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Research paper

Circulating miR-221-3p as a novel marker for early prediction of acute myocardial infarction

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ABSTRACT

Recent studies have reported circulating microRNAs (miRNAs) as novel biomarkers for cardiovascular diseases including acute myocardial infarction, heart failure, diabetes mellitus, stroke, and acute pulmonary embolism. The aims of this study were 1) to compare the plasma expression levels of miRNAs in patients with acute coronary syndrome (ACS) and control subjects and in ST-elevation myocardial infarction (STEMI) and non-STEMI 2) to evaluate miRNAs potential to be used as novel diagnostic biomarkers for ACS.

Twenty seven consecutive patients, admitted to emergency department of a training and research hospital between January–December 2013 with acute chest pain and/or dyspnea and diagnosed with ACS, and 16 non-ACS control subjects were included in this study. miRNA profiling was performed by using real time polymerase chain reaction. Functions of dysregulated miRNAs were evaluated by computerized-pathways analysis.

miR-221-3p was one of the two most dysregulated miRNAs with a fold regulation of 3.89. It was significantly positively correlated with both Troponin and GRACE and Synthax Score. Moreover, miR221-3p was found to be significantly inversely correlated with left ventricular ejection fraction. miR-221-3p was the most prominent biomarker candidate with an area under curve (AUC) level of 0.881 (95% confidence interval: 0.774–0.987; $p = 0.002$). The present study is the first to report an increased expression levels of miR-221-3p in AMI. Since miR-221-3p has a high discriminative value and significant relations with Troponin, GRACE and Synthax score and left ventricular systolic function, it may be a potential biomarker for early prediction of AMI.

1. Introduction

Acute myocardial infarction (AMI), which is subdivided as ST-elevation myocardial infarction (STEMI), non-ST elevated myocardial infarction (NSTEMI), and unstable angina pectoris, is the leading cause of mortality and morbidity worldwide (Thygesen et al., 2012; Anderson et al., 2013). The major pathophysiologic events for the development of AMI are partial or complete coronary artery occlusion caused by vulnerable atherosclerotic plaque rupture and thrombus formation or acute occlusion of a coronary artery by coronary emboli or vasospasm (Oerlemans et al., 2012; Oerlemans et al., 2013).

Early and precise diagnosis of AMI and urgent revascularization of culprit coronary lesion are crucial to prevent or reduce ischemic myocardial damage, which results in limited adverse cardiac remodeling and heart failure. The diagnosis of AMI is based on patient history and clinical symptoms like acute or subacute typical chest pain, dyspnea, nausea and vomiting, syncope and acute dynamic changes on the electrocardiogram (ECG) or new pathological Q waves and changes in the levels of biomarkers, This is a challenging procedure and requires the clear cognitive function of the patient and the experience of the clinician (Anderson et al., 2013; Oerlemans et al., 2012). Moreover,

Abbreviations: ABLIM1, Actin binding LIM protein 1; ACS, acute coronary syndrome; AMI, acute myocardial infarction; APE, acute pulmonary embolism; CKMB, creatine kinase MB isoenzyme; HF, heart failure; miRNA, microRNA; LVS, left ventricular systolic; LVEF, left ventricle ejection fraction; NFATC3, nuclear factor of activated T-cells; Non-STEMI, non–ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention; PPP3CB, protein phosphatase 2B; ROCK2, Rho-associated protein kinase 2; RT-PCR, real-time PCR; STEMI, ST-segment elevation myocardial infarction; SYNTAX, The synergy between PCI with Taxus; TIMI, Thrombolysis in Myocardial Infarction.

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interpretation of the ECG is quite subjective and sometimes patients with ACS may show normal ECG findings on emergency admission. Troponin (cTnI/T) is generally known as the most reliable and preferable biomarker, but cannot precisely confirm or exclude the diagnosis of ACS (Oerlemans et al., 2012; Oerlemans et al., 2013). In addition, the time limitation, and liability from non-ACS settings are the main disadvantages of Troponin. Hence, it is necessary to investigate more sensitive and specific novel biomarkers for the early diagnosis of AMI.

MicroRNAs (miRNAs, miRs) are endogenous, non-coding, single-stranded RNAs of 19–25 nucleotides in length, which regulate gene expression at the post-transcriptional level. They lead to mRNA degradation or translational inhibition resulting in the termination of protein synthesis (Suzuki and Miyazono, 2010; Xiao and Chen, 2010; Sibley and Wood, 2011). miRNAs play crucial roles in physiological and pathological processes such as development, metabolism, cellular differentiation, proliferation, migration, conduction, cell death and stress response (Bartel, 2004; Ambros, 2004; Plasterk, 2006). Recent studies have reported circulating miRNAs as novel biomarkers for cardiovascular diseases including acute myocardial infarction, congestive heart failure (HF), coronary artery disease, diabetes mellitus, stroke, essential hypertension, and acute pulmonary embolism (APE) (Xu et al., 2012; Cakmak et al., 2015). Cardiomyocyte-enriched miRNAs (miR-1, miR208a, miR-208b, miR-133a, miR-133b, miR-499) have been presented as potential diagnostic biomarkers in an acute myocardial infarction according to their raised sensitivity and specificity (Wang et al., 2010; D'Alessandra et al., 2010). There are some clinical studies in the literature evaluating a diagnostic role of miRNAs in AMI by using real time polymerase chain reaction (RT-PCR) or microarray techniques with different expression levels and controversial results (Deddens et al., 2013). Hence, there is still a need for new miRNA based researches, which may lead to a decrease in the time span between first medical contact and balloon time to improve prognosis in this setting.

The aims of the present study were 1) to compare the serum expression levels of miRNAs in patients with AMI and control subjects with an acute atypical chest pain/dyspnea and in patients with STEMI and non-STEMI 2) to evaluate their potential to be used as novel diagnostic biomarkers for AMI in patients admitted to emergency service for acute chest pain and/or dyspnea 3) to investigate the relations between the serum levels of miRNAs with the serum levels of previously validated biomarkers, namely troponin I, cardiac risk scores and post-MI left ventricular functions.

2. Material and methods

2.1. Study participants

Twenty seven consecutive patients, who were admitted to emergency department of a training and research hospital between January 2013 and December 2013 with acute chest pain and/or dyspnea and diagnosed with AMI underwent primary percutaneous coronary intervention or early coronary angiography procedure, and 16 control subjects were included in this case-control study.

The inclusion criteria for patients with AMI were according to "2012 European Society of Cardiology Universal Definition of MI Guideline" (Thygesen et al., 2013). It was diagnosed by acute ischemic-type chest pain with an accelerating pattern or a prolonged duration (>20 min) or recurrent episodes at rest or with minimal effort, acute ischemic ECG changes with such as ST-segment elevation, ST-segment depression of 0.1 mV, or T-wave inversion in at least two contiguous ECG leads and changes for level of previously validated biomarkers (Troponin I) and emergent coronary angiography procedure. Control group were composed of individuals, who admitted to emergency service with acute atypical chest pain and/or dyspnea without any acute dynamic ECG changes and/or rise of cardiac enzymes.

The exclusion criteria of the study were as follows: age >80 years, history of previous myocardial infarction, heart failure or arrhythmia, history of PCI or coronary artery bypass graft surgery, history of cardiogenic shock, severe valvular heart disease, congenital heart disease, paced rhythm, presence of bundle branch blocks, patients died within the first 48 h, renal or hepatic dysfunction, malignancy, acute or chronic inflammatory or infectious disease.

Baseline demographic, clinic and laboratory characteristics of the study groups were recorded from hospital records. ECG was recorded for each patient just after hospital admission to obtain the MI type. Cardiac risk scores, which are useful for predicting in-hospital, short and long term prognosis for AMI patients, such as "The Thrombolysis in Myocardial Infarction" (TIMI) and "Global Registry of Acute Coronary Events" (GRACE) (Sianos et al., 2005; Cheitlin et al., 2003) were calculated from patient files and coronary angiography procedure images by one expert interventional cardiologist who was unaware from data of this study. Moreover, "The synergy between PCI with Taxus and cardiac surgery" (SYNTAX) score, which reflects culprit coronary artery lesion complexity and severity and guides revascularization strategy especially in NSTEMI patients, were calculated from the coronary angiography procedure images (Sianos et al., 2005; Vlachos et al., 2012). Moreover, height and weight of each study participant were measured, and body mass index (BMI) was calculated as body weight in kilograms divided by the square of the height in meters (kg/m2).

Chewable acetylsalicylic acid (300 mg) and a loading dose of clopidogrel (600 mg) were given to AMI patients without contraindications before primary PCI procedure. Primary PCI was initiated using standard techniques. The access approach was either transfemoral or transradial. During the procedure, non-ionic, low-osmolality contrast media were used and the coronary artery was confirmed to be clinically significant if its stenosis was >50%. An angiographic evaluation was made by visual assessment.

All study subjects underwent transthoracic echocardiographic examination (Vivid 3; General Electric, Milwaukee, Wisconsin, USA), which was performed by an experienced operator in a left lateral supine position in order to determine left ventricle ejection fraction (LVEF) values. LVEF was determined using Simpson's method of discs in two dimensional echocardiography (Cheitlin et al., 2003).

Eligible patients were between 18 and 80 years of age and all were able to provide written informed consent, which was a prerequisite for enrolment. The study complies with the Declaration of Helsinki, and the trial protocol was approved by the Istanbul University local Ethics Committee (January 2011/05111).

2.2. Plasma separation

Five milliliters of whole blood were obtained from study subjects within 4 h of onset of clinical symptoms (chest pain and/or dyspnea) and plasma was separated within 45 min of collection via centrifugation at 3000 rpm for 10 min. Following centrifugation, supernatant was transferred to DNase/RNase free tubes and stored at −80 °C until further analysis.

2.3. RNA isolation

RNA was isolated using miRNeasy Serum/Plasma Kit (Qiagen, Hilden, Germany, Cat. No: 217004) according to manufacturer's instructions. Nanodrop 2000c spectrophotometer was used to determine the purity and concentration of RNA samples. RNA integrity was assessed by agarose gel electrophoresis. Samples with insufficient concentration and quality were re-isolated.

2.4. Reverse transcription and real-time qPCR analysis

1 μg of isolated RNA was used for cDNA synthesis with miScript II RT Kit (Qiagen, Hilden, Germany, Cat. No: 218161) according to manufacturer's instructions. For each sample three different predesigned commercial PCR arrays with 384 well format and