Original Article

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Dentistry Section

Evaluation of Salivary Cardiac Troponin-I as Potential Marker for Detection of Acute Myocardial Infarction

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ABSTRACT

Introduction: Serum cardiac troponin-I is a specific biomarker and raised significantly after Myocardial Infarction. Saliva is a non-interventional bio-fluid which contains portion of serum constituents and can be key in diagnosis of MI.

Aim: To estimate and correlate the level of cardiac Troponin-I (cTnI) in un-stimulated whole saliva and serum in acute MI patients and control group.

Materials and Methods: Total of 60 individuals were enrolled and equally divided in to study (group I) and control group (Group II). Informed consent form was taken from all the subjects. Saliva and blood samples were obtained from patients with ECG features suggestive of acute MI within 24 hours. Serum and saliva samples were processed further for cTnI. The results obtained were then statistically analysed.

Results: The mean cTnI level in serum of group I and group II were found to be 4.27±1.79 mg/L and 0.158±0.05 mg/L respectively. The mean cTnI level in saliva of group I and group II was found to be 0.67±0.10ng/L and 0.160±0.05 ng/L respectively. On analysing serum values and saliva values, p-value was found to be significant'.

Conclusion: The saliva levels of cTnI were directly associated with serum levels demonstrating a highly significant strong positive relation and confirms the diagnostic ability of saliva for detection of cTnI.

Keywords: Cross-sectional studies, Diagnostic biomarkers, Heart muscle, Necrosis

INTRODUCTION

Myocardial Infarction (MI) is an ishaemic heart disease with irreversible damage (necrosis) of heart muscle due to lack of oxygen supply. Contemporary universal definition of MI states that the criteria for diagnosis of acute MI is detection of rise and/or fall of cardiac biomarkers with at least one value above the 99th percentile of the upper reference limit together with symptoms of ischemia or ECG changes indicative of new ischemia [1].

Acute Myocardial infarction can be divided in to ST Elevated MI (STEMI), Non ST Elevated MI (NSTEMI) and unstable angina. STEMI is diagnosed when there is an evidence of complete occlusion of coronary artery leading to myocardial necrosis with cardiac symptoms whereas in NSTEMI, partial/incomplete blockage of Coronary artery occurs with no or mild clinical signs and/or ECG changes. The diagnosis of NSTEMI majorly depends on detection of serum cardiac biomarkers however, salivary cTn I has not been studied separately for STEMI and NSTEMI. Patients with typical MI may have fatigue, chest discomfort and malaise as symptoms in the days preceding the event [2].

The diagnosis of MI is based upon *Electrocardiography* (ECG), various cardiac biomarkers and cardiac imaging. The biomarkers of cardiac necrosis are of great importance in the diagnosis of MI [3]. Among all the proteins, the commonly used biomarker in acute cardiac care is natriuretic peptides, C-Reactive Protein (CRP), Creatine Kinase (CK) and cardiac troponin [4]. The American College of Cardiology/American Heart Association (ACC/AHA) and the European Society of Cardiology (ESC) guidelines recommend that cardiac biomarkers should be measured at presentation in patients with suspected MI [5].

The troponin complex is located on the thin filament of striated muscle and is composed of three subunits, Troponin T, Troponin-I and Troponin C. Troponin T attaches the troponin complex to tropomyosin. Troponin-I modulates the interaction of actin and myosin by inhibiting actomyosin adenosine triphosphatase activity, and Troponin C is the calcium-binding subunit of the troponin complex [6].

Troponin's sub types, Troponin-I & T are genetically and immunologically different in cardiac muscle and skeletal muscles. Troponin C in cardiomyocytes is identical to striated skeletal muscle troponin C. Hence cardiac specific troponin has nearly complete myocardial tissue specificity and high clinical sensitivity [7,8].

The cardiac specific troponins are detectable in the serum within 4 to 12 hours after the onset of myocardial necrosis, and depending on the duration of ischaemia and reperfusion status, peak values occur 12 to 48 hours from symptom onset. Cardiac Troponin offer a wide temporal diagnostic window (4 hours to 14 days) as their levels remain elevated in serum for up to 14 days after myocardial injury, allowing for diagnostic confirmation even when patients delay their presentation to medical care after onset of symptom [9,10].

Human saliva is a plasma ultra filtrate containing proteins derived from blood or from salivary glands. Saliva also plays an important role in maintenance of oral tissues [11]. Compared to serum, saliva demonstrated more accurate, inexpensive, and convenient results.

Saliva unlike plasma, does not clot over a period of time thus can be easily used for laboratory testing. Saliva collection involves non-invasive approach which makes this biological fluid an inexpensive, useful alternative to blood and urine testing [12].

Various studies have been carried out on the significance of troponin-I in serum. However, fewer studies correlate the estimation of troponin-I in saliva. Therefore, saliva being an important biological fluid, we planned to study the role of saliva in estimation of Troponin-I in myocardial infarction patients.

MATERIALS AND METHODS

A cross-sectional study of one year duration was planned & carried out at King Georges Medical University, Lucknow. The study was conducted from March 2015 to March 2016. The study protocol was approved by Institutional ethical committee of King George's

Medical University and written informed consent was obtained from all participants (consent was taken from patients as sample was derived between 12h-24h of clinical symptoms). The study included two groups of 30 individuals each on the basis of the formula, n=16 x (σ^2/δ^2) + 1, where σ is standard deviation (estimated) and d is the difference in effect of two interventions which is required (estimated effect size)

Detailed inquiries were made for all participants (subjects and controls) via questionnaire about their medical history, including recognized diseases, medical interventions, medications, and other health problems.

Inclusion criteria were patients clinically diagnosed with ST Elevated Myocardial Infarction (STEMI) (according to universal criteria of diagnosis) and gave their consent to participate in the study. Non ST Elevated Myocardial Infarction (NSTEMI) patients, unstable angina patients, immune system disorders, stroke patients, patients with any organ failure/complication, patients on steroidal therapy; patients who refused to participate in study were excluded from the study.

The study included two groups of 30 individuals each. Group I (study group) consisted of patients admitted in the last 24 hours to the Intensive Care Unit (ICU) of Department of Cardiology with typical chest pain and electrocardiographic characteristics of acute cardiac injury/necrosis, while group II (control group) consisted of age and sexes matched individuals with no documented heart disease.

We have also compared the relationship of serum and salivary cTn I with habit of smoking, hypertension, diabetes mellitus and familial history of cardiac disease in study and control groups.

Serum and Saliva Sampling

Procedure

For resting saliva sampling, subjects were asked to rinse their mouths with water followed by swallowing of all their oral fluids. Thereafter, individuals were asked to expectorate 2-3 ml of their resting whole saliva in a graded plastic tube. This procedure was continued for 5 minutes unless 2-3 ml of saliva was collected.

Blood samples were obtained from patients and divided in group I & group II. Two ml of venous blood was drawn immediately from antecubital vein after saliva sampling.

The serum and saliva samples were then carried to the Department of Biochemistry in an ice box and centrifuged at 3800 g for 10 minutes. The serum and saliva supernatants were isolated and divided into aliquots. The aliquots were put in a cryobox and stored at -70°C till analysed. All samples were processed within 24 hours of storage

Commercially available RayBio cardiac troponin-I Elisa kit was used for analysis of cardiac troponin-I levels in serum and saliva as per the manufacturer's instructions.

The intensity of the color (absorbance) for each well was measured at 450 nm in a micro plate reader and concentrations of cTnI were determined.

STATISTICAL ANALYSIS

The obtained data was statistically analysed by using SPSS software (version 20, 2008). Data was summarized as Mean±SD. Groups (cases v/s controls) were compared by unpaired or independent Student's t-test. Pearson's correlation coefficients were used to determine the relationship amongst the parameters. While correlation was defined as a measure of the strength and direction of a linear relationship between two scale variables.

RESULTS

The present study included total 60 individuals with age range from 44-81 years. Subjects were divided into two groups, study group (Group I) and control group (Group II).

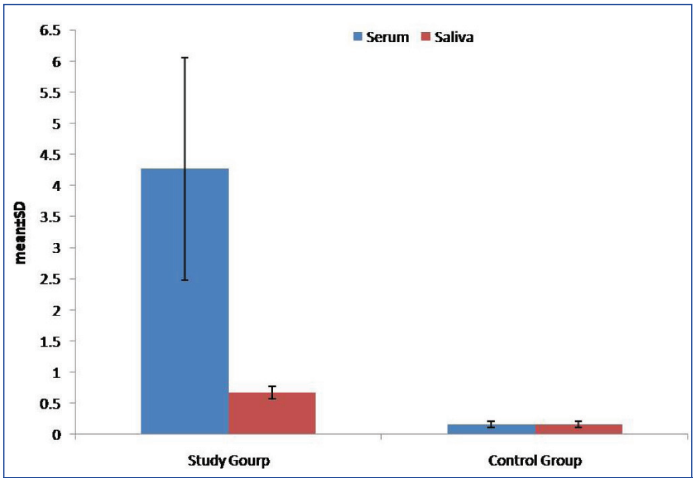
The mean age of study group was 64.10±10.3 years, whereas the mean age of control group was 63.72±9.7 years. Both group comprised of 25 (83.3%) males and 5 (16.7%) females.

The mean cardiac troponin-I level in serum of group I and group II was found to be 4.27±1.79 mg/l and 0.158±0.05 mg/l respectively. The mean cardiac troponin-I level in saliva of group I and group II was found to be 0.67±0.10 ng/l and 0.160±0.05 ng/l respectively [Table/Fig-1].

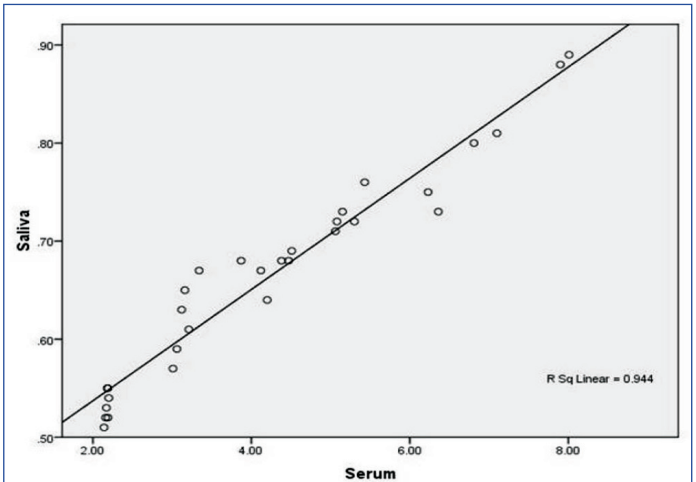
Comparing the mean of two groups (study versus control), t-test showed statistically significant difference ($p<0.001$) for the levels of cardiac troponin-I in both serum and saliva. The mean value of cardiac troponin-I was found to be higher in serum as compared to saliva for both study and control group. Similarly, Serum levels of cardiac troponin-I were directly associated with saliva levels and demonstrated a highly significant strong positive relation ($r=0.972$, $p<0.001$) [Table/Fig-2].

We have also evaluated the serum and salivary cTn I with respect to various other parameters such as habit of smoking, Diabetic condition, Hypertensive state and family history of cardiac diseases. [Table/Fig-3] shows the frequency distribution of the parameters in cases and control group. [Table/Fig-4,5] summarizes the levels of serum and salivary cardiac troponin-I in cases and control groups respectively with respect to other parameters (Habit of smoking, hypertensive state, diabetic history, and familial history of cardiac disease).

On further analysis, we found that in group I (cases), none of the parameter showed statistical significant difference between absence and presence of disposing factors in salivary and serum cardiac troponin-I levels However, in group II (controls), statistical significant



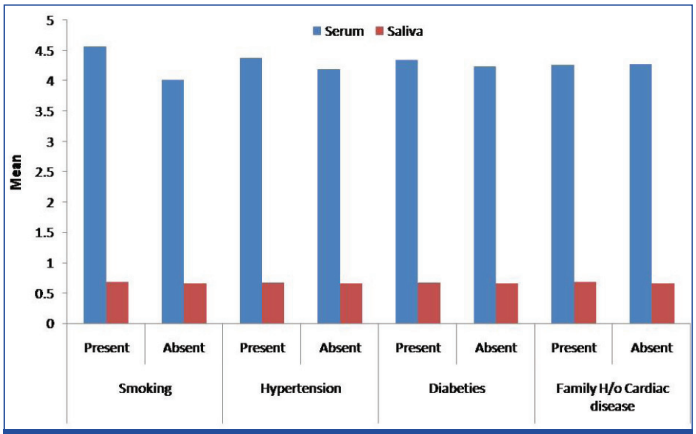
[Table/Fig-1]: Bar diagram showing mean cTnI levels of serum and saliva in groups I & II.



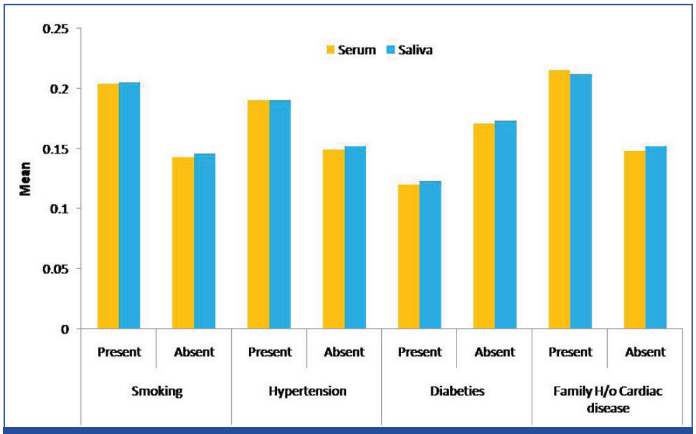
[Table/Fig-2]: Serum levels of cardiac troponin-I were directly associated with saliva levels and demonstrate a highly significant strong positive relation (r=0.972, p=<0.001)

Parameters		Study (N=30)	Control (N=30)
History of smoking	Yes	14	7
	No	16	23
History of Diabetics	Yes	10	8
	No	20	22
History of Hypertension	Yes	13	6
	No	17	24
Family history of cardiac disease	Yes	7	4
	No	23	26

[Table/Fig-3]: Frequency distribution of habits, associated diseases and family history of cardiac disease between study and control groups.



[Table/Fig-4]: Serum and Salivary levels of cTnI in group I (Cases) with respect to various parameters



[Table/Fig-5]: Serum and Salivary levels of cTnI in group II (Controls) with respect to various parameters

difference was observed in cTn I levels of subjects with and without smoking, diabetes and familial history of cardiac disease. In control group, we also noted that the levels of cTn I decreases in all variables except in diabetic patients where the level of cTn I are more in non diabetic patients.

DISCUSSION

Identification of patients with high risk for major cardiac events is a common and difficult challenge. Of late, cardiac troponins have seen as potential markers for early detection of acute myocardial injury. Previous studies have demonstrated the prognostic value of serum cTnI for risk stratification in patients with unstable angina and MI [13,14].

Saliva as a diagnostic specimen, can give almost similar information as serum testing. Changes in saliva quality and quantity are indicative of the wellness of the patient. Human saliva contains number of biomarkers specific for the different pathogenesis of oral and systemic conditions. On the other hand serum holds more proteins than saliva thus, non specific interference in assaying trace amount of factors will be more pronounced and there are increased chances of interaction among them. Therefore considering salivary biomarkers for the detection of abnormality in systemic illnesses is of importance [15]. Approximately, 27% of salivary proteins resemble plasma proteins and similar proteins present in both saliva and plasma are very valuable in monitoring of both disease progression and therapeutic treatments [16].

Miller and co-workers conducted a study to determine levels of cardiac markers in salivary secretions of acute MI patients and found that salivary myoglobin levels were significantly higher within 48 hours of chest pain onset in patients with acute MI. However, they did not mention elevation of CK-MB or cTnI levels in saliva of MI patients in their results. Moreover, they could not found any significant correlation between saliva and serum cTnI levels [17].

Iraj Mirzaii-Dizgah & Esmail Riahi found that unstimulated saliva and serum concentrations of cTnI were significantly higher in patients with acute MI than in controls. They also demonstrated that unstimulated saliva cTnI concentrations correlated significantly with serum cTnI levels [18].

The saliva levels of cTnI were directly associated with serum levels demonstrating a highly significant strong positive relation and confirms the diagnostic ability of saliva for detection of cTnI.

These findings were consistent with the study of Habib Haybar et al., who found no significant difference in the concentration of troponin-I in blood and saliva signifying the relative and consistent increase of this biomarker in saliva and blood. They concluded that use of saliva instead of blood, can be used acceptably in determination of cardiac troponins in patients with MI [19].

In the study group, the habit of smoking, diabetes, hypertension and family history were not found to be significantly affecting the levels of cTnI (above a minimum diagnostic value) in both serum and saliva. However, habit of smoking showed a much stronger correlation as compared to other variables.

In the control group, habit of smoking and family history was found to be significantly affecting the cTnI levels in both serum and saliva. This infers that these factors might be causing some degree of myocardial damage as shown by increased cTnI levels in these subjects as compared to subjects with absence of these factors. Also, other factors like tachycardia, severe aortic stenosis, gastrointestinal bleeding, sepsis, left ventricular hypertrophy, myocardial contusion, hypertensive emergency, diabetic keto-acidosis, chronic obstructive pulmonary disease exacerbation and coronary spasm can lead to increased cardiac troponin-I without any coronary artery disease [20].

Diabetic patients also showed significant difference in cardiac troponin levels however, the levels were more in non diabetic patients. This could be attributed to the fact that more number of individuals in non diabetic group had familial history of cardiac diseases and caused surge in the reading of cardiac troponin-I levels. There was no statistically significant effect of hypertension on cTnI levels in control group.

These results varied with that of Iraj Mirzaii-Dizgah & Esmail Riahi who in their study, conducted in 2013, found no statistically significant difference of cTnI levels in smokers and diabetics [18].

This current study suggests that habit of smoking and familial history of cardiac diseases is independent factors for cardiac inflammation and should be checked and brought under control to prevent any cardiac complications in the future. Subjects with a diabetic history should be screened thoroughly for their cardiac condition and subsequent measures must be taken to arrest the progress of any further myocardial damage.

As advantages of saliva as a medium to detect various biological markers is already established in literature, this current study, once again, confirms the role of saliva in patients with cardiac diseases.

LIMITATION

Firstly, the concentration of cTnI is much lower in saliva as compared to serum that is why salivary samples were not routinely used as an adjunct to serum analysis of cardiac troponin-I therefore tests with higher sensitivity and specificity should be used to precisely determine the salivary cTnI levels. Secondly, present study was carried out with nominal sample size and in control group statistical result cannot be extrapolated to the general population therefore; further studies with larger sample size should be followed.

CONCLUSION

This current study indicates a strong positive correlation between serum and un-stimulated salivary levels of cTnI. Therefore, we propose that highly sensitive saliva-based assays can be used as diagnostic marker of acute MI.

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