

## ORIGINAL ARTICLE

## Unstimulated whole saliva creatine phosphokinase in acute myocardial infarction

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**OBJECTIVE:** Accurate and rapid diagnosis of acute myocardial infarction (MI) is of major medical and economic importance. The objective of this study was to identify unstimulated whole saliva creatine phosphokinase (CPK) in patients with acute MI.

**SUBJECTS AND METHODS:** A case–control study was carried out in 30 normal healthy individuals and 30 patients with acute MI were hospitalized in CCU of Kamkar hospital, Qom, Iran. CPK levels were assayed in serum and unstimulated whole saliva at the first and the second days of acute MI by IFCC method. Statistical analysis of the Student's *t*-test and Pearson correlation coefficient was performed.

**RESULTS:** The mean saliva and serum levels at both the first and the second days of acute MI were significantly higher in patients with acute MI compared with healthy individuals. They were significantly greater in the first day than in the second day. Saliva CPK concentration correlated significantly with serum CPK level in the first day ( $r = 0.442$ ,  $P < 0.01$ ) and in the second day of acute MI ( $r = 0.268$ ,  $P < 0.01$ ).

**CONCLUSION:** Results suggest that salivary CPK can be used as an alternative to serum CPK for diagnosis and monitoring of myocardial infarction.

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cules when the integrity of the cellular membranes is compromised. These molecules enter the bloodstream, and circulating cardiac biomarkers are measured to detect or exclude myocardial injury/necrosis. Therefore, measurements of serologic markers are an important component of diagnosing acute myocardial infarction (MI). They are appealing in part because they are relatively inexpensive and easy to interpret.

It has been determined that a number of markers are present in saliva; therefore, its use as a diagnostic fluid could have significant diagnostic and logistical advantages when compared with serum. As a diagnostic medium, saliva has several advantages – its collection is safe, non-invasive, inexpensive, and simple, and it may be collected repeatedly without discomfort to the patient (Tabak, 2001; Bigler *et al*, 2002; Kaufman and Lamster, 2002; Lawrence, 2002; Agha-Hosseini *et al*, 2006). It is used to aid in the diagnosis of diseases and assessment of the severity of some illnesses (Agha-Hosseini *et al*, 2007a,b).

Serologic creatine phosphokinase (CPK) has been used as biochemical markers for early diagnosis of acute MI by observing its rise and fall activity (Zhang, 2005). In acute MI, CPK starts to rise in 4 h, peaking around 12 h, and returning to normal in 24–72 h (Short and Clements, 1993). The purpose of the current study was to examine the relationship between serum and saliva levels of CPK and to compare saliva CPK between healthy individuals and patients with acute MI.

## Introduction

Myocardial infarction is the major cause of death worldwide (Mueller *et al*, 2008). Accurate and rapid diagnosis is of major medical and economic importance. Myocardial necrosis is accompanied by the release of structural proteins and other intracellular macromole-

## Subjects and methods

*Subjects*

This study was designed as a case–control survey in Kamkar Hospital (Qom, Iran) to investigate correlation between serum and salivary levels of CPK in patients with acute MI and apparently healthy people. Thirty patients who were admitted to the emergency department with a typical ischemic chest pain, electrocardiographic ST segment elevation, and a rise in serum biomarkers of MI as case group (23 men, 7 women; aged 38–84 years) and 30 age- and sex-matched individuals with no documented heart disease (22 men, 8 women;

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aged 38–81 years) as control group were included in the study. Controls were selected from hospital staff or individuals who accompany patients referred to the hospital. People with lesion(s) in their mouths or with muscular trauma were excluded from the study. Basal clinical characteristics and biochemical parameters of study groups were summarized in Table 1. The protocol was approved by the Review Board of AJA University of Medical Sciences, and written informed consent was obtained from all patients and control subjects.

#### Saliva and serum collection

Venous blood and saliva were collected simultaneously from each case participant on the first and the second morning after occurrence of MI. For saliva sampling, all participants received detailed information about the collection protocol. They were asked to avoid eating, drinking, cigarette smoking, and brushing teeth at least for 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 preweighed 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 (Mirzaei-Dizgah and Agha-Hosseini, 2010).

Two milliliters of venous blood was drawn immediately after saliva sampling. Upon completing sample collection, the specimens were centrifuged at 3800 g for 10 min, and then the serum and saliva supernatants were isolated and divided into aliquots. The aliquots were stored in  $-70^{\circ}\text{C}$  for later analysis of CPK.

#### Analysis of saliva

CPK concentration was analyzed by International Federation of Clinical Chemistry (IFCC) method (Horder *et al*, 1991) using commercially available kits (Parsazmoon, Tehran, Iran).

#### Statistical analysis

For statistical analysis, the data are presented as a mean  $\pm$  SEM. Comparison of means between groups

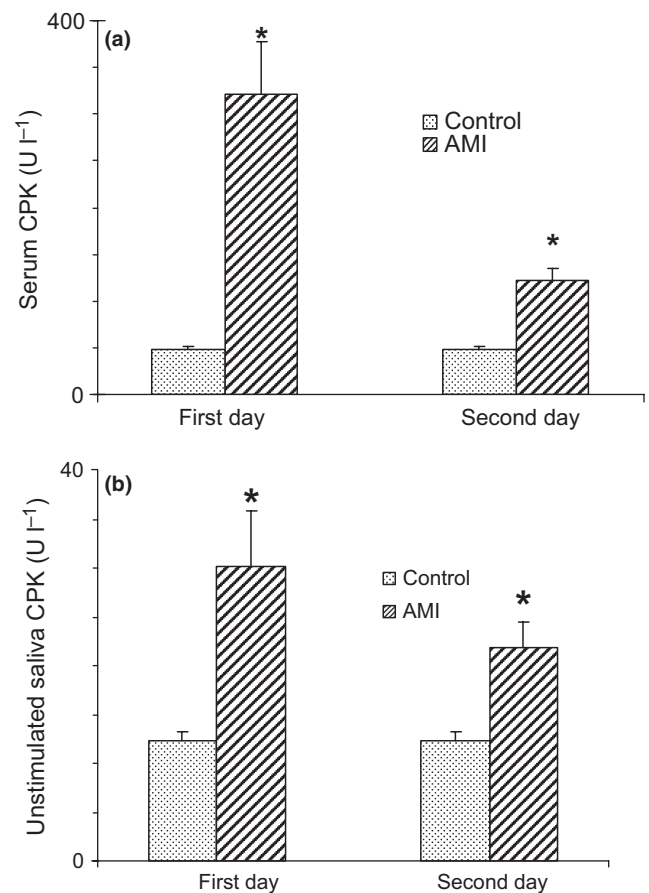
was carried out with unpaired two-tailed student's *t*-test. The Pearson correlation test was applied to determine association between serum and salivary concentration of CPK. Results were considered statistically significant if  $P < 0.05$ . Analyses were performed using SPSS software version 16 (SPSS Inc. Chicago, IL, USA).

#### Results

There was no significant difference in baseline unstimulated saliva flow rate between two groups (first morning:  $0.50 \pm 0.04 \text{ ml min}^{-1}$  in controls versus  $0.60 \pm 0.13 \text{ ml min}^{-1}$  in MI patients; second morning:  $0.51 \pm 0.04 \text{ ml min}^{-1}$  in controls versus  $0.53 \pm 0.06 \text{ ml min}^{-1}$  in MI patients), confirming the integrity of salivary gland function (Arregger *et al*, 2008).

As anticipated, the mean serum concentration of CPK, a biomarker of myocardial necrosis, was higher in patients than that of controls in both the first and the second days of acute MI (Figure 1a).

Salivary concentration of CPK proved to be significantly higher in patients with acute MI compared to people without ischemic heart diseases in both the first and the second days of acute MI (Figure 1b).



**Figure 1** Concentrations of creatine phosphokinase (CPK) in (a) serum and (b) unstimulated saliva in patients with acute myocardial infarction (AMI) and control individuals. \* $P < 0.05$

**Table 1** Basal clinical characteristics and biochemical parameters of subjects

Characteristics	Acute MI	Controls
Current smoking (%)	42	21
Hypertension (%)	46	21
Diabetes mellitus (%)	46	43
Serum enzymes		
Triglycerides ( $\text{mg dl}^{-1}$ )	$187 \pm 120$	—
Total cholesterol ( $\text{mg dl}^{-1}$ )	$190 \pm 89$	—
LDL cholesterol ( $\text{mg dl}^{-1}$ )	$104 \pm 25$	—
HDL cholesterol ( $\text{mg dl}^{-1}$ )	$35 \pm 8$	—
LDH ( $\text{U l}^{-1}$ )	$678 \pm 350$	—
SGOT ( $\text{U l}^{-1}$ )	$37 \pm 25$	—
SGPT ( $\text{U l}^{-1}$ )	$27 \pm 21$	—
ALP ( $\text{U l}^{-1}$ )	$170 \pm 55$	—

Statistical evaluation of data using Pearson analysis indicated a moderate correlation between salivary concentration of CPK and its serum concentration in the first day ( $r = 0.442$ ,  $P < 0.01$ ) and in the second day ( $r = 0.268$ ,  $P < 0.01$ ) of acute MI.

## Discussion

Cardiovascular disease, having enormous health, social, and economical consequences, is the leading cause of death in developed countries. So, accurate and rapid diagnosis is of major medical and economic importance. The current study investigated the unstimulated whole saliva CPK level in patients with acute MI. We found that patients with acute MI had significantly higher saliva CPK levels than controls, and such as serum, unstimulated whole saliva CPK levels rise and fall in the first and second days of acute MI, respectively. Significantly, the positive correlation of saliva with serum CPK level was also seen.

The main purpose of this study was to investigate possible changes of CPK levels in the saliva following occurrence of acute MI. To the best of our knowledge, this is the first report on the increase in saliva CPK levels after acute myocardial necrosis.

The CPK system of isoenzymes consists of CK-MM, CK-MB, and CK-BB. Each subunit of the dimeric CPK is regulated by a distinct gene and expressed in a tissue-specific manner. In humans and animals, CK-MB is found predominately in the myocardium, with concentration ranges from 5% to 30% of the total CPK activity of the heart (Apple, 1999). Serum CPK and its MB isoenzyme fraction have been widely used as biochemical markers for early diagnosis of acute MI and other cardiac injury. In MI, they usually increase in serum within 4–8 h after MI onset and return to normal 48–72 h later (Zhang, 2005), which was in agreement with our study. However, in a previous study, it has been shown that the saliva CK-MB do not show a significant difference between acute MI patients and controls (Floriano *et al*, 2009).

The underlying mechanism of saliva CPK increase is not known; nevertheless, plasma is obviously the main source of salivary secretions, and any change in the blood of CPK levels can lead to a similar, although to a lesser extent, modification in salivary content of this necrotic biomarker.

Saliva as a diagnostic specimen can give not only the same information as serum testing but also additional or new information that cannot be obtained from serum (Hofman, 2001). Saliva is a clear colorless liquid, whereas serum may become milky when lipemic, red when blood cells are hemolyzed because of trauma, and icteric in the presence of liver disease. Because serum possesses more proteins than saliva, assaying trace amounts of factors may result in a greater risk of non-specific interference and a greater chance for hydrostatic interactions between the factors and serum proteins. Because of these significant characteristics, finding biomarkers in saliva for the detection of serious systemic illnesses is of great interest for most salivary researchers.

The unstimulated whole saliva concentration of CPK revealed a significant correlation with serum CPK concentration. The level detected in the saliva was in the range 10% of that found in the serum. It is in agreement with prior studies on correlation between saliva and serum levels of progesterone (Walker *et al*, 1979), testosterone (Landman *et al*, 1976), 17B-estradiol (Agha-Hosseini *et al*, 2009a), parathyroid hormone (Agha-Hosseini *et al*, 2009b), cancer antigen (CA) 15-3 (Agha-Hosseini *et al*, 2009c), CA125 (Agha-Hosseini *et al*, 2009d), and steroid hormone-binding globulin (Hammond and Langley, 1986).

## Conclusion

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 CPK just as what occurs in serum. The core of the present study is the suggestion that salivary CPK can be used as an alternative to serum CPK for diagnosis and monitoring of myocardial infarction. Further studies need to be carried out to make this suggestion come true.

## Author contributions

Both authors contributed in all stages of work.

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