 0.015; *r*= -0.306, p= 0.023, respectively) with no significant correlation with the age or HDL-C (*r*= -0.125, p= 0.363; *r*= 0.038, p= 0.781, respectively). **Fig. 3** shows the significant positive correlation between *SIRT1* expression and *PGC-1α* expression (r= 0.279, p= 0.039).

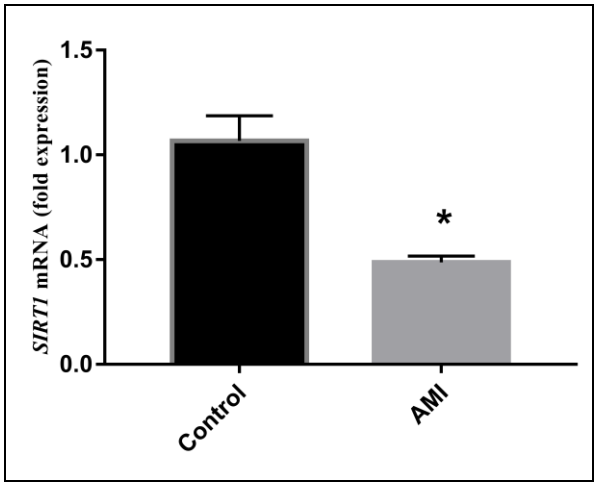

Fig.1. Blood *SIRT1* expression in the studied groups

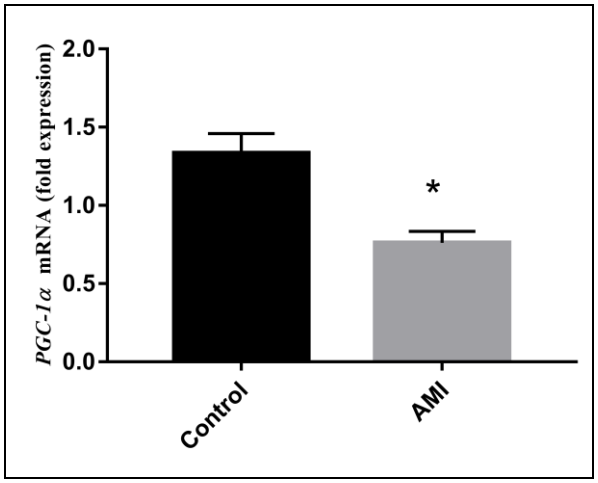

Fig.2. Blood *PGC-1α* expression in the studied groups

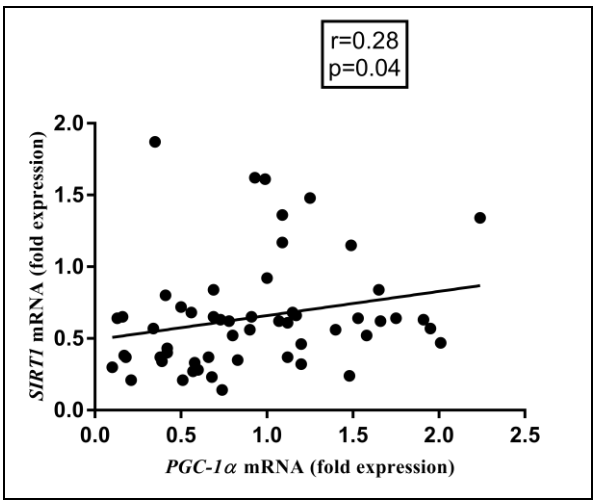

Fig.3. Correlation of blood *SIRT-1* expression with blood *PGC-1α* expression in the studied groups

Table 1. Demographic and clinical characteristics of the studied groups

Groups (n)/Factor	Control -15	AMI -40	p-value
Age (years)	47.47±1.05	50.80±0.81	0.017
BMI (kg/m^2)	27.73±0.27	27. 62±0.17	0.761
TAG (mg/dl)	104.20±6.86	148.43±5.22	0
TC (mg/dl)	180.60±3.91	248.75±5.22	0
LDL-C (mg/dl)	120.40±2.81	178.40±4.47	0
HDL-C (mg/dl)	38.93±67	35.77±0.93	0.008
Diabetes (%)	0 (0%)	22 (55%)	NA
HTN (%)	0 (0%)	16 (40%)	NA
Current smoking (%)	0 (0%)	15 (37.5%)	NA
STEMI (%)	0 (0%)	27 (67.5%)	NA

Numerical data expressed as mean ± standard error, categorical data expressed as number (percentage). AMI, acute myocardial infarction; BMI, body mass index; HDL-C, high density lipoprotein-cholesterol; HTN, hypertension; NA, not applicable; TAG, triacylglycerol; TC; total cholesterol; LDL-C, low density lipoprotein-cholesterol; STEMI; ST segment elevation myocardial infarction.

Table 2. Correlation of *SIRT1* and *PGC-1α* expression with the age and lipid profile parameters of the studied groups

Factor	*SIRT-1* expression	*PGC-1α* expression
Age (years)	-0.276*	-0.125^{NS}
TAG (mg/dl)	-0.258^{NS}	-0.369**
TC (mg/dl)	-0.498**	-0.327*
LDL-C (mg/dl)	-0.461**	-0.306*
HDL-C (mg/dl)	0.100^{NS}	0.038^{NS}

* Significant correlation at p<0.05; ** significant correlation at p<0.01; NS, non-significant; HDL-C, high density lipoprotein-cholesterol; TAG, triacylglycerol; TC; total cholesterol; LDL-C, low density lipoprotein-cholesterol; *PGC-1α*, Peroxisome proliferator-activated receptor-gamma coactivator-1α; *SIRT1*, silent mating type information regulation 2 homolog 1.

4. DISCUSSION

Early identification of AMI is necessary for early treatment interventions, which can significantly reduce the mortality rate. Many studies have been conducted to seek potential molecular biomarkers for AMI detection[2]. Ischemia reduces cellular NAD^+ due to the downregulation of nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme in the NAD^+ synthesis, which leads to the decreased expression of *SIRT1*[19]. This mechanism may probably explain the low blood *SIRT1* expression in AMI patients when compared to the control group. Consistently with our results, Chan *et al.* claimed decreased *SIRT1* mRNA and protein expression in monocytes isolated from patients with CAD[20]. Breitenstein *et al.* showed that peripheral *SIRT1* expression was

significantly lower in both AMI and CAD patients when compared to the healthy subjects, but with no significant difference between both patients groups[21].

Interestingly, the expression of *PGC-1α* in blood was found to be correlated with its myocardial expression. Thus, detection of blood expression of *PGC-1α* may provide a non-invasive method to monitor metabolic changes in the heart during myocardial ischemia[22]. We found lower blood *PGC-1α* in AMI patients when compared with the control group. The myocardial tissue of experimental animals had lower *PGC-1α* expression levels after AMI[23]. In contrary to our study, Fabregat-Andres *et al.* revealed that *PGC-1α* expression is induced in the monocytes of patients with AMI [24].

In this study, a significant negative correlation was observed between *SIRT1* expression and the age of the studied groups. Previously, *SIRT-1* showed significantly lower expression in vascular smooth muscle cells obtained from old donors when compared with young donors with a strong significant negative correlation with the age of the donors[25]. These age-related changes contribute to plaque instability and trigger AMI[26].

Dyslipidemia is a major risk factor for AMI, even more, detrimental for AMI risk in men than in women[27]. *SIRT1* mRNA was negatively correlated with TC and LDL-C. *SIRT1* regulates hepatic lipid metabolism. It inhibits sterol regulatory element binding protein-1c (SREBP-1c) with subsequent inhibition of lipogenic genes which results in inhibition of lipid synthesis and fat storage[28]. Concomitantly, *SIRT1* activates liver X receptor (LXR), thus, enhances reverse cholesterol transport[29]. Therefore, low *SIRT1* expression may be accompanied by dyslipidemia.

The role of *PGC-1α* in augmenting FA oxidation is prominently identified. It stimulates FA transporters expression as well as upregulates the expression of enzymes involved in FA oxidation[30]. Therefore, less FA is available for esterification and TAG formation. This fact comes in agreement with the present study in which *PGC-1α* expression showed a negative correlation with TAG. On the other hand, hepatic cholesterol synthesis was reduced in relation to activation of the *PGC-1α*[31]. The former study may explain the negative association between blood *PGC-1α* and TC levels.

In conclusion, peripheral expression of *SIRT-1* and *PGC-1α* may provide diagnostic keys for patients with AMI. Their pathophysiologic role of the *SIRT-1/PGC-α* pathway in relation to dyslipidemia in AMI patients should be evaluated.

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