Original Article

Evaluate the Levels of Cardiac Troponin I in Saliva and Serum of Acute Myocardial Infarct Patients

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ABSTRACT

Objective: The objective of this research was to evaluate the levels of cTnI in saliva between 12-24 hours and correlate the levels of saliva and serum cardiac Troponin I in AMI patients.

Study Design: A cross sectional analytical study

Place and Duration of Study: This study was conducted at the Departments of Cardiology Civil Hospital and Ojha campus Karachi from April, 2021 to August, 2021.

Materials and Methods: Sixty Myocardial infarct patients between the age of thirty to seventy were included in this research. Participants were clinically examined for cardiac and dental complications. Blood and saliva samples were collected between 9 -11 am between 12-24 hours and analyzed by sandwich ELISA technique.

Results: The cTnI was significantly increased in saliva and serum with mean values (0.1609 ng/dl and 14.494 ng/dl in serum) in patients with acute MI. We have positive statistical correlation i.e. spearman roh (r= 0.647) with p-value .001.

Conclusion: Present results reveals positive correlation of Troponin I in saliva and serum which indicates a constant release of troponin I in saliva. Hence, saliva can be used as a noninvasive approach for detection of troponin I at low value in myocardial infarct patients as point of care testing for early and rapid diagnosis.

Key Words: Myocardial infarction, Cardiac troponin-I, Saliva, Serum, ELISA

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INTRODUCTION

Heart diseases are increasing progressively in the world population due to excessive food consumption or genetic background. It is the primary source of morbidity and mortality in different parts of the world [1]. Pakistan is a growing South Asian country with four major territories, and its evaluated population is more than 200 million. Cardiovascular hazard for example, hypertension, diabetes mellitus, and weight[2] are expanding in Pakistan and are the leading cause of morbidity and mortality[3]. The most frequent type of CHD is myocardial infarction (MI).

It is responsible for over 15% of mortality consistently, in which the majority of people experiencing a non-ST-segment elevated myocardial infarction (NSTEMI) than ST-segment elevation myocardial infarction (STEMI) [4]. In the emergency setting, patients associated with having an AMI get an electrocardiogram (ECG) and proportions of serum biomarkers to recognize or exclude myocardial necrosis. For MI, cardiac Troponin-T (cTnT) and cardiac troponin-I (cTnI) are observed as sensitive whereas Cardiac troponin-I (cTnI) is considered a gold standard biomarker and ultimate laboratory analysis for the diagnosis of acute MI. The cardiac troponin-I could be detected within 4 to 12 hrs. in serum after the beginning of myocardial ischemia and acheive its peak from 12 hours to 2 days. It can remain high up to two weeks after myocardial injury[5]. Currently, various devices have become available for point-of-care testing for most established cardiovascular markers that takes less than 20 minutes. Whole blood, plasma as well as the serum is being tested as a specimen, but novel Nano-biochip technology which is based on saliva testing consisting of 21 biomarkers, revealed another enormous capability to diagnose acute MI[6]. Body fluids such as serum, sputum, saliva are likewise being utilized for the appraisal of these biomarkers[7]. These biological markers are being determined and checked for their levels through different strategies like enzyme-linked immunosorbent assay (ELISA), immuno-histochemistry (IHC), western blot, and others. Out of these methods, ELISA and IHC are ordinarily utilized techniques to identify proteins in body fluids and tissues separately due to their reasonable, reproducible, and cost-effective properties [8]. For early diagnosis and

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to reduce the stress level of the patients, early sample collection for detection of biomarkers is mandatory, and for that reason, saliva collection is the fundamental focal point of testing which incorporates its ease, inexpensive, non-intrusiveness, and non-clotting nature, unlike blood and negligible danger of cross-contamination[9]. Oral saliva is a unique bio-fluid containing various components secreted from three major (paired) salivary glands named parotid, submandibular and sublingual glands, and hundreds of minor salivary glands distributed all over the oral cavity[10]. For such reasons saliva is gaining attention as a fluid of choice in the detection of multiple diseases like inflammatory conditions, metabolic disorders, cardiac myopathies, neurological and malignant conditions [11,12].

MATERIALS AND METHODS

2.1. Study Participants: A total of 60 patients between the ages of 30-70 years in the emergency department of cardiology having complained of chest pain, shortness of breathing, history of diabetes, and hypertension went through ECG and lab investigations. Standard clinical records were collected from all participants on questionnaire about their biographic data, medical history, oral hygiene status, medication, and other health issues. OPD, CCU, ICU, unstable critical patients, and patients on the ventilator were excluded from the study.

2.2. Serum and Saliva Sample Collection: Saliva sampling was collected between 9-11 am. Participants were asked to refrain from anything at least two hours prior sampling and then allowed to rinse their mouth with water and swallow saliva for 2- 3 minutes. Later they were asked to collect 3-5 mL unstimulated saliva for 10 minutes into 15 mL sterile plastic falcon tube placed on ice. After saliva collection, immediately 3cc blood was drawn by a phlebotomist and stored in vacutainers. All samples were centrifuged at 4000 rpm for 15 minutes, and aliquots were stored at -20 ˚C for further analysis. ELISA (ELISA Kit (Cloud-clone Crop.SEA478Hu) was performed to analyze cardiac Troponin-I in the laboratory of the National Center for Proteomics, University of Karachi to measure the concentration of cTnI in samples of saliva and serum according to the manufacturer's guideline.

2.4. Statistical Analyses: The data was entered and analyzed in SPSS version 21. The frequencies were analyzed and correlation of serum and saliva samples, was established accordingly. The nonparametric test of spearman roh was applied. p-value=0.05 were considered significant. Chi-square was applied to determine the association of gender with risk factors (hypertension, DM, smoking and stress).

RESULTS

The number of patients calculated for the study was (n=60). Four (n=4) patients were dropped due to dry months and inability to give the required quantity of saliva. Therefore, the total samples analyzed were (n=56). About 80% of the subjects were male with mean age was 55 years. Participants (n=36) showed non-ST elevation on ECG readings, whereas only (n=20) showed ST elevation as shown in Fig.1. The frequency of mild to moderate gingivitis was 98.2% of the patients and 83.9% of patients have mild to moderate periodontitis, while only 32% of patients presented carious teeth (Fig. 1 and Fig. 2).

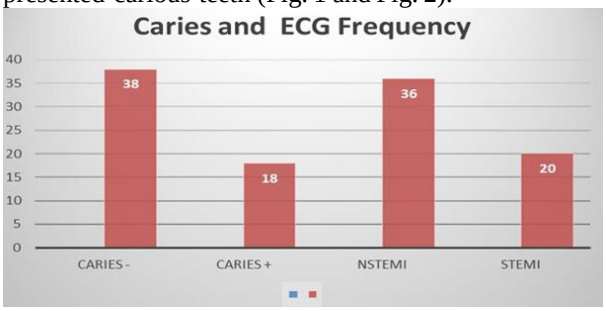

Figure No.1: Statistical presentation of presence (+) and absence (-) of caries and non-ST/ST elevation myocardial infarction by ECG readings

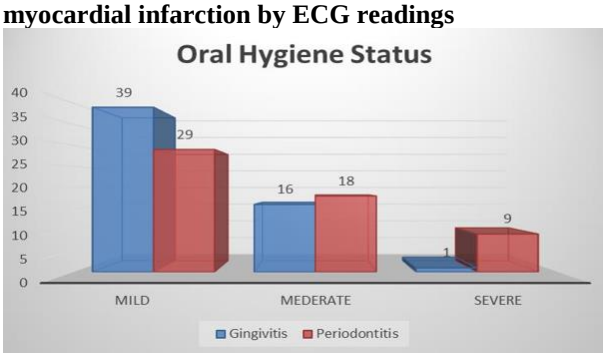

Figure No.2: Statistical presentation of mild, moderate and severe levels of gingivitis and periodontitis

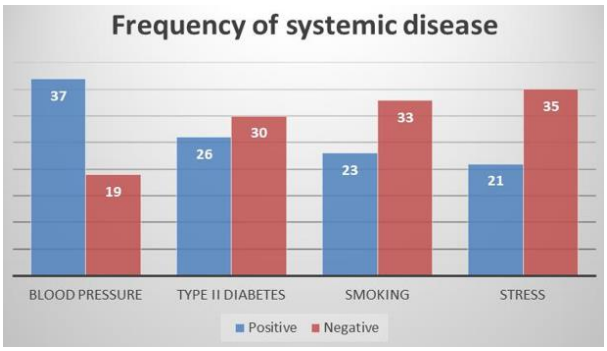

Figure No.3: Statistical presentation of systemic diseases with occurrence (positive) and absence (negative) of the respective disorder

The risk factor found in the study group was blood pressure (BP) which was analyzed to be 66% followed by diabetes at 46%, smoking at 39% and stress was 37%.as shown in fig 3.

When the saliva troponin-I level was correlated with risk factors, a weak positive correlation (r=0.112) was observed between salivary troponin-I and diabetes only. When the troponin-I was statistically analyzed, the patients were having different values in serum from a lower limit of 0.18 ng/ml up to 45 ng/dl (the cutoff value in serum is 0.04 ng/dl), while the lowest value to be found in saliva was from 0 to 0.45 ng/dl.

Table No.1: Statistical analysis of cardiac troponin-I values obtained in serum and saliva of myocardial infarction patients (N= 56 cases)

Samples	N	Minimum	Maximum	Mean	Std. Deviation
Serum Troponin I	56	0.18	45.00	14.0188	9.93341
Saliva troponin I	56	0.00	0.45	0.1609	0.14806
Valid N (listwise)	56				

The mean value of cTnI in saliva was 0.16 ng/dl and in serum was 14.01 ng/dl with a standard deviation of 9.93 and 0.14 in serum and saliva, respectively (Table 1). The Spearman correlation between the values in salivary and serum troponin I and the correlation was found to be positive (r=0.647) with a significant p-value of 0.001.

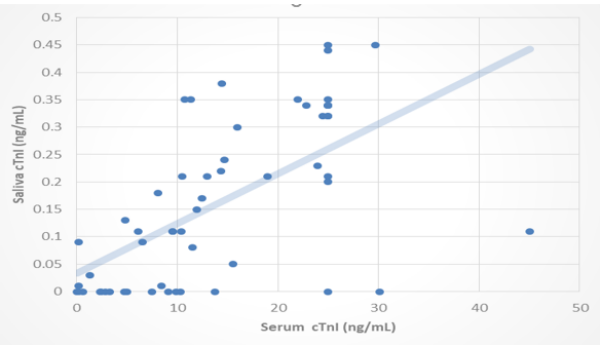

Figure No.4: Serum levels of cardiac Troponin-I in correlation with saliva levels demonstrate a moderate positive relation (r=0.643)

DISCUSSION

In this study, we analyzed cardiac troponin-I in acute myocardial infarct patients by using the ELISA technique. We tested the troponin-I cardiac marker in saliva and correlated its results with serum troponin-I. The correlation between serum and saliva was found to be (r=0.647) according to non-probability test of the Spearman correlation which depicts a moderate positive correlation. This finding is in agreement with a previously reported study that showed a positive correlation between serum and saliva troponin concentrations (r=0.56)[13]. This also indicates the presence and continuous release of the troponin-I biomarker in saliva as well. Another study conducted in 2013 by Iraj Mirzaii-Dizgah also found a positive correlation (r=0.45) of cardiac troponin-I that suggested point of care testing at the preclinical stage, which is time-saving and helpful to start immediate treatment. The evaluation of troponin-I with high sensitivity assay in acute myocardial patients showed that the value of troponin-I increases in saliva with respect to the increase in serum levels in 24 hours. However, the value drops but remain significant in the 24 to 48 hour period[14]. The evaluation of different biomarkers including troponin-I from saliva was reported by CS Miller and co-workers who concluding that the serum markers are more sensitive than saliva. However, combining the salivary biomarker such as c-reactive protein (CRP) detection with clinical information such as an ECG, can provide sensitivity of 80% with 100% specificity for acute myocardial infarction[6]. As mentioned above that we have found a moderate positive correlation of cTnI values in saliva and serum samples taken within 24 hours of MI, whereas, Vaibhav Mishra and associates found a strong positive relation between the levels of cTnI in serum and saliva samples taken during the same time[15]. During the analysis of saliva, it is important to have an idea related to the oral disorders that may or may not interfere with the sample analysis. The percentages of diseases such as gingivitis, periodontitis and caries have already been mentioned in our results (Fig. 1 and 2). These conditions may develop as a result of the negligence of oral hygiene by MI patients. All of the patients in the study were affected by gingivitis and periodontitis at different severity levels. Therefore, it is contributory to discuss briefly about these oral conditions with respect to cardiovascular disease. Generally, periodontal disease (PD) seems to be associated with no more than a modest increase (~20%) in cardiovascular risk in the overall population[16]. The underlying mechanism might be the signaling pathways in human gingival fibroblasts leading to periodontal disease, which in turn offers a biological burden of endotoxin and inflammatory cytokines such as thromboxane A2, prostaglandin E2, interleukin (IL), and tumor necrosis factor-β. These factors may lead to thrombus formation as well as towards atherogenesis[17,18]. Systematically, diabetes and hypertension were noted to be the two main disorders present in the study subjects. In Pakistan there is an increasing trend of hypertension in both urban and rural areas and in both genders with respect to time. The urban areas have higher prevalence[19]. Our analysis showed that 66% of the patients were suffering from hypertension. For diabetes mellitus it is known to have a direct association with coronary artery disease and the risk of coronary death is higher in women as

compared to men [20, 21]. Our data showed 46% of the cases were diabetic, the second prevailing systemic complaint. This is the first report of cTnI evaluation in the saliva samples from local population. Several modified approaches can be adopted for further evaluation and validation of this study in the future starting from adopting a standard method of saliva collection rather than the classical method of passive drooling. This is necessary for keeping the consistency of saliva samples which is altered from one individual to another and negatively impacts when analyzing secreted biomarkers in nano measuring levels like in this study. Additionally, thermo-sensitivity of saliva proteins, saliva secretion and its flow rates[22,23] are also few parameters to be considered. To establish saliva as an alternative medium of diagnosis to various other biological fluid such as plasma, is a highly attractive research topic. Our study also presents an idea that saliva has the potential to detect biomarkers and by developing a highly sensitive tool through available molecular technologies a saliva based assay can be developed for cTnI detection in MI patients, a direction that has started to be explored [24].

CONCLUSION

Myocardial infarction is a life-threatening condition and needs to be diagnosed very quickly and accurately. In this analytical study, most saliva samples showed a significant association between the unstimulated saliva and blood serum concentrations of cardiac troponin-I. Although collecting saliva is non-invasive and a less stressful tool but an important cardiac biomarker such as troponin-I, is detected in minimal values during our analysis as compared to serum. However, saliva based assay can be developed for cTnI detection requiring a large sample size and a high sensitive cTnI assay.

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Author's Contribution:

Concept & Design of Study:	Bhunesha Devi
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Final Approval of version:	Bhunesha Devi

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Bahbah EI, Noehammer C, Pulverer W, Jung M, Weinhäusel A. Salivary biomarkers in cardiovascular disease: An insight into the current evidence. The FEBS J 2020.
2. Khowaja S, Karim M, Zahid M, Zahid A, Ahmed S, Kazmi K, et al. Impact of Temperature Variation on Acute Myocardial Infarction in Karachi, Pakistan. Cureus 2019;11(10).
3. Rehman S, Li X, Wang C, Ikram M, Rehman E, Liu M. Quality of Care for Patients with Acute Myocardial Infarction (AMI) in Pakistan: A Retrospective Study. Int J Environmental Research and Public Health 2019;16(20):3890.
4. Anderson JL, Morrow DA. Acute myocardial infarction. New England J Med 2017;376(21): 2053-64.
5. Bavia L, Lidani KCF, Andrade FA, Sobrinho MIAH, Nisihara RM, de Messias-Reason IJ. Complement activation in acute myocardial infarction: An early marker of inflammation and tissue injury? Immunol Letters 2018;200:18-25.
6. Miller C, Foley III J, Floriano P, Christodoulides N, Ebersole J, Campbell C, et al. Utility of salivary biomarkers for demonstrating acute myocardial infarction. J Dental Research 2014;93(7_suppl): 72S-9S.
7. Csősz É, Kalló G, Márkus B, Deák E, Csutak A, Tőzsér J. Quantitative body fluid proteomics in medicine—A focus on minimal invasiveness. J Proteomics 2017;153:30-43.
8. Comakli S, Sağlam YS, Timurkan MÖ. Comparative detection of bovine herpesvirus-1 using antigen ELISA, immunohistochemistry and immunofluorescence methods in cattle with pneumonia. Turkish J Veterinary Animal Sciences 2019;43(3).
9. Ngamchuea K, Chaisiwamongkhol K, Batchelor-McAuley C, Compton RG. Chemical analysis in saliva and the search for salivary biomarkers–a tutorial review. Analyst 2017;143(1):81-99.
10. Khurshid Z, Warsi I, Moin SF, Slowey PD, Latif M, Zohaib S, et al. Biochemical analysis of oral fluids for disease detection. In Advances in Clinical Chemistry; Elsevier: London, UK;2021.p.205–253.
11. Meleti M, Cassi D, Vescovi P, Setti G, Pertinhez TA, Pezzi ME. Salivary biomarkers for diagnosis of systemic diseases and malignant tumors. A systematic review. Medicina Oral, Patología Oraly Cirugía Bucal 2020;25(2):e299.
12. Khurshid Z, Zafar MS, Khan RS, Najeeb S, Slowey PD, Rehman IU. Role of salivary biomarkers in oral cancer detection. Advances Clin Chem 2018;86:23-70.
13. 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 2012;1(5):8602:2.
14. Mirzaii-Dizgah I, Riahi E. Salivary troponin I as an

indicator of myocardial infarction. Ind J Med Res 2013;138(6):861.
15. Mishra V, Patil R, Khanna V, Tripathi A, Singh V, Pandey S, et al. Evaluation of Salivary Cardiac Troponin-I as Potential Marker for Detection of Acute Myocardial Infarction. J Clin Diagnostic Research 2018;12(7).
16. Pattnaik NK, Das SN, Biswal BN. Cardiovascular Diseases and Periodontal Diseases: Review and Update. Int J Scientific Study 2017;5(1):239-44.
17. Brinson CW, Lu Z, Li Y, Lopes-Virella MF, Huang Y. Lipopolysaccharide and IL-1β coordinate a synergy on cytokine production by upregulating MyD88 expression in human gingival fibroblasts. Molecular Immunol 2016;79:47-54.
18. Moss JW, Ramji DP. Cytokines: roles in atherosclerosis disease progression and potential therapeutic targets. Future Med Chem 2016;8(11):1317-30.
19. Mahendra J, Rao AN, Mahendra L, Fageeh HN, Fageeh HI, Balaji TM, et al. Genetic Polymorphisms of NLRP3 (rs4612666) and CARD8 (rs2043211) in Periodontitis and Cardiovascular Diseases. Biol 2021;10(7):592.
20. Fuchs FD, Whelton PK. High blood pressure and cardiovascular disease. Hypertension 2020; 75(2):285-92.
21. Mendis S, O'Brien E, Seedat YK, Yusuf S. Hypertension and diabetes: entry points for prevention and control of the global cardiovascular epidemic. Int J Hypertens 2013;1-3.
22. Criado C, Chaya C, Fernández-Ruíz V, Álvarez MD, Herranz B, Pozo-Bayón MÁ. Effect of saliva composition and flow on inter-individual differences in the temporal perception of retronasal aroma during wine tasting. Food Research Int 2019;126:108677.
23. Pedersen AML, Sørensen CE, Proctor G, Carpenter G, Ekström J. Salivary secretion in health and disease. J Oral Rehabilitation 2018; 45(9):730-46.
24. Westreich R, Neumann Y, Deutsch O, Krief G, Stiubea-Choen R, Zager D. Development of saliva-based cTnI point-of-care test: a feasibility study. Eur Heart J 2020 Nov;41(Supplement_2):ehaa946-1693.