Increased microRNA-21 serum level reflects the process of cardiac necrosis rather than plaque vulnerability in patients with acute coronary syndrome

– a pilot study

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Increased microRNA-21 serum level reflects the process of cardiac necrosis rather than plaque vulnerability in patients with acute coronary syndrome – a pilot study

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SHORT TITLE: MicroRNA-21 in cardiac necrosis.

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CONFLICT OF INTEREST: none declared.

Introduction

Stable atherosclerotic plaque manifests clinically as stable coronary artery disease (SCAD), whereas unstable or vulnerable plaque – as acute coronary syndrome (ACS) which is further divided into unstable angina (UA) and myocardial infarction with and without ST-segment
elevation (i.e. STEMI and NSTEMI). Rupture of a vulnerable plaque leads to thrombus generation, coronary artery occlusion and subsequent cardiac muscle necrosis with dynamically increased serum troponin level, which is clinically defined as type 1 myocardial infarction [1]. The process of plaque destabilization and cardiac necrosis is associated with extracellular matrix alterations, in which metalloproteinases like matrix metalloproteinase-9 (MMP-9) play a pivotal role [2]. The MMP-9 is upregulated epigenetically by microRNA-21, which downregulates the expression of the MMP-9 inhibitors – reversion-inducing cysteine-rich protein with Kazal motifs (RECK) and the tissue inhibitor of metalloproteinase-3 (TIMP-3) [3, 4]. We aim to investigate whether miRNA-21 expression is enhanced in ACS patients (with and without cardiac necrosis) comparing with SCAD patients as well as healthy control group.

Methods

Study design and inclusion criteria

In this pilot study, the subjects were classified into the following groups: STEMI, NSTEMI, UA, SCAD and healthy volunteers (HV), according to the corresponding European Society of Cardiology Guidelines (SCAD – 2013; UA/NSTEMI – 2015, STEMI – 2017). Moreover, in all groups (except for HV), the presence of coronary atherosclerotic plaque was confirmed by coronary angiography and plaque burden was assessed by Gensini Index (GI)[5]. The patients from STEMI and NSTEMI groups presented type 1 myocardial infarction. Study has been approved by the local Research Ethics Committee in Medical University of Warsaw (KB/55/2016 and KB/26/A/2017).

Exclusion criteria

Patients presenting at least one of the following criteria were excluded from the study:

- malignant neoplastic, active autoimmune or rheumatic disease
• surgery or invasive intervention (6 months)
• eGFR level (MDRD) < 45 ml/min/1.73 m²
• diabetes mellitus
• active infection (3 months)

Additional exclusion criteria for the SCAD group were:

• previous ACS
• previous PCI with stent implantation

Study population

A total of 60 patients and volunteers were initially recruited to the study. All persons have given informed consent for participation in the study. Finally, 43 persons were eligible and enrolled:

• ACS (n = 25):
  o STEMI (n = 8),
  o NSTEMI (n = 9)
  o UA (n = 8)

• SCAD (n = 8)

• HV (n = 8)

Blood samples

Blood samples for miRNA analysis and blood measurements (hemoglobin, lipid panel, creatinine) were taken within the first 24 hours of admission before percutaneous coronary intervention (PCI). Blood samples in EDTA tubes were centrifuged at 1200 × g for 10 minutes to separate plasma from blood cells and then the plasma was aliquoted into microcentrifuge tubes, frozen on dry ice and immediately stored at −80°C. Blood troponin in
ACS patients was measured on admission (1st troponin) and after at least 8 hours from admission, after PCI (2nd troponin).

**qRT-PCR detection of hsa-miR-21-5p**

The miRNA particles measured in the plasma samples were: cel-miR-39-3p (a “spike-in” control), hsa-miR-93-3p, hsa-miR-191-5p (control microRNAs) and hsa-miR-21-5p (study miRNA). MicroRNA isolation and quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) were done according to the manufacturer’s protocol, with the use of TaqMan Advanced miRNA Assays. The obtained Ct (cycles of threshold) values for hsa-mir-21-5p and hsa-mir-191-5p, hsa-mir-93-3p as endogenous controls were normalized by Ct of cel-miR-39-3p and were used to calculate relative expression using the $2^{-\Delta\Delta Ct}$ method.

**Statistical analyses**

All analyses were performed using STATISTICA 13, StatSoft Inc. Values are presented as mean and standard deviation (SD), standard error of the mean (SEM) or median values and interquartile ranges according to the normality of distribution (Shapiro–Wilk test). The Student’s t-test, Cochrane-Cox test (for non-equal variances) and the ANOVA F-test were conducted for normally distributed continuous variables, whereas the Kruskal–Wallis test, Mann–Whitney U-test and Spearman’s Rank correlation coefficient ($r_s$) – for non-normally distributed continuous variables. Categorical variables were compared with the use of the $\chi^2$ test. p-values < 0.05 were considered statistically significant.

**Results and Discussion**

**Baseline characteristics**

The groups have been compared in terms of demographic parameters (age and sex), BMI, history of hypertension, active smoking and LDL cholesterol serum level, i.e. crucial risk
factors for atherosclerosis. In the 1st analysis (ACS vs SCAD vs HV) the SCAD group presented lower LDL levels than both ACS and HV and less active smokers than ACS group. HV patients were younger and were predominately females, without active smokers. The 2nd analysis (STEMI vs NSTEMI vs UA vs SCAD vs HV) has revealed only a significant difference between UA and STEMI groups in terms of active smokers. No difference in BMI and hemoglobin was shown both in the 1st and in the 2nd analysis. The GI values (SD) for the patients’ groups were: STEMI - 59,1 (27,3); NSTEMI - 86,8 (39,3); UA - 66,3 (31,0); SCAD - 65,9 (54,5); without significant intergroup difference. In the entire study population 3 patients presented carotid stenosis (2 from NSTEMI and 1 from UA group) and one patient – peripheral artery disease (from UA group). Among myocardial infarction patients, 2 STEMI (25%) and 6 NSTEMI (66,7%) patients presented chest pain onset more than 12 hours before admission.

**Relative hsa-miR-21-5p expression difference between ACS, SCAD and HV groups**

The distribution of hsa-miR-21-5p expression levels in the ACS group rejected normality. Patients from the ACS group presented non-significantly increased hsa-miR-21-5p relative expression levels than patients from the SCAD and HV groups (2.47 [1.46-4.68] vs 1.90 [1.02-3.25] vs 1.81 [0.99 – 2.60]; p = 0.15).

**Relative hsa-miR-21-5p expression difference between STEMI, NSTEMI, UA, SCAD and HV groups and correlation with troponin I**

The reciprocal analysis of hsa-miR-21-5p relative expression level (mean [SEM]) revealed that the STEMI group presented significantly increased levels of this miRNA than the UA (5.96 [1.43] vs 2.31 [0.46], p = 0.04) and SCAD (5.96 [1.43] vs 2.32 [0.51], p = 0.04) groups. The relative expression level of hsa-miR-21-5p did not differ between the UA and SCAD groups (2.31 [0.46] vs 2.32 [0.51], p = 0.99). The STEMI group, unlike the UA group,
differed significantly in hsa-miR-21-5p relative expression level from the HV group. There was also no significant correlation between the SCAD and HV groups (2.32 [0.51] vs 1.79 [0.35], p = 0.43) (Figure). The distribution of hsa-miR-21-5p in NSTEMI group rejected normality (median: 1.49, interquartile range: [1.44-3.49]) and no significant differences were shown between NSTEMI and other groups. Moreover, in STEMI and NSTEMI patients there is an average, albeit non-significant correlation between 2nd serum troponin I and hsa-miR-21-5p relative expression level ($r_s = 0.53$), with neglectable correlation in 1st troponin and expression level of the investigated micro-RNA.

Figure 1

**Hsa-miR-21-5p increased in acute coronary syndrome with cardiac necrosis**

Darabi et al. has shown, that hsa-miR-21-5p relative expression level is elevated in ACS comparing with SCAD patients and correlates positively with serum MMP-9 and hs-CRP level [6]. However, our results have demonstrated increased hsa-miR-21-5p serum level in STEMI patients comparing with both UA and SCAD, without difference between UA and SCAD group. There is some evidence, that hsa-miR-21-5p play a role in the process of extracellular matrix regulation during myocardial necrosis. The study on C57BL/6 mice has confirmed increased hsa-miR-21-5p mice analogue expression in the infarcted zone of mice hearts [7]. In a human study, elevated hsa-miR-21-5p serum expression, measured 5 days after STEMI in 198 patients, was significantly correlated with the probability of left ventricular remodeling [8]. However, another study demonstrated negative correlation between hsa-miR-21-5p and both LDL-C and total cholesterol in NSTE-ACS patients, which suggests, that this miRNA might participate in regulation of lipid homeostasis in this population of patients [9]. In conclusion, different physiological and pathophysiological processes within the heart (like e.g. differentiation from cardiomyoblasts to cardiomyocytes, fibrosis, hypertrophy) depends on miRNA particles, which might function as “molecular
switches” promoting the pathways, that are either beneficial or detrimental[10]. The overexpression of certain miRNA particles might be associated with particular clinical situations. For instance, hsa-miR-124 serum expression level distinguishes patients with occluded from patients with patent infarct-related coronary artery (area under the curve value of 0.787 in the receiver-operator characteristic analysis, study upon 43 patients)[11]. Therefore, miRNA particles might be promising diagnostic and therapeutic tools and should be investigated not casually, but as elements of certain cardiac molecular pathways.

References


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Figure 1. The reciprocal relations between the STEMI, UA, CAD and HV groups in hsa-miR-21-5p relative expression level (values expressed as - mean [SEM]).