Salivary Creatine Kinase MB in myocardial infarction

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SUMMARY

Introduction: Most biomarkers in the blood and urine can also be detected in salivary samples.

Aims and objectives: To determine the relationship between serum and salivary levels of Creatine Kinase MB in patients with acute myocardial infarction.

Design: In a case-control study, forty-one patients diagnosed with myocardial infarction and forty- two age and sex- matched controls were enrolled.

Methods: Saliva sampling by the spitting method was performed 12 to 24 hours after myocardial infarction, and in controls, between 9am and 12 noon. Salivary Creatine Kinase MB levels were measured by the photometric method. Mann Whitney U test and Spearman coefficient were used to analyze the data.

Results: There was no significant difference between patients with myocardial infarction and the controls in terms of the salivary levels of creatine kinase MB (case: 24 U/l vs. control: 19.5 U/l, p=0.30). Patients showed no significant difference in median levels of salivary creatine kinase MB in terms of sex (p=0.69) and previous history of myocardial infarction (p=0.31). In all patients there was a weak positive relationship between serum and salivary levels of creatine kinase MB (rs=0.14, p= 0.39).

Conclusions: Salivary creatine kinase MB level cannot be an indicator for diagnosis of myocardial infarction.

Keywords: Creatine Kinase MB, saliva, myocardial infarction

ACRONYMS

AMI:	acute myocardial infarction
CK-MB:	Creatine Kinase MB
ESC:	European Society of Cardiology
ACCF:	American College of Cardiology Foundation
AHA:	American Heart Association
WHF:	Womens' Heart Foundation

INTRODUCTION

Acute myocardial infarction (AMI) is considered as the leading cause of death and disability in many countries.[1] In Iran (place of the present study), about 138 000 deaths occur annually due to coronary heart disease of which MI accounts for about half.[2] Early diagnosis of MI with high accuracy is a medical priority, saving lives and reducing treatment costs.[3] According to the recent guideline set forth by the ESC/ACCF/AHA/WHF Task force for the redefinition of myocardial infarction, the main criteria for diagnosis of AMI are: 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 based on one of the following: ECG changes indicative of a new ischemia, development of pathological Q waves, imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.[4] The Task Force has emphasised the importance of cardiac biomarkers as a necessary prerequisite for the diagnosis of MI. Although troponin has been recommended as the preferred biomarker, creatine kinase MB (CK-MB) was proposed as the best alternative where troponin assays are not available.[5] In the 1960's serum CK-MB was demonstrated as a highly specific marker of MI.[6] In the 1970's, CK-MB considered as a standard for the diagnostic and quantitative assessment of MI.[7] In a recent study focused on early detection of MI by using cardiac biomarkers, the sensitivity and specificity of the CK-MB isoforms were measured as 95.5% and 93.9%, respectively.[8] In 1980s, the use of CK-MB markers revolutionised the diagnosis of acute MI which turned out to be more specific than an accurate clinical history and myoglobin, and more reliable than ECG pattern recognition.[5] As a consequence, CK-MB became the gold standard for the identification of cardiac injury. Myocardial necrosis gives rise to appearance of different proteins released into the circulation from the damaged heart cells.[5] CK-MB levels rise within 3-5 hours of injury and reach a peak in 24 hours.[3] In recent years, there has been an increasing interest in salivary- based analyses because saliva offers increased flexibility, cost effectiveness, convenience, and is less invasive compared with serum sampling.[9-11] Most of biomarkers in the blood and urine can also be detected in salivary samples.[9] According

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Table 1: Distribution of study variables in case and control groups

Group	Age (mean± SD)	Sex		Salivary flow rate (ml/min)	Level of CK-MB (U/l)
		Men	Women		
Case	59.9 ± 13.16	28 (68.2%)	13 (31.8%)	0.93 ± 0.35	24
Control	60.1 ± 13.31	29 (69%)	13 (31%)	0.81 ± 0.21	19.5
P value	0.94*	0.75**		0.10*	0.30†

* Student's t-test **Chi-square test †Mann-Whitney U test

to the recent findings from the National Institutes of Health, proteomic studies have shown more than 1 000 proteins and 19 000 unique peptide sequences in saliva.[12] However, the clinical application for salivary diagnostics in the evaluation of systemic diseases has remained elusive.[12] Having regard to the relatively high incidence of cardiovascular diseases in Iran[13,14] and recognizing the importance of early diagnosis and monitoring to the prognosis of patients, this investigation was aimed at comparing salivary CK-MB between MI patients and healthy individuals. The study sets out to determine the correlation between serum and saliva levels of CK-MB in MI and in healthy patients. Further, the possible role of gender and a previous history of MI on levels of salivary CK-MB in MI patients will be considered.

METHODS

This case-control study was performed in Baghiatollah Hospital, Tehran, Iran. The group constituted of 41 patients (28 (68.2%) men, 13 (31.8%) women, aged 27-89 years) admitted to the emergency department with a typical ischemic chest pain, electrocardiographic ST segment elevation, and a rise in serum biomarkers of MI. Forty two (29 (69%) men, 13 (30%) women, aged 27-88) age and sex-matched individuals with no history of heart disease selected from hospital staff or persons who accompanied patients were enrolled as a control group. People with oral lesions or with muscular trauma were excluded from the study.

The study received approval by the Institutional Review Board (IRB) of the Shahid Beheshti University of Medical Sciences, (#3219,Oct.2013). Before saliva sampling, all persons received detailed information about the aim of the study and collection protocol in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments. Informed written consent was obtained from all participants whose identity remained anonymous.

They were asked to avoid eating, drinking, smoking and brushing teeth for at least two hours before sampling. One minute after mouth rinsing with tap water, the participants swallowed all their oral fluid, and thereafter, they expectorated 2-3ml of resting whole saliva into a dry, pre-weighed plastic tube. The saliva sample weight was calculated as the difference in the weights of the dry and the filled tubes. The salivary flow rate could then be determined. The tubes containing the saliva samples were centrifuged at 3800g for 10 minutes and then were stored at a temperature of -70°C for later analysis of CK-MB levels.[5] In the group of patients, salivary collection was done 12 to 24 hours after MI and in the control group, the salivary samples were collected between 9am and 12 noon. Serum levels of CK-MB and troponin were measured periodically for these patients in the hospital and these data were available for the study from the patient records.

The tests were all done in the same laboratory using the same standardized procedures. The times of collection of samples for serum and for salivary CK-MB testing were matched as closely as possible. Salivary CK-MB levels were measured by a photometric method using a Pars-Azmoon commercial kit (Tehran, Iran).

The Mann-Whitney U test was used to compare the salivary CK-MB levels in the MI patient and the control groups. The data was also examined to determine whether gender and previous history of MI (positive and negative) had an influence. A Spearman test was used to analyze the correlation between serum and saliva levels of CK-MB in MI patients. Results were considered statistically significant if $p<0.05$. Analyses were performed using SPSS software version 16.

RESULTS

There were no statistically significant differences in the data between study groups in terms of age, sex, and unstimulated whole salivary flow rate (Table 1).

As salivary CK-MB levels between the case and the control groups were not normally distributed, the median of variables was used for statistical analysis by means of a Mann-Whitney U test. There were no significant differences between MI patients and healthy controls in terms of salivary CK-MB levels ($p=0.30$) (Table 1).

In the MI patient group, there were no significant differences in median levels of salivary CK-MB in terms of gender (28 men, CK-MB: 21 U/l vs. 13 women, CK-MB: 24 U/l, $p=0.69$).

The same finding was also obtained in terms of the previous history of MI in the group of affected patients. (14 positive history, median CK-MB level: 12.5 U/l vs. 27 negative history, median CK-MB level: 24 U/l), $p=0.31$). The mean serum CK-MB level in the MI case group was (76.4 ± 64.1 U/l).

According to Spearman's r-correlation test, only very weak correlations and no statistically significant differences were found between serum and salivary levels of CK-MB in patients with MI for both men ($r=0.17$, $p= 0.38$) and women ($r=0.43$, $p= 0.14$). Similar results were seen when the genders were pooled ($r=0.14$, $p= 0.39$).

DISCUSSION

Plasma has been described as the main source of salivary secretions and it is therefore possible that any changes in the CK-MB level in blood could lead to a similar modification in the salivary content of this biomarker of necrosis. Hence it could be surmised that salivary CK-MB

may serve as an easy-to-use diagnostic tool for point of care testing for AMI.[5]

In this study salivary flow rates as well as salivary CK-MB levels were compared between MI patients and healthy individuals. No significant differences were demonstrated in salivary flow rate between the groups, as was also found by Mirzaii-Dizgah *et al*. in two different studies.[5,15]

The results demonstrated no significant differences between case and control group in terms of salivary CK-MB levels, an outcome in agreement with previous similar studies.[16,17] Although CK-MB and troponin are excellent serum biomarkers of AMI, they did not demonstrate discriminatory capacity in unstimulated whole saliva for the identification of AMI.[6] Salivary CK-MB levels were found to rise only about 0.4-fold above baseline at 16 hours post-septal ablation. It was also noted that salivary CK-MB drifted upward early, returned to baseline levels, and remained thereafter below healthy control level at all time points.[17] In somewhat stark contrast, another case control study on 30 MI patients concluded that in comparison with healthy controls patients with MI had an about 8-fold increase in salivary CK-MB level.

These differences in results might be related to variations in sample size, demographic characteristics of patients, study design and methodology in detection of CK-MB. For example, the immuno-inhibition method, which was used in a similar study, is described as a method of low efficacy to detect salivary and serum CK-MB.[5]

According to current results, sex and previous history of MI did not affect salivary CK-MB levels. No evidence was found in the literature indicating the effect of these two variables on salivary CK-MB levels in MI patients. However, a higher percentage of serum CK-MB alterations and positive ECG were found in men than women.[18] In addition, despite the fact that age and sex are regarded as predisposing factors for heart disease; they had no significant effect on cardiac biomarkers such as troponin concentrations in saliva and blood in MI patients.[19]

Contrary to Mirzaii-Dizgah *et al*.[5] who found a strong correlation between serum and salivary levels of CK-MB, the present study revealed a very weak correlation.

There were some limitations to the study. Sequential measurements of salivary CK-MB were not possible, as participants declined this procedure. There is little evidence in the literature regarding the alteration of salivary CK-MB levels during episodes of MI, and no data about the timing of CK-MB levels rising in salivary secretion after the onset of MI was discovered.

As patients with suspected, but not established MI, were not included in this study, no judgment regarding salivary CK-MB levels in patients with unstable angina could be established.

CONCLUSIONS

This study has not shown that alteration of salivary CK-MB levels could be a possible indicator in the diagnosis of myocardial infarction. However, in the light of contradictory evidence, further studies are warranted to clarify this finding.

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