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Salivary Heart Fatty Acid Binding Protein - A Novel Biomarker of Myocardial Damage

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Abstract

Aim: Early diagnosis of acute myocardial infarction can greatly reduce the mortality rate of patients suffering from cardiovascular diseases. Cardiac biomarkers are slowly replacing the traditional diagnostic methods for diagnosis of acute myocardial infarction. These enzymes are released into the blood as early as 3 hours from the onset of the initial symptoms. Saliva offers an alternative to serum in the detection of these enzymes. This study is carried out to detect the levels of the cardiac biomarker, heart fatty acid binding protein in the serum and saliva of patients with acute myocardial infarction.

Materials and Methods: The analysis was performed using Enzyme linked immunosorbent assay.

Results and Conclusion: The results demonstrated significant alteration in the levels of the protein in the serum and saliva of patients suspected of acute myocardial infarction.

Keywords: Myocardial infarction; Electrocardiogram; Heart fatty acid binding protein; Creatinine kinase myocardial band; Cardiac troponins

Introduction

Acute myocardial infarction (AMI) is a major cause of morbidity and mortality worldwide. The prolonged imbalance between the oxygen supply and oxygen demand in myocardial cells leads to myocardial infarction [1]. The highest risk of death occurs within the initial hours of onset of AMI. Thus early diagnosis of cardiac ischemia is critical for the effective management of patients with acute myocardial infarction. The most common cause of myocardial ischemia is the rupture of an atherosclerotic lesion in a coronary artery that forms a thrombus and stops the blood supply. Early diagnosis allows clinicians to enable appropriate and successful treatment. The electrocardiogram (ECG) alone is insufficient for the diagnosis of AMI since ST-segment elevation may be observed in other conditions. Cardiac enzymes are now being widely used as frontline diagnostic tools. Cardiac troponins (cTnI), creatinine kinase myocardial band (CK-MB), and myoglobin are the routinely used biomarkers to detect myocardial injury [2]. Heart fatty acid binding protein (H-FABP) is an emerging biomarker of myocardial necrosis and injury [3]. It is a small cytosolic protein found abundantly in the heart and to a lesser extent in the brain, kidney and skeletal muscle. H-FABP is released very early into the serum after the onset of chest pain [4,5]. Levels of H-FABP are detectable in the blood as early as 1-3 hours and reach a peak concentration in about 6-8 hours and returns to baseline values within 30 hours [6]. Studies have reported that H-FABP can contribute as an early biological marker of post thrombolysis due to its rapid return to baseline values [7]. The established markers like CK-MB and myoglobin has limited ability because of its presence in the skeletal muscle and also lacks specificity. Furthermore, the gold standard, the cardiac troponin I that is highly specific for myocardial injury is detectable in the blood in substantial quantity approximately around 6–8 h after the onset of acute myocardial infarction making early diagnosis difficult [8]. Hence H-FABP can independently diagnose AMI during the early hours of myocardial injury. Furthermore, the advantages of H-FABP as a biomarker include its augmented expressions in myocardium, cytoplasmic confinement, molecular size, tissue specificity and early release into the plasma and urine [9-11].

Salivary biomarkers has helped clinicians, researchers and community health care workers to better diagnose and monitor disease progression to improve the overall well-being of the public [12]. Several studies have demonstrated the increased expression of biomarkers of myocardial injury like CK-MB and Troponin I in the saliva of patients with MI [13-16]. Thus saliva can be used as an alternative to serum in the diagnosis of AMI. The aim of this study is to quantify the levels of H-FABP in serum and saliva of patients with MI to necessitate easy and early diagnosis. The purpose of this study was to assess the level of this protein in the saliva of patients suffering from AMI.

Materials and Methods

Patients reporting to the Department of Accident and Emergency Medicine, Sri Ramachandra University within 6 hours after the onset of chest pain were recruited for participation in the study. The study group included 25 patients diagnosed as suffering from AMI by an attending cardiologist and admitted to the hospital based on clinical symptoms, ECG findings and evaluation of the cardiac biomarkers, troponin and CK-MB. All the procedures were performed with appropriate informed consent from all patients and healthy controls and the study protocol was approved by the Institutional Ethical Committee and Review Board of Sri Ramachandra University.

Materials: The un-stimulated saliva (2 ml) and peripheral blood (2.5 ml) were collected by a qualified phlebotomist. The study group comprised of 25 patients (mean age 58 years; 19 male and 6 female) and the control group comprised of 15 healthy individuals (mean age

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40 years; 9 male and 6 female). Patients, who were less than 18 years of age, or had previous history of cardiac surgery, muscular disorders and renal insufficiency, were excluded from the study. The number of samples was restricted to 25 based on the availability of samples.

Sample preparation and ELISA for H-FABP: Patients and healthy individuals were advised to collect the saliva in the floor of the mouth and spit into sterile containers. The collected blood samples and saliva samples were centrifuged at 1500 rpm for 5 minutes. The serum and the supernatant of the saliva samples were transferred into eppendorf tubes and stored at -80°C till further analysis. The analysis of H-FABP was performed by an enzyme linked immunosorbent assay (ELISA) using assay kit (R&D Laboratories, H-FABP Duoset) as per manufacturer instruction. Briefly, 100µl of the serum or saliva samples from the study group and control group were added to the capture antibody pre-coated plates and incubated at 37°C for two hours. The plate was washed thrice with 200 µl of the washing buffer and the detection antibody (100 µl) was added and incubated further for 2 hours. Following incubation, the wells were washed with the washing buffer and 100 µl of the Streptavidin-HRP conjugate was added and incubated at room temperature for 20 minutes. Then the plate was washed again and the substrate was added and incubated in the dark for 20 minutes. After 20 minutes, the reaction was arrested by addition of 50 µl stop solution and the optical density was observed at 450 nm and 570 nm wavelengths. The optical density at 570 nm was subtracted from the optical density at 450 nm.

Results

A total of 25 patients and 15 healthy individuals were included in the study. Samples for H-FABP analysis was collected during the time of admission of patients. The patients underwent routine ECG and analysis of troponin I at the time of admission. Out of the 25 patients, 3 patients had negative troponin values. 18 patients had previous history of type 2 diabetes and 10 patients gave history of hypertension. The healthy individuals did not have past history of diabetes or hypertension. Results of H-FABP analysis are presented as mean ± SEM. p values < 0.05 were considered statistically significant (Tables 1 and 2). The study demonstrated the expression of H-FABP in serum and saliva at the early hours (within 6 hours) of acute MI and compared with healthy individuals. The mean concentration of H-FABP in the serum and saliva were found to be elevated in acute MI patients when compared with healthy individuals (Figure 1). There was no association between the increased concentration of H-FABP in the serum and saliva of patients.

Discussion

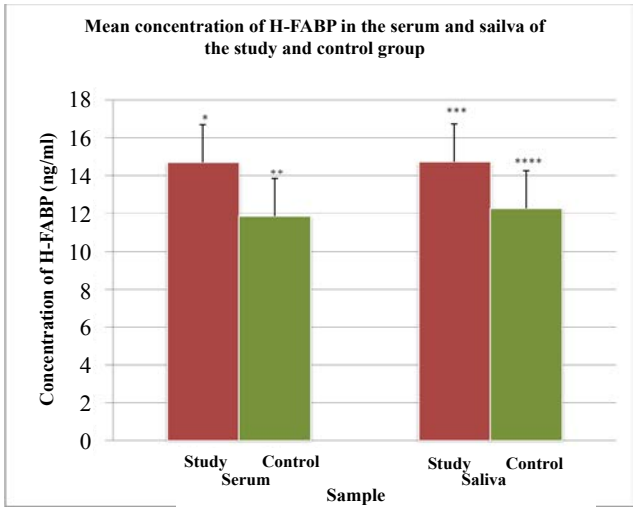
The diagnosis of myocardial infarction is based on the assessment of clinical symptoms, ECG changes and elevated levels of cardiac biomarkers [17]. Biomarkers are routinely used to assist in the timely diagnosis of these conditions while the novel markers have been used to predict outcome following an acute myocardial infarction [18]. Cardiac enzymes have replaced the older diagnostic tools in the detection of myocardial injury caused by myocardial ischemia. Serial measurements of biochemical markers of myocyte injury are advised for patients with acute myocardial infarction. Elevated levels of CK-MB, troponin T and troponin I have been regarded as biochemical markers of myocyte necrosis. HFABP and myoglobin have greater sensitivities than those of CK-MB and TnT. Therefore, H-FABP has better diagnostic values for the detection of myocardial injury. Troponin is a widely used biomarker but it lacks sensitivity during the early phase (1-3 hours) of acute myocardial injury [19]. Previous studies done by Junnichi Ishii et al. [20] and Junnichi Ishii et al. [21] demonstrated the augmented H-FABP expression in the acute MI group when compared with the non-AMI and control groups. The sensitivity, specificity, and predictive accuracy of H-FABP is significantly higher than those of myoglobin for the detection of acute myocardial infarction in the initial hours of onset of chest pain, but was found to be similar to myoglobin concentration from 3 to 6 h of onset. H-FABP concentration was similar to myoglobin concentration in patients with unstable angina, but there was increase in myoglobin concentration in noninfarcted patients also. Thus, H-FABP is a more sensitive and specific marker than myoglobin, CK-MB and troponin I for the detection of AMI within 3 h, after the onset of chest pain [22,23]. Elevated levels of heart fatty acid binding protein are associated with adverse cardiovascular events following the acute phase [24]. In our study, the H-FABP analysis showed increased H-FABP levels in the serum and saliva of patients when compared with healthy individuals. Salivary diagnostics would enable clinicians to monitor diseases frequently and easily and would have impact on the future medical research and therapy [25,26]. Analysis of saliva has proved the expression of proteins like immunoglobulins, amylase, prolactin-inducible protein, glycoprotein and cystatins, interleukin-1 receptor antagonist, von Ebner's gland protein (lipocalin-1) and calgranulin A and B (S100A8 and A9), apolipoprotein A, glutathione

Statistical analysis of the concentration of H-FABP in the serum			
Sample	**Group**	**Mean (± Std. Deviation)**	**p value**
Serum	Study group (N=25)	14.72 ± 1.369	.000
	Control group (N=15)	11.85 ± 0.912	

Table 1: Statistical analysis of the concentration of H-FABP in the serum.

Statistical analysis of the concentration of H-FABP in the saliva			
Sample	**Group**	**Mean (± Std. Deviation)**	**p value**
Saliva	Study group (N=25)	14.77 ± 0.869	.000
	Control group (N=15)	12.3 ± 0.542	

Table 2: Statistical analysis of the concentration of H-FABP in the saliva.



*- mean concentration of H-FABP in the serum of the study group
**- mean concentration of H-FABP in the serum of the control group
***- mean concentration of H-FABP in the saliva of the study group

Figure 1: Mean concentration of H-FABP in the serum and saliva of patients with MI (Study) and healthy individuals (Control).

S-transferase P and fatty acid binding protein [27,28]. H-FABP analysis in the saliva and serum by serial measurements in a larger sample population can further improve the diagnostic and prognostic value of H-FABP for AMI.

Conclusion

This preliminary case study showed significant increase of H-FABP in serum and saliva of patients with MI suggesting that H-FABP in the saliva can be used for the diagnosis of AMI. The limitations of this study were the sample size, serial measurements of samples and analysis of other biochemical parameters. Further studies will enable us to give a diagnostic cutoff value for H-FABP in saliva for diagnosing acute myocardial infarction and to assess its sensitivity and specificity in saliva.

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