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Correlation of Salivary Troponin I among High Risk Patients of Myocardial Infraction

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Abstract: ***Cardiovascular disease is the major complication of systemic illness like diabetes mellitus, hypertension, hypercholesterolemia and leading cause of death globally.. According to Framingham study, silent myocardial infraction is higher in diabetic patients than others. Salivaomics has emerged as a new branch of diagnostics paving way for early detection of diseases. The aim of the study is to quantify and compare the Salivary Troponin I among normal, high risk patients of myocardial infractions and post myocardial infract patients. Materials and Methods-The study included 30 individuals divided into three groups. Group I consisted of 10 normal participants, Group II consisted of 15 patients of chronic systemic illness like type 2 diabetes mellitus (T2DM) and hypertension and Group III consisted of 15 post myocardial infract patients (both T2DM and hypertension) who were under anticoagulant therapy at Mahatma Gandhi post graduate institute of dental science, Puducherry. Un stimulated saliva was collected and subjected to estimation of salivary troponin I using ELISA.. Result: The mean salivary troponin I levels in group I, II and III were 0.08pg/ml, 0.85pg/ml and 0.22pg/ml respectively. Correlation of salivary cardiac troponin I levels and random blood sugar was highly significant with a 'p' value of 0.001 in group II and 0.003 in group III . Conclusion: The salivary cardiac troponin I was increased in patients who are at high risk of developing cardiac events in the future (group II) when compared to the other groups. Serial salivary cardiac troponin I testing can reduce the morbidity and mortality in patients with chronic systemic illness.***

Keywords: Cardiovascular disease, Pathophysiology of myocardial infraction, Chronic systemic illness, Salivary Troponin I

1. Introduction

Cardiovascular disease (CVD) is globally considered as the leading cause of death in low and middle income countries like India[1] . It is expected that by 2020, CVD would prevail as the leading cause of death and disability over infectious diseases globally[2].

Classic risk factors contributing to the development of cardiovascular disease according to Framingham risk score are age, gender, body mass index, altered lipid metabolism, hypertension, diabetes mellitus and smoking.

Cardiovascular diseases include a broad spectrum of disease of which myocardial infraction is a major manifestation and is of paramount importance as its association with mortality rate is higher.

2. Literature Survey

Myocardial infraction (MI) develops secondary to prolonged ischemia leading to irreversible necrosis of the heart muscle. MI results due to perfusion mismatch between oxygen supply and demand. It is characterized by loss of normal cardiac myocytes structure like myocytolysis, coagulative necrosis, inflammatory cell infiltrate and fibrosis. Myocardial infraction may present as a minor event in chronic illness and remain undetected or it may lead to sudden mortality due to haemodynamic mismatch. Detection of MI is made from the presentation of symptoms, ECG findings and enzyme detection from biochemical analysis. But in recent times

advent of sensitive molecular diagnostic techniques has facilitated detection of biomarkers corresponding to even smaller grams of myocardial necrosis [3] . Cardiac specific biomarkers such as Cardiac Troponin I and T exhibit more sensitivity and specificity for myocardial damage in clinical setting[4]. It is considered as a gold standard for myocardial damage. Assays for cTnI were first described by Cummins et al. Detection of rise or fall of the biomarkers is essential to the diagnosis of acute myocardial infraction[4].Troponin biomarkers remain elevated for upto two weeks following the myocardial damage[5]

Diabetes mellitus is responsible for a spectrum of cardiovascular disease. The best known complications arise from endothelial dysfunction, oxidation, inflammation, and vascular remodelling which contribute to atherogenesis.[6]]Hypertension is a frequent finding in patients with acute myocardial infarction (AMI) and its association with female sex, diabetes, older age, smoking and vascular co morbidities, composes a risk profile. [7]

Cardiac Troponin I (cTnI) contains 210 amino acids, of which 31 are unique to cardiac muscle and are not found in skeletal troponin. Unlike other markers of myocardial injury, troponins can be released in reversible myocardial injury and the event of myocardial necrosis alone does not mandate for the troponins to be released from myocytes. Reversible injury related changes in myocyte membrane are considered sufficient for the release of cardiac troponins from the free cytosolic pool, whereas in case of irreversible myocardial injury the source of troponin release is the structural damage of the myocytes. The changes in myocyte membrane permeability resulting from the injury could be enough for the release of cardiac troponins from the free cytosolic pool of myocytes without structural damage[8].

Salivary diagnostics is emerging as a new detection system of biologically important markers. Salivary testing is inexpensive, non-invasive, safer, involves easier sample collection methods, facilitates screening at home and paves way for chair-side diagnosis. Salivary constituents are believed to be derived from the blood and hence it can be considered as a reflection of physiological function of the body[9].

Salivary Troponin levels have been observed to be elevated in acute myocardial infraction with corresponding elevation of serum levels. Several studies has concluded that salivary troponin levels can be used for diagnosis and further monitoring of the disease progression.

As Troponin I is released even due to subclinical damage of myocardium in individuals with long standing history of systemic illness, this study was conducted to demonstrate and compare the cardiac Troponin I (cTnI) in saliva of both normal and diseased individuals and propose a novel risk indicator in patients with chronic systemic illness thereby decreasing the morbidity and mortality.

3. Materials and Methods

A cross-sectional study was conducted in the Department of oral pathology and microbiology, Mahatma Gandhi Post Graduate Institute of Dental Science, Puducherry during March 2019. A total of 40 participants was included in the study and were divided into three groups. Group I consisted of 10 normal participants who were willing to take part in the study and consisted mostly of hospital staffs, Group II consisted of 15 patients of chronic systemic illness like type 2 diabetes mellitus (T2DM) and hypertension and who were at an increased risk of developing myocardial infraction and Group III consisted of 15 post myocardial infract patients (both T2DM and hypertension) who were under anticoagulant therapy. Group 2 and 3 participants were recruited from the Department of General Medicine of our institution. Participants with congenital heart disease, chronic renal failure and recent infection were excluded from the study. Purpose of the study was explained to the study participants and informed consent was obtained from all the participants. A Questionnaire for recording demographic data, personal, past history and medical intervention was utilized and the details were recorded for all the study participants.

3.1 Sample Collection

Sample collection was done under aseptic environment using sterilized armamentarium between 9 to 11 AM when the diurnal variations were minimum and in normal room temperature. Patients were asked to refrain from any oral habits before one hour of collection and were asked to rinse the mouth thoroughly with distilled water before salivary sample collection. They were instructed to sit in a relaxed position with slight forward advancement of mandible to facilitate pooling of saliva in the floor of the mouth. 2ml of saliva was collected by spitting method in collecting tubes and immediately stored at -20 degree Celsius to prevent proteolysis of proteins. Salivary samples were collected in accordance with protocol proposed for standardized sample collection by Wong et al. The collected samples were centrifuged at 1000rpm for 20 minutes at 2-8degree celsius and the supernatant was subjected to estimation of salivary cTnI by Enzyme Linked Immunosorbent Assay (ELISA) immediately.

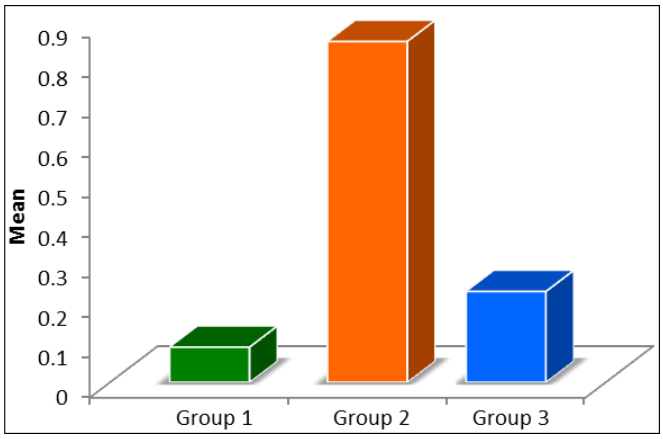
3.2 Assay Procedure

Human Cardiac Troponin I ELISA kit manufactured by MyBiosource based on sandwich enzyme-linked immune-sorbent assay technology was used in this study. The sensitivity of the kit is lesser than 2pg/ml. Anti-cTnI antibody was precoated in well plates. A biotin conjugated anti-cTnI antibody was used as detection antibodies. A standard curve was plotted with relative optical density 450nm of each standard solution versus the respective concentration of the standard solution. The optical density was read at 450nm in a microplate reader, then the concentration of cTnI was calculated from the standard curve.

4. Result

The present study included 40 individuals within the age group of 40-65 years. The mean age of normal patients and diseased were 43 and 56 years respectively. The control group (Group I) consisted of 6 female and 4 male. The diseased group (Group II and III) consisted of 19 female and 11 male. Table 1 describes the frequency distribution of diabeted mellitus and hypertension between Group II and Group III.

The mean salivary troponin I levels in group I, II and III were 0.08pg/ml, 0.85pg/ml and 0.22pg/ml respectively from our study (Table 2) and was found to be highly significant when correlated with each group.



Correlation of salivary cardiac troponin I levels and random blood sugar was highly significant with a ‘p’ value of 0.001 in group II and 0.003 in group III respectively (Table 3). Correlation of salivary troponin I and the duration of diabetes mellitus and hypertension in diseased groups were not significant. (Table 4)

The salivary cardiac troponin I was increased in group II patients who are at high risk of developing cardiac events when compared to the other groups.

The mean salivary cardiac troponin I levels were compared with the mean serum troponin I levels of study with similar groups conducted by Karar T et al[10] which revealed a serum troponin I level of 0.15µg/ml both in normal subjects (Group 1)and in patients with chronic illness(Group 2) while 0.19µg/ml in patients who experienced myocardial infraction(Group 3) (TABLE 5). When the serum cardiac troponin I levels (from study conducted by Karar T) and salivary cardiac troponin I levels (from the present study) were compared, 'p' value was highly significant in group I and group II.

Table 1: Frequency distribution of Diabetes mellitus and hypertension between Group II and Group III

Parameters		Group II	Group III
Diabetes mellitus	Yes	12	15
	No	3	0
Hypertension	Yes	10	10
	No	5	5

Table 2: Correlation of Salivary Cardiac Troponin I among all Three Groups

Group	N	Mean pg/ml	SD	F value	P value
Group 1	15	0.088	0.068	13.483	0.000**
Group 2	15	0.852	0.649		
Group 3	15	0.227	0.106		

Table 3: Correlation of Salivary Cardiac Troponin I and Random Blood Sugar in Group II and III

S. No	Groups	Salivary cTnI (pg/ml)	RBS (mg/dl)	Pearson's Correlation	'p' value
1	II	0.852	225	0.777	0.001
2	III	0.227	213	0.716	0.003

Table 4: Correlation of Salivary Cardiac Troponin I and duration of Diabetes Mellitus

S.No	Groups	Salivary cTnI (pg/ml)	Duration (In Years)	Pearson's correlation	'p' value
1	II	0.852	11.7	0.308	0.264
2	III	0.227	13.1	0.264	0.157

Table 5: Comparison of Salivary and Serum Troponin I among Three Groups

Parameters	Group I	Group II	Group III
Salivary cTnI (pg/ml)	0.08 ± 0.06	0.85 ± 0.64	0.22 ± 0.10
Serum cTnI (ng/ml)*	0.15 ± 0.04	0.15 ± 0.04	0.19 ± 0.01

*(The reference values for serum troponin I level for all the three groups has been obtained from the study by Karar et al [10])

5. Discussion

This is a first kind of study wherein salivary cTnI was estimated in patients with chronic systemic illness who were at a risk of developing myocardial infraction in the future.

Cardiac troponin I (cTnI) test is performed as a routine protocol in patients suspected with acute coronary syndrome[8]. Troponin I elevation without clinical signs in chronic condition does not indicate Acute Myocardial Infraction (AMI), but reveals a potential subclinical myocardial damage. cTnI testing is more organ specific than disease specific and is associated with the prediction of mortality in chronic conditions like diabetes mellitus, hypertension and hypercholesterolemia.

Cardiac Troponin I (cTnI) is expressed in 3 isoforms – Fast skeletal fsTnI, slow skeletal ssTnI and cardiac cTnI. Each troponin isoform is encoded by separate genes. During embryonic development ssTnI is expressed exclusively in place of cTnI in heart. The expression is switched to cTnI isoform at 8 – 9 months postnatally. After myocardial damage, the released cTnI is either in free unbound form or occur predominantly as a complex with Troponin C. 2 to 4 % of cTnI is unbound in cytosol or loosely bound to sarcomere. The N and C terminal region of cTnI is more susceptible to proteolysis and the central region of cTnI forms the calcium binding domain for troponin C and is more stable. This region is targeted for most of the assays. The release of cTnI is multifactorial and is not confined to that of event of myocardial infraction. Increased cTnI are recorded during increased wall tension and ventricular strain, excess catecholamine and impaired renal clearance.[8]

Brian R Wel et al observed that in events of ischemia an intracellular pool of unbound cTn could become packaged and released from viable myocytes in response to myocardial stresses insufficient to produce necrosis[10].

Harvey D white et al in his review stated 6 potential mechanisms of troponin elevation. They are myocyte necrosis, apoptosis, normal myocyte cell turnover, cellular release of proteolytic troponin degradation products, increased permeability of the cell membrane without cell necrosis, formation and release of membranous blebs. With repeated episodes of short lived ischemia, there is increased activity of calcium dependent protease calpain leading to chronic proteolytic degradation of myofibrillar cTnI. This proteolytic degradation of cTnI leads to fragmentation and easy passage of it through the intact plasma membrane. With reversible damage to myocardium there is functional recovery of the patient with no overt clinical signs. Patient with chronic systemic illness may be subjected to such repeated reversible myocardial damage when the underlying disease status is not controlled[12].

Saliva is an emerging medium to be explored for health and disease surveillance and serves as a major mandate to detect and monitor systemic diseases by the clinicians[13].

In the present study, the serum and salivary cTnI levels are greater in patients with chronic systemic illness like diabetes mellitus, hypertension (group 2) when compared to group comprising of normal individual (group 1) and post infract patients(group 3). The post infract patients are stable and are already under medication (anti coagulants) and hence potential myocardial damage is controlled. While in patients

with chronic disease(group II), the ongoing unfavourable cardiac events leads to the increase in salivary cardiac troponin I levels when compared to other groups.

Tarig Karar et al in their study concluded that there is significant increase of serum cTnI in diabetes individual and indicated the progression of cardiovascular disease in them[10]. Horwich et al in their study demonstrated that patients with T2DM(Type 2 Diabetes Mellitus) and no atherosclerotic disease, had myocardial damage as detected by high sensitivity (hs) TnI. This suggests that T2DM contributes to subclinical myocardial injury as patients with T2DM have increased arterial stiffness due to increased oxidative stress, increased endothelial cell apoptosis, endothelial dysfunction and depletion of endothelial progenitor cells all contributing towards ischemic events. Decreased compliance of the aorta leads to increase of systolic pressure and left ventricular preload thereby elevating stress on the left ventricular myocardium during the systolic phase. These events may predispose the patients to develop left ventricular hypertrophy that is associated with an elevated high-sensitivity troponin level[14].

Mirzaii Dizgah et al assayed cTnI in serum and saliva 12 hour and 24 hour of acute myocardial infraction by ELISA method and concluded that saliva can be used for measurement of cTnI in patients with acute myocardial infraction [15]

Habib Haybar et al conducted a study comprising 80 patients diagnosed with myocardial infraction and found no correlation between saliva and serum troponin [16]. Mishra et al also conducted a similar study among myocardial infract patients and concluded with a significant correlation between salivary and serum troponin I levels. Their study also suggested that diabetic patients should be screened thoroughly for their cardiac conditions[17].

F Chekin et al conducted a study to detect serum and salivary troponin I by nitrogen doped porous reduced graphene oxide electrode and concluded that cTnI levels in saliva are lower than that of serum and the sensor indicated that patient with acute myocardial infraction had a salivary cTnI level of 675pg/ml and thus propagated application of sensitive screening and routine monitoring of cTnI[18].

6. Conclusion

The salivary cardiac troponin I was increased in group II patients who are at high risk of developing cardiac events in the future when compared to the other groups.

7. Future Scope

The present study specifies the importance of standardising salivary Troponin I in normal and diseased individual. As release of cardiac troponin I is organ specific, other causes of myocardial ischemia should be explored and established. Salivary cardiac Troponin I testing in such patients should be of great use in decreasing mortality. Future studies should be targeted with larger sample and should include other risk factors of myocardial infraction.

References

[1] Gupta S, Gudapati R, Gaurav K, Bhise M. Emerging risk factors for cardiovascular diseases: Indian context. Indian journal of endocrinology and metabolism. 2013 Sep;17(5):806.
[2] Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study.Murray CJ, Lopez ADLancet. 1997 May 24; 349(9064):1498-504
[3] Thygesen K, Alpert JS, White HD. Universal definition of myocardial infarction. Journal of the American College of Cardiology. 2007 Nov 27;50(22):2173-95.
[4] Jaffe AS, Babuin L, Apple FS. Biomarkers in acute cardiac disease: the present and the future. Journal of the American College of Cardiology. 2006 Jul 4;48(1):1-1.
[5] Ferguson JL, Beckett GJ, Stoddart M, Walker SW, Fox KA. Myocardial infarction redefined: the new ACC/ESC definition, based on cardiac troponin, increases the apparent incidence of infarction. Heart. 2002 Oct 1;88(4):343-7.
[6] Marwick TH. 1 Diabetic heart disease. Postgraduate medical journal. 2008 Apr 1;84(990):188-92.
[7] Pedrinelli R, Ballo P, Fiorentini C, Denti S, Galderisi M, Ganau A, Germanò G, Innelli P, Paini A, Perlini S, Salvetti M. Hypertension and acute myocardial infarction: an overview. Journal of cardiovascular medicine. 2012 Mar 1;13(3):194-202.
[8] Park KC, Gaze DC, Collinson PO, Marber MS. Cardiac troponins: from myocardial infarction to chronic diseases. Cardiovascular research. 2017 Sep 14;113(14):1708-18.
[9] Javaid MA, Ahmed AS, Durand R, Tran SD. Saliva as a diagnostic tool for oral and systemic diseases. Journal of oral biology and craniofacial research. 2016 Jan 1;6(1):67-76.
[10] Karar T, Elfaki EM, Qureshi S. Determination of the serum levels of troponin I and creatinine among Sudanese type 2 diabetes mellitus patients. Journal of natural science, biology, and medicine. 2015 Aug;6(Suppl 1):S80.
[11] Weil BR, Young RF, Shen X, Suzuki G, Qu J, Malhotra S, Canty JM. Brief myocardial ischemia produces cardiac troponin I release and focal myocyte apoptosis in the absence of pathological infarction in swine. JACC: Basic to Translational Science. 2017 Apr 24;2(2):105-14.
[12] White HD. Pathobiology of troponin elevations: do elevations occur with myocardial ischemia as well as necrosis?.
[13] Wong DT. Salivaomics. The Journal of the American Dental Association. 2012 Oct 1;143:19S-24S
[14] Horwich TB, Fonarow GC. Glucose, obesity, metabolic syndrome, and diabetes: relevance to incidence of heart failure. Journal of the American College of Cardiology. 2010 Jan 26;55(4):283-93.

[15] Haybar H, Yousefimanesh H, Ahmadzadeh A, Malekzadeh H, Assare A. Relation of saliva and blood troponin levels in patients with myocardial infarction: cross-sectional clinical study. Int J Cardiovasc Res 1: 5. doi: http://dx. doi. org/10.4172/2324. 2012;8602:2.
[16] Haybar H, Yousefimanesh H, Ahmadzadeh A, Malekzadeh H, Assare A. Relation of saliva and blood troponin levels in patients with myocardial infarction: cross-sectional clinical study. Int J Cardiovasc Res 1: 5. doi: http://dx. doi. org/10.4172/2324. 2012;8602:2.
[17] Mishra V, Patil R, Khanna V, Tripathi A, Singh V, Pandey S, Chaurasia A. Evaluation of Salivary Cardiac Troponin-I as Potential Marker for Detection of Acute Myocardial Infarction. Journal of Clinical & Diagnostic Research. 2018 Jul 1;12(7).
[18] Chekin F, Vasilescu A, Jijie R, Singh SK, Kurungot S, Iancu M, Badea G, Boukherroub R, Szunerits S. Sensitive electrochemical detection of cardiac troponin I in serum and saliva by nitrogen-doped porous reduced graphene oxide electrode. Sensors and Actuators B: Chemical. 2018 Jun 1;262:180-7.