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ORIGINAL ARTICLE

Saliva-based creatine kinase MB measurement as a potential point-of-care testing for detection of myocardial infarction

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Abstract Myocardial infarction (MI) is the main cause of death all over the world. Biomarkers of cardiac necrosis are of great importance in the diagnosis of MI. The aim of this study was to determine probable changes of creatine kinase MB isoform (CK-MB) levels in saliva of patients with acute MI. A case–control study was carried out on 30 patients with acute MI who were hospitalized in Kamkar-Arabnia Hospital of Qom City and 30 healthy control subjects. CK-MB levels were measured by immunoinhibition assay in saliva and serum of patients and healthy individuals. Statistical analysis of the Student's *t* test and Pearson correlation coefficient was used. CK-MB levels showed a significant elevation in saliva and serum of patients with acute MI compared to healthy controls. Furthermore, there was a strong correlation between salivary levels of CK-MB and its serum values. Subsequent to an acute MI, there is a rise in salivary levels of CK-MB just as what occurs in the serum. Moreover, salivary levels of CK-MB reflect well its serum values. It seems that cardiac biomarker CK-MB is measurable in the saliva of patients with acute MI. Salivary CK-MB may serve as an easy-to-use diagnostic tool for point-of-care testing of acute MI.

Keywords Myocardial infarction · Creatine kinase MB · Unstimulated saliva

Abbreviations

MI	Myocardial infarction
ECG	Electrocardiogram
CK-MB	Creatine kinase MB isoenzyme

Introduction

Myocardial infarction (MI) is the major cause of death throughout the world. With the access we have to effective treatments, accurate and quick diagnosis is of major medical and economic importance [1].

According to the current guideline which has been set forth by ESC/ACCF/AHA/WHF Task Force for the redefinition of myocardial infarction, the criteria for diagnosis of acute MI were recommended as follows: detection of rise and/or fall of cardiac biomarkers (preferably troponin) with at least one value above the 99th percentile of the upper reference limit together with evidence of myocardial ischemia with at least one of the following: (1) symptoms of ischemia, (2) ECG changes indicative of new ischemia (new ST-T changes or new left bundle branch block), (3) development of pathological Q waves in the ECG, and (4) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality. Obviously, the Task Force has highlighted the importance of cardiac biomarkers as a necessary perquisite for detection of acute MI. Although troponin has been

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suggested as the preferred biomarker, CK-MB mass assay was proposed as the best alternative in conditions where troponin assays are not available [2].

Myocardial necrosis gives rise to the appearance in the blood of different proteins released into the circulation from the damaged heart cells, including myoglobin, cardiac troponins T and I, creatine kinase, lactate dehydrogenase, as well as many others. In the clinical setting of acute ischemia, a rise in blood levels of sensitive and specific biomarkers, such as cardiac troponin and CK-MB, is recognized as MI [3].

Having nearly absolute myocardial tissue specificity, as well as high sensitivity, cardiac troponin (I or T) is the biomarker of choice for identifying even minute zones of myocardial necrosis [3]. Nevertheless, CK and more particularly CK-MB still have a formal place in defining myocardial infarction [4]. In the 1970s and 1980s, CK-MB revolutionized the diagnosis and treatment of acute MI. At that time, CK-MB turned out more specific than an accurate clinical history, more reliable than ECG pattern recognition, and has more specificity than myoglobin. As a consequence, it became the gold standard for identification of cardiac injury [5], and from then on, it has been widely used in clinical practice. CK-MB may rise within 3 to 4 h of injury and reach its peak in 24 h, and then it dwindles to normal levels 24 to 36 h later [3, 6].

In recent times, there has been an increasing interest in saliva-based analyses because saliva collection methods are simple and noninvasive. Oral fluid sampling is safe for both the operator and the patient and has easy and low-cost storage. Since the saliva was put forth as a potential diagnostic tool, its use for surveillance of disease and general health has become a highly desirable goal in healthcare and medical research [7, 8].

Increasing attempts to establish saliva as a diagnostic matrix have compelling reasons behind. In this regard, it clearly offers an inexpensive, noninvasive, and easy-to-use screening method. In addition, it has several advantages over serum and urine in terms of collection, storage, shipping, and voluminous sampling. Moreover, the handling of oral fluid during laboratory procedures is far easier than blood because it does not clot, thus reducing the number of required manipulations. Furthermore, the noninvasive nature of saliva collection approach could dramatically reduce anxiety and discomfort and thereby increase patients' willingness to continue health-related examinations over time [8, 9].

To investigate if saliva can be used as a diagnostic specimen in clinical practice for detection of myocardial infarction, we compared salivary and serum levels of CK-MB in patients with acute MI with those of apparently healthy people. Here we show that salivary levels of CK-MB correlate perfectly with its serum concentrations.

Methods

Study design and population

This study was designed as a case–control survey in Kamkar-Arabnia Hospital of Qom Medical University to investigate the correlation between serum and salivary levels of CK-MB in patients with acute MI and apparently healthy people. In this study, saliva and blood samples were obtained from 32 patients who were admitted to the emergency department with a typical ischemic chest pain, electrocardiographic characteristics of acute heart injury/necrosis, and a rise in serum biomarkers of MI as case group. For the control group, 32 age- and sex-matched individuals with no documented heart disease were included in the study. Healthy controls were selected from hospital staff or individuals who accompany patients referred to the hospital. Informed consent was obtained from all study participants. People with lesion(s) in their mouths or with muscular trauma were excluded from the study.

Sample collection

Saliva and blood samplings were performed on the first morning after occurrence of MI. For saliva sampling, all participants received detailed information about the collection protocol. They were asked to avoid from eating, drinking, cigarette smoking, and brushing teeth at least 2 h before sampling. Two minutes after rinsing their mouth with tap water, the subjects swallowed all their oral fluid, and thereafter, they collected 2–3 ml of resting whole saliva into a pre-weighed and dry plastic tube by spitting method. By subtracting the empty tube weight from the saliva-filled one, saliva sample weight was determined to calculate the salivary flow rate. The flow rate was calculated in grams per minute, which is almost equivalent to milliliters per minute [10]. The timing of saliva gathering was recorded in order to calculate saliva flow and salivary output of CK-MB. Salivary output of CK-MB (unit per minute) was computed as saliva flow rate (milliliters per minute) multiplied by salivary concentration of CK-MB (unit per milliliters).

Two milliliters of venous blood was drawn immediately after saliva sampling. Upon completing the sample collection, the specimens were centrifuged at 3,800×*g* for 10 min, and then the serum and saliva supernatants were isolated and divided into aliquots. The aliquots were stored at −70°C for later analysis of CK-MB.

Laboratory measurements

CK-MB levels were measured by immunoinhibition assay using commercial kits purchased from BioSystems Com-

pany (Barcelona, Spain). The measurements were performed by an autoanalyzer on the wavelength of 340 nm.

Statistics

Unless otherwise specified in descriptive statistics, results are presented as mean±SEM. Comparison of means for detection of between-group differences was carried out with unpaired two-tailed Student's *t* test. The Pearson correlation test was applied to determine association between serum and salivary concentration of CK-MB. Results were considered statistically significant if $P<0.05$. Analyses were performed using SPSS software version 16.

Results

As it was mentioned in the "Methods" section, we closely matched control individuals for sex and age with case subjects, who were experiencing an acute myocardial infarction, so that the number of men and women and also their age were nearly equal in both study groups. In each group, 22 men and 10 women by the age of 36–83 (median—56 years for patients and 55 years for healthy subjects) were recruited to the study. There was no significant difference in baseline unstimulated saliva flow rate between the two groups (0.39±0.02 ml/min in controls vs. 0.44±0.05 ml/min in MI patients), confirming the integrity of salivary gland function [11]. As anticipated, the mean serum concentration of CK-MB, a biomarker of myocardial necrosis, was higher in patients suffering from acute MI than that of the controls (118.22±19.10 vs. 13.82±0.89 U/l, respectively; $P=0.0001$; Fig. 1a).

Salivary concentration of CK-MB proved to be significantly higher in patients with acute MI compared to people without ischemic heart diseases (72.41±14.15 vs. 8.16±0.54 U/l, respectively; $P=0.004$; Fig. 1b). In comparison to healthy controls, patients with acute MI showed an about eightfold increase in both salivary and serum concentrations of CK-MB. Taking saliva flow rate into account to calculate salivary output of CK-MB revealed that there was still a significant difference between the two study groups, with the patients with acute MI having a greater amount of salivary CK-MB output (0.0265±0.0041 U/min in MI patients vs. 0.0032±0.0003 U/min in healthy controls; $P=0.001$; Fig. 1c).

Statistical evaluation of data using Pearson analysis indicated a strong correlation between salivary concentration and output of CK-MB and its serum concentration. Pearson correlation coefficient, *r*, was 0.92 for salivary concentration of CK-MB ($P<0.001$) and 0.86 for salivary output of CK-MB ($P<0.003$).

Discussion

The present study has demonstrated that salivary concentration and output of creatine kinase MB isoform, a

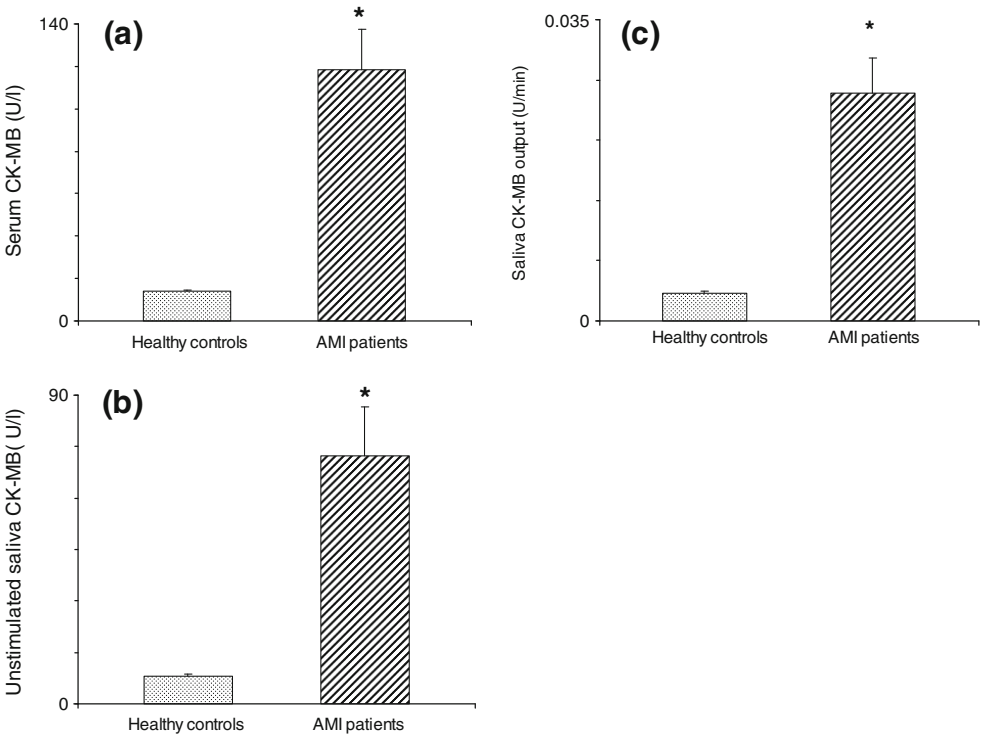

Fig. 1 Concentrations of CK-MB in **a** serum, **b** unstimulated saliva, and **c** saliva output in patients with acute myocardial infarction (*AMI*) and healthy individuals. *$P<0.05$

biomarker of cardiac necrosis, are increased in patients with acute myocardial infarction compared to people with no documented ischemic heart diseases. Furthermore, the salivary levels and output of CK-MB correlate well with its serum concentration.

Modern diagnosis of MI includes the use of blood biomarkers, which can also be used for risk assessment and for guiding interventional and noninterventional therapies [5]. On the basis of recommendations made by current guidelines, markers of myocardial necrosis such as cardiac troponin and CK-MB are used as the gold standard in the detection of acute MI [2]. The results of our study are consistent with current knowledge of biomarkers of cardiac tissue necrosis, in that, there was a significant difference between serum CK-MB values of patients with acute MI and those of apparently healthy people. Patients suffering from acute MI had higher levels of CK-MB in serum than healthy individuals.

The main purpose of this study was to investigate the possible changes of CK-MB levels in saliva following the occurrence of acute MI. To the best of our knowledge, this is the first report on the increase of saliva CK-MB levels after acute myocardial necrosis. In this regard, a recent sophisticated study on saliva-based assays as point-of-care testing for detection of acute MI evaluated a broad range of established and novel cardiac biomarkers in the serum and saliva of patients with acute MI, but it failed to show a significant rise of CK-MB in saliva of MI patients in comparison to control subjects [12]. However, our study clearly showed a significant rise of CK-MB in the saliva of MI patients. The underlying mechanism of this increase is not known; nevertheless, plasma is obviously the main source of salivary secretions, and any change in the blood of CK-MB levels can lead to a similar, though to a lesser extent, modification in salivary content of this necrotic biomarker. Given that CK-MB has relatively high degrees of cardiac specificity [13, 14], a saliva specimen with an increased value of CK-MB can be roughly interpreted as taken from a patient with acute MI. This interpretation requires an eligible cutoff point defined for saliva-based CK-MB assays. Comprehensive studies with adequate number of participants and with complete range of ischemic heart diseases should be done to define standard reference values for salivary CK-MB.

In 1994, Jones and his/her coworkers accomplished a study to determine levels of platelet activating factor (PAF) in the saliva of patients admitted to the CCU with acute MI. They found that PAF was significantly raised in the saliva of patients with ischemic heart disease. Nonetheless, they did not find any relationship between salivary PAF and serum levels of creatine kinase, a nonspecific marker of heart muscle damage [15]. However, they were not in search of a salivary biomarker of cardiac necrosis to set up a saliva-based assay for diagnosis of acute MI.

Saliva is being approved as a diagnostic fluid of the future. Much of the attention saliva receives as a biological specimen is due to the quick, uncomplicated, and noninvasive nature of sample collection [16]. To establish saliva as an alternative medium to plasma for various biological assays, there must be a high correlation between plasma and saliva levels of measured parameters [7]. As our results indicated, there was a strong correlation between serum and salivary levels of CK-MB, and also between serum concentration and salivary output of this marker. In other words, salivary levels of CK-MB reflect perfectly its serum concentration, and based on this observation, we can put forward this idea that saliva-based assays may have the potential to be used as a point-of-care testing to detect acute MI by measuring salivary CK-MB. Supporting the idea of using saliva-based assays for detection of acute MI is the recent study of Floriano and coworkers, proposing a salivary biomarker panel of C-reactive protein, myoglobin, and myeloperoxidase which in conjunction with ECG yielded strong screening capacity for acute MI [12].

Several lines of evidence have consistently validated and proposed using salivary assays for diagnosing, monitoring, or predicting prognosis of diseases. In this regard, it has been shown that several biochemical molecules can be measured in oral fluids of diseased patients, for example, steroid hormones such as cortisol [17], CA15-3 [18], CA125 [19], hydroxyprogesterone [20], progesterone [10, 21], 17β-estradiol [22], aldosterone [23], and catecholamines; protein/polypeptide hormones such as growth hormone, prolactin, insulin-like growth factor I, melatonin [7], and parathyroid hormone [24]; antibodies such as HIV antibodies; antibodies for herpes viruses, hepatitis B virus, Epstein–Barr virus, and ameba (*Entamoeba histolytica*) infection; and drugs including alcohol, amphetamines, barbiturates, benzodiazepines, cocaine, a variety of inhalants, lysergic acid diethylamide, marijuana, opioids, phencyclidine, and tobacco [25].

There were some limitations to the present study. For example, we applied the immunoinhibition method to evaluate CK-MB activity in serum and saliva specimens obtained from the study participants. This method has lower efficacy for detecting CK-MB than CK-MB mass measurement [26]. Moreover, we did not include patients with suspected but not established MI in our study; thus, we cannot judge about the salivary levels of CK-MB in patients with unstable angina. Furthermore, because we anticipated and experienced resistance from the study participants, we did not perform sequential assessment of salivary CK-MB, and hence, there are no data about the timing of CK-MB rise in salivary secretions after the onset of MI.

Based upon the findings of this study, it can be concluded that subsequent to an acute MI, there is a rise in salivary levels of CK-MB just as what occurs in serum. Salivary levels of CK-MB reflect serum values, hence confirming the epithet "mirror of the body" [27]. The core of the present study is the suggestion that salivary CK-MB can be used for developing an easy-to-use diagnostic tool for point-of-care testing of acute myocardial infarction. Further studies need to be done to make this suggestion come true.

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Conflict of interest There was no conflict of interest involved in this study.

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