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Effects of perilipin-5 on lipid metabolism and high-sensitivity cardiac troponin I

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SUMMARY

OBJECTIVE: Heart attack is one of the most common causes of sudden death in adults. Therefore, early detection of heart attack and investigation of potential new biomarkers are of great importance. We investigated whether perilipin-5 is a potential biomarker by examining changes in perilipin-5 serum levels along with high-sensitivity cardiac troponin I during a heart attack.

METHODS: The subjects were divided into two groups: (1) control group and (2) patients with heart attack, with 150 people in each group. High-sensitivity cardiac troponin I, perilipin-5, total oxidant status, malondialdehyde, reduced glutathione, and superoxide dismutase levels in serum samples were measured. In addition, perilipin-5 mRNA expressions and protein levels were analyzed.

RESULTS: There was no overall statistical difference between the demographic characteristics of the groups. However, high-density lipoprotein, creatine kinase, Creatine kinase myocardial band, aspartate amino transferase, lactate dehydrogenase, and calcium levels were higher in the heart attack group compared to the control group. We found that the high-sensitivity cardiac troponin I and perilipin-5 levels increased in the patients with heart attack ($p<0.0001$) compared to control. Although there was an insignificant increase in malondialdehyde levels in the heart attack group ($p>0.05$), there was a 35.9% increase in total oxidant status levels and a 33.5 and 24.1% decrease in glutathione and superoxide dismutase levels, respectively ($p<0.01$), compared to control. Perilipin-5 mRNA and protein levels in heart attack patients increased by 48.2 and 23.6%, respectively, compared to the control group ($p<0.01$).

CONCLUSION: Our results showed that perilipin-5 together with high-sensitivity cardiac troponin I could be a promising biomarker in heart attack.

KEYWORDS: Perilipin-5. Troponin I. Heart disease. Oxidative stress. Lipid peroxidation.

INTRODUCTION

Cardiac troponins (cTns) and creatine kinase (CK) MB are the most sensitive and specific biochemical markers used in the diagnosis of myocardial damage, cardiac risk stratification, and prognosis[1]. cTns are protein structures responsible for performing the physiological functions of the heart muscle[2]. In the presence of myocardial damage, serum cTn levels are above the reference range[3]. In addition, elevated troponin levels are universally an important component of the diagnosis and treatment of heart conditions, such as myocardial infarction (MI), coronary syndrome, and cardiac ischemia.

High cTns levels can be caused by various non-coronary causes, such as alkaline-phosphatase interaction, fibrin interaction, hemolysis, kidney failure, and instrumentation failure[4]. Along with analytical improvements, high-sensitivity cTn (hs-cTn) allows early detection of cardiomyocyte damage accurately at lower levels and in a shorter time[5]. Although hs-cTn values are important in terms of diagnosis and prognosis, they may lead to elevated hs-cTn and false-positive results in many cardiac and non-cardiac failures[6].

In most tissues, fatty acids (FAs) are converted to triacylglycerol (TAG) and stored as lipid droplets (LDs), comprising a single layer of phospholipid[7]. Perilipin (PLIN) family proteins, which have a tissue-specific expression, consist of five members (i.e., PLIN1, PLIN, PLIN3, PLIN4, and PLIN5) with ~100 amino acid sequence homology at the N-terminals[8]. Of these, PLIN5 plays a significant role in regulating FA metabolism in oxidative tissues, such as the heart, liver, and skeletal muscle[9]. In in vivo study, PLIN5 expression, which enables the storage of LDs in the heart under normal physiological conditions, was shown to cause an increase in pathological conditions and abnormal lipid accumulation in the heart[10].

Biomarkers, such as CK-MB, troponins, and lactate dehydrogenase (LDH), which cause an increase in serum with cardiomyocyte damage accompanying a heart attack, sometimes cause false-positive results. This case prevents the detection of the current situation or causes misdiagnosis. Since PLIN5 is a heart-specific molecule, it is possible to expect an increase in serum levels with cellular damage during heart attack once other

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conditions (as stated in the exclusion criteria) that may cause interference are eliminated. Thus, in this study, we hypothesized that PLIN5 levels during heart attack increased and released into the circulation with myocardial damage.

METHODS

This study included 300 subjects (age median [min–max]: 45 [36–58] years) who visited Duzce University Medical Faculty Hospital in the period January to November 2019. The subjects are divided into two groups, with 150 participants in each group: (1) control group (healthy subjects) and (2) patients with heart attack. Subjects in Group 1 were selected from those who came to the hospital for routine control, had no acute or chronic disease, and were not previously diagnosed with the disease or were not using drug. In Group 2, we included patients who are over 30 years old and who have had a heart attack suspicion with chest pain (acute MI) applied to the emergency department 6 h after symptom onset. Patients with heart attacks coming to the emergency department with clinical symptoms were identified by surface electrocardiogram (ECG) and/or biochemically cardiac biomarkers. In the diagnostic criteria, there was chest pain lasting for about an hour, the Q-wave was 25% wider than the R wave, and the ST elevation was greater than 1 mm on ECG. According to ECG and biochemistry results, 150 patients with non-ST-elevation MI were included in the study. All subjects were informed verbally and in writing about the experimental design and possible risks and voluntarily included in the study. The Clinical Research Ethics Committee of the Duzce University approved the study protocol (no: 2019/273), and the study was carried out following the current Helsinki Declaration and Good Clinical Practice principles. Informed consent was obtained from the patients included in the study.

The blood samples were centrifuged at 5000×*g* for 10 min at room temperature and then routine biochemistry analyses [i.e., glucose, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, CK, CK-MB, aspartate amino transferase (AST), alanine amino transferase (ALT), gamma-glutamyl transferase (GGT), LDH, creatinine, blood urea nitrogen (BUN), urea, calcium, alkaline phosphatase (ALP), and total bilirubin) were performed using the Cobas 600 autoanalysis. hs-cTnI levels were measured using Elecsys 2010 system (Roche Diagnostics, Germany).

The PLIN5 levels in serum samples were measured with Uscn Life Sciences PLIN5 ELISA kit according to the manufacturer's instructions (ELISA Kit for PLIN5 – Human SEE039Hu). Absorbance reading was done on Chromate 4300 brand ELISA reader device.

Malondialdehyde (MDA), total oxidant status (TOS), reduced glutathione (GSH), and copper/zinc superoxide dismutase (Cu/Zn SOD) levels in serum samples were determined using a commercially available (LS-F27741, Rel Assay Diagnostics, MBS265674 and BMS222, respectively). Measurements were made colorimetrically with a microplate reader according to the manufacturer's instructions.

Perilipin-5 (PLIN5) expression and protein levels were analyzed by quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) and Western blot analyses, respectively, in whole blood samples according to our previous work[11]. The PLIN5 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers were as follows:

PLIN5 forward 5′-TGT GCA CAG TGC AGC CGA GGA-3′ and PLIN5 reverse 5′-GCT GCA CGA GCA AGG GAA GAC-3′; GAPDH forward 5′-AGC CAC ATC GCT CAG ACA C-3′ and GAPDH reverse 5′-GCC CAA TAC GAC CAA ATC C-3′. The fold changes in PLIN5 and GAPDH mRNA levels were determined according to the $2^{-\Delta\Delta CT}$ formula after amplification. The GAPDH mRNA levels were used as an internal standard.

Two independent samples t-test was used for univariate analyses as well as for normally distributed variables. Pearson's chi-square test was used for the analysis of categorical data. A p-value <0.05 was considered statistically significant. Results were analyzed using the GraphPad Prism software 7 (GraphPad Software, San Diego, CA, USA).

RESULTS

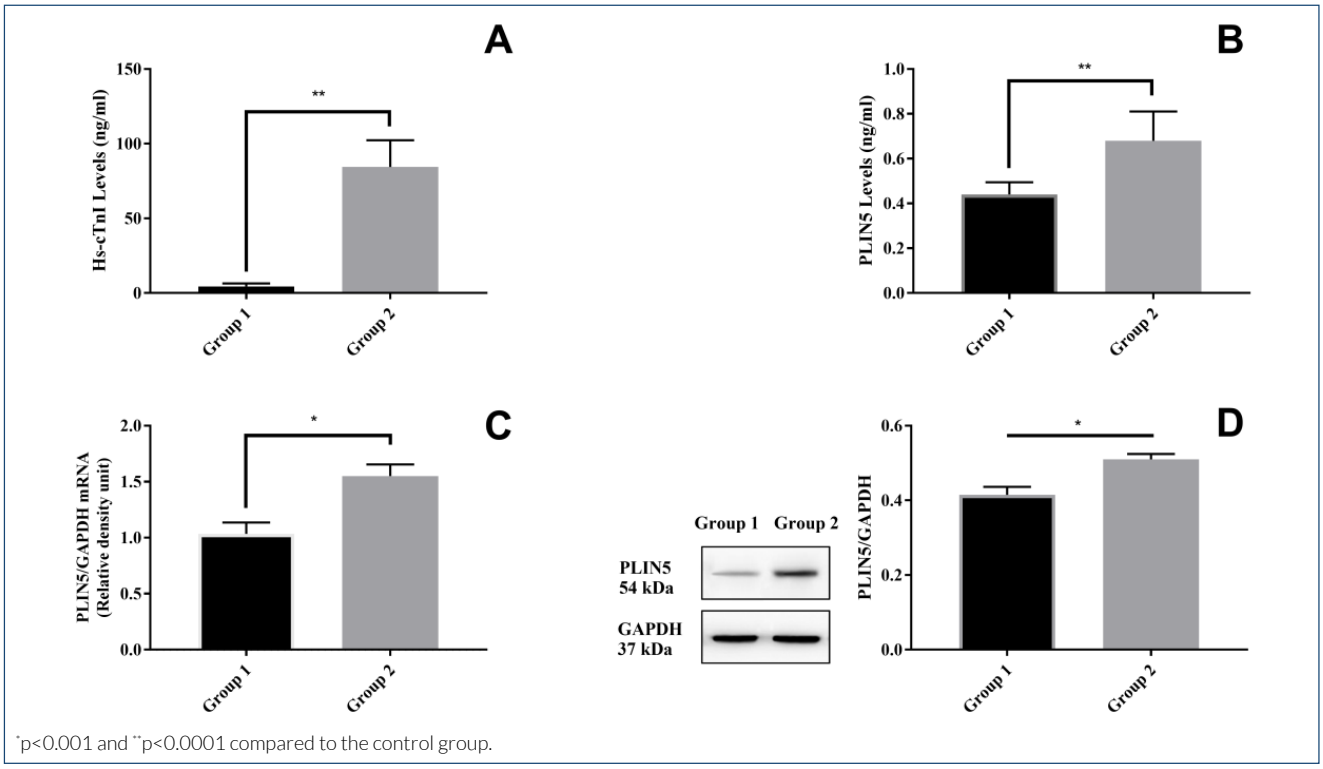
The demographic characteristics and biochemistry results of the subjects are shown in Table 1. We found that HDL, CK-MB, AST, and calcium levels in the patients with heart attack were higher than in the control group (p<0.01). There was an increase in HDL, CK, CK-MB, AST, LDH, and calcium levels in Group 2 (35.8, 27.9, 644.5, 73.7, 41.8, and 87.5%, respectively) compared to the control group.

The patients with heart attack showed a significant increase in hs-cTnI levels compared to the control group (Figure 1). In the patients with heart attack, an increase in hs-cTnI levels is accompanied by an increase in serum PLIN5 levels (Figures 1A, B). The enhancement in hs-cTnI and PLIN5 levels in the patients with heart attack was 1361 and 58.2%, respectively, compared to the control group (p<0.0001). In addition, hs-cTnI and PLIN5 levels showed a positive correlation in both Group 1 and Group 2 (r=0.714; p<0.01).

Table 1. Basic characteristics and biochemical parameters of the subjects.

	Control group (n=150)	Patients with heart attack (n=150)	p-value
Age#	45 (37–58)	41 (36–55)	<0.01
Male*	50 (58)	50 (50)	>0.05
Female*	50 (42)	50 (50)	>0.05
Body mass index	25±2.51	24±1.08	>0.05
Glucose (mg/dl)	80.45±5.72	78.52±6.09	>0.05
Total cholesterol (mg/dl)	153.44±26.84	155.61±21.38	>0.05
HDL (mg/dl)	39.81±4.95	53.36±2.58	<0.01
LDL (mg/dl)	50.33±12.91	51.06±13.82	>0.05
Triglycerides (mg/dl)	104.68±21.75	106.24±20.56	>0.05
Creatine kinase (CK)	79.92±11.61	101.42±15.84	<0.01
CK-MB (IU/L)	8.62±4.95	58.11±8.25	<0.01
AST (U/mL)	15.48±10.53	26.69±8.06	<0.01
ALT (U/mL)	19.24±8.15	20.36±5.65	>0.05
GGT (U/L)	38.51±10.18	40.42±11.17	>0.05
LDH (U/L)	177.39±21.64	248.55±17.49	<0.01
Creatinine (mg/dl)	0.7±0.14	0.8±0.25	>0.05
BUN (mg/dl)	13.62±5.08	12.75±3.71	>0.05
Urea (mg/dl)	27.13±4.15	28.59±6.12	>0.05
Calcium (mg/dl)	8.04±1.67	15.02±3.84	<0.01
ALP (U/L)	71.35±16.58	69.55±6.43	>0.05
Total bilirubin (mg/dl)	0.61±0.14	0.58±011	>0.05

#Numbers are presented as median. *Numbers are presented as (%).



*p<0.001 and **p<0.0001 compared to the control group.

Figure 1. hs-cTnI and PLIN5 levels during heart attack. (A) hs-cTnI levels, (B) PLIN5 levels, (C) PLIN5 mRNA levels, (D) PLIN5 protein levels. Group 1: Control group. Group 2: Patients with heart attack. hs-cTnI: high-sensitivity cardiac troponin I; PLIN5: perilipin-5; GAPDH: glyceraldehyde 3-phosphate dehydrogenase.

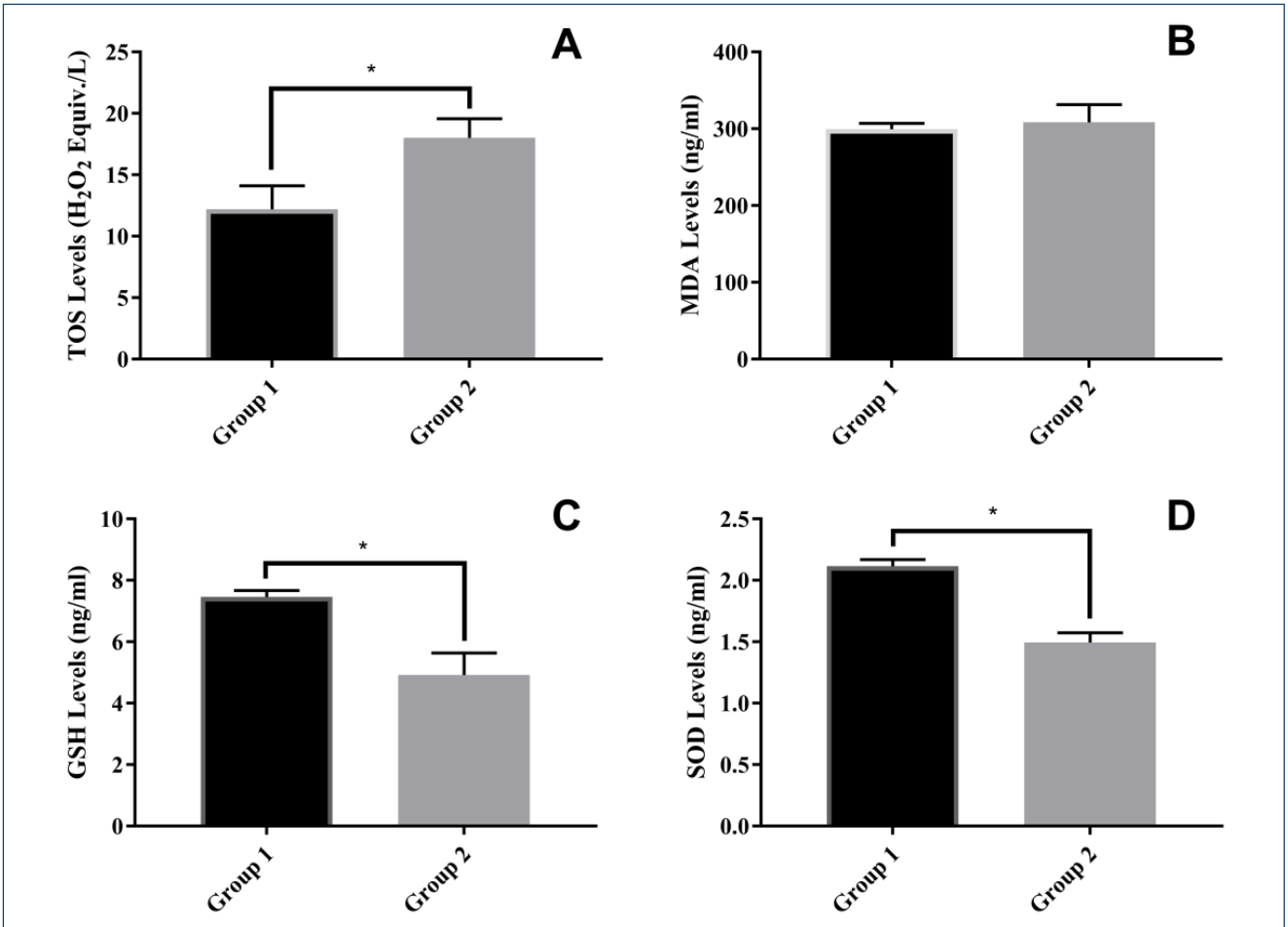
Perilipin-5 (PLIN5) mRNA levels in Group 1 and Group 2 were measured by RT-PCR. PLIN5 mRNA levels in the Group 2 were significantly higher than that in Group 1 (Figure 1C). PLIN5 mRNA levels in the patients with heart attack increased by 48.2% compared to the control group ($p<0.01$). PLIN5 protein levels in healthy subjects (Group 1) and heart attack patients (Group 2) were visualized by Western blot analysis. We found a significant increase in PLIN5 protein levels in Group 2 compared to the Group 1 (Figure 1D).

The TOS and MDA levels in both Group 1 and Group 2 were shown in Figure 2. TOS levels in the patients with heart attack demonstrated a statistically significant increase of 35.9% compared to the control group ($p<0.01$; Figure 2A). The MDA levels in the patients with heart attack showed a statistically insignificant increase of 3.7% compared to the control group ($p>0.05$; Figure 2B). In addition, patients with heart attack in Group 2 exhibited statistically a decrease in GSH and SOD levels by 33.5 and 24.1%, respectively, compared to the control group ($p<0.01$; Figures 2C, D).

DISCUSSION

One of the key findings of this research was that the expression levels of PLIN5 during heart attacks increased with hs-cTnI. Since the increase in hs-cTnI levels was also accompanied by increased serum PLIN5 levels, it is reasonable to say that PLIN5 may be important for detecting cardiac damage status. It is also suggested that despite increased oxidative stress during a heart attack, the lack of an insignificant increase in MDA levels was due to the increase in PLIN5 levels.

Perilipin-5 (PLIN5) has an important role in maintaining heart functions by providing the energy balance of the heart[12].



TOS: total oxidant status; MDA: malondialdehyde; GSH: reduced glutathione; SOD: superoxide dismutase. *p<0.01 compared to the control group.

Figure 2. Oxidation and antioxidant levels during heart attack. (A) TOS levels, (B) MDA levels, (C) GSH levels, (D) SOD levels. Group 1: Control group. Group 2: Patients with heart attack.

Wang et al. demonstrated that the increase in PLIN5 expression levels in cardiac diseases regulated cardiac energy metabolism by stabilizing cardiac lipid droplets[13]. In addition, heart function and metabolism in PLIN5-deificied mice decreased significantly after MI, accompanied by significantly decreased survival[14]. In this study, we showed that PLIN5 expression levels increased in patients with heart attacks, and circulating PLIN5 levels enhanced with cardiac damage.

High-sensitivity cardiac troponin I (hs-cTnI), which is free in myocyte cytosol and bound to myofilaments, is released into the circulation after myocyte damage caused by various heart diseases[15]. The presence of heterophile antibodies and auto-antibodies in serum may affect the analysis process, leading to false hs-cTnI quantification[16]. In this study, we found that patients with heart attack showed an increase in hs-cTnI and PLIN5 levels. This suggested that PLIN5 levels in the serum increased with cardiac damage, because the subjects involved in this study did not have muscle or liver disease. Besides, combined results of blood analysis indicated a high positive correlation between PLIN5 and hs-cTnI. According to our results, when hs-cTnI and PLIN5 results were evaluated together, we clearly found that PLIN5 expression levels were increased in all patients with heart attack. Based on the test results, increased hs-cTnI and PLIN5 levels might provide more accurate prediction in the evaluation of cardiac problems.

Oxidative stress induced by reactive oxygen and reactive nitrogen species (ROS and RNS) has been shown to play a key role in the pathogenesis of various cardiac diseases such as acute MI, coronary syndrome, atherosclerosis, and cardiac ischemia17. A previous study reported that oxidative mechanisms were induced in cases of various cardiac problems18. Similar to our study, Karabacak et al. revealed that there was a strong correlation between cardiac dysfunction and oxidative stress levels19. In the study, we showed that TOS levels in the patients with heart attack were higher than the control. Polidori et al. reported that lipid peroxidation showed a positive correlation with oxidative stress in patients with heart disease20. Wang et al. suggested that the increase in PLIN5 expression may protect against cardiac dysfunction by upregulating the Nrf2 antioxidative pathway13. Likewise, in this study, we found that there was an increase in TOS levels and a decrease in GSH and SOD levels in patients with heart attack, but there was no significant difference in MDA levels. These results may be related to the effect of increased PLIN5 expression levels in the patients with heart attack.

Since our study is a preliminary study, we first tested the accuracy of our hypothesis. Notably, the fact that no statistically significant difference was observed in lipid peroxidation despite the oxidative damage caused by the pro-oxidant/oxidant imbalance during heart attack is also an important data for our study. In addition, when we eliminated interferences (as in the exclusion criteria), we found that PLIN5 increased with troponins in the acute phase during heart attack. Therefore, one of the most important limitations of our study is that although the relationship between PLIN5 and hs-cTnI levels in patients with heart attack in the acute phase was investigated, PLIN5 and hs-cTnI levels in patients hospitalized after heart attack were not evaluated. Another is to assess whether results from a larger patient population to which exclusion criteria were gradually included are consistent with current results. Ultimately, the clinical applicability of our study is not yet available.

CONCLUSION

Although there are significant data showing that PLIN5 increases in serum during heart attack, it should be measured in more patients and over a wider time period to investigate whether it will be a potential biomarker in heart diseases.

AUTHORS' CONTRIBUTIONS

IES: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Validation. **CH**: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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