Salivary Biomarkers in Cardiovascular Diseases

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ABSTRACT

Human saliva is a dynamic biological fluid secreted by salivary glands into the oral cavity. Over the last decade, human saliva has attracted attention as a liquid biopsy for the detection of diseases. Many of the biomarkers in the blood enter saliva through blood via passive diffusion, active transport or extracellular ultra-filtration. Therefore, saliva can be a good reflection of the physiological function of the body. Nearly 31% of deaths globally are reported to be caused by cardiovascular diseases (CVDs). Currently, the diagnosis of CVDs is based on time-significant multiple biomarkers. Biomarkers are defined as a biological molecules found in blood, saliva and other body fluids, or tissues that are a sign of a normal or abnormal process, or of a condition or disease. Cardiovascular diseases can be detected through biomarkers such as Cardiac troponin (cTn), Creatine phosphokinase MB (CK-MB) etc. Hence this paper aims to provide a key update on salivary biomarkers in diagnosis of cardiovascular diseases.

Keywords: Salivary Biomarkers; Cardiovascular Disease; Cardiac Troponin; Creatine Phosphokinase

INTRODUCTION

Cardiovascular disease (CVD) is a term describing all types of diseases affecting the blood circulatory system, including the heart and vasculature, which respectively displaces and conveys the blood. This disorder encompasses numerous congenital and acquired maladies[1]. Globally 31% of deaths are caused by cardiovascular diseases and is recognized to be one of the leading causes of death annually by the World Health Organization (WHO). Multiple diseases are included in CVDs, but the most vicious is acute myocardial infarction (AMI). The pathology occurs because of cardiac ischemia leading to necrosis of the area involved. AMI accounts for around 50% of all CVDs and is one of the most frequent causes of death. Currently, the diagnosis of CVDs is based on time-significant multiple biomarkers. Biomarkers are defined as biomolecules found in serum, saliva and other body fluids, or tissues that are a sign of a normal or disease.

Human saliva is a dynamic biological fluid secreted by salivary glands into the oral cavity. Over the last decade, human saliva has gained popularity as a liquid biopsy for the detection of diseases. Many of the biomarkers in the blood enter saliva through blood via passive diffusion, active transport or extracellular ultra-filtration. So, saliva can be a good reflector of the physiological function of the body[2].

Cardiovascular diseases (CVDs) can be detected through biomarkers such as Cardiac troponin (cTn), Creatine phosphokinase MB (CK-MB), Myoglobin (MYO), Brain natriuretic peptide (NT-proBNP), C - reactive protein (CRP), Matrix metalloproteinase- (MMP-8, MMP-9) and tissue inhibitor of MMP-9, Myeloperoxidase (MPO)[2].

SALIVARY BIOMARKERS IN CARDIOVASCULAR DISEASE DETECTION

CARDIAC TROPONIN (CTN):

Troponins are a group of proteins located on the thin filament of both skeletal and myocardial myocytes. The troponin complex has 3 subunits namely TnC - the calcium binding component, TnI - maintains the structural position of the troponin-tropomyosin complex and TnT - the tropomyosin binding subunit. Interestingly, both TnT and TnI subunits have distinct isoforms for every muscle type, hence there is a specific cardiac isoform. Cardiac troponins cTnT and cTnI are now recognized as the most tissue-specific biomarkers associated with cardiac damage and are included as a diagnostic criterion for several cardiac-related pathologies. This success is associated with unique troponins position and function and generation of specific monoclonal antibodies against both cTnT and cTnI which are tissue-specific biomarkers of myocardial injury that are not detected in healthy individuals[4].

CARDIAC TROPONIN I (CTNI) AND ACUTE MYOCARDIAL INFARCTION

Cardiac TnI was first reported in 1992 as a biochemical marker of myocardial injury and is a very sensitive and specific for the diagnosis of AMI. cTnI (24 KDa) is unique containing 31 amino acids that makes it different from sTnI or fTnI (19 KDa). During foetal development both sTnI and cTnI are expressed in the myocardium; however, the only isoform present in the myocardium at birth is cTnI. cTnI has not been shown to be expressed in any kind of skeletal muscle during either development or disease. This makes it specific for myocardial tissue and detection of myocardial injury in serum. It has similar release kinetics to CK-MB and cTnT. It does not provide an earlier detection for AMI within the first 6 hours after symptom onset. cTnI peaks between 12-

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36 hours after the onset of AMI and remains elevated for 3-7 days after AMI. The half-life is < 2 hours and the prolonged diagnostic window is due to the continuous release of this marker from the myofibril[5].

Salivary cTnI

Mirzaii-Dizgah I et al conducted a study to identify salivary levels of cTnI in patients with acute MI. Levels were analysed in both serum and saliva 12 and 24 h after acute MI. The study concluded that saliva can be used for measurement of cTnI in patients with acute MI. Further research is needed for salivary cTnI for being employed in point-of-care testing for early detection of MI in pre-clinical settings[6].

Vaibhav Mishra et al conducted a study to estimate and correlate the level of cTnI in un-stimulated whole saliva and serum in AMI patients and control group. Serum and saliva samples were obtained within 24 hours from patients with ECG features suggestive of acute MI. The levels of cTnI in saliva were directly associated with serum levels demonstrating a highly significant strong positive relation and confirms the diagnostic ability of saliva for detection of cTnI[7].

CARDIAC TROPONIN T (CTNT) AND ACUTE MYOCARDIAL INFARCTION

Cardiac-TnT (34 KDa) was first introduced as a marker for AMI in 1989. It appears in the serum within 12 hours after AMI symptom onset. It shows similar release kinetics to CK-MB and cTnI, but does not provide an earlier detection for AMI than CKMB or cTnI within the first 6 hours after symptom onset. Once in the circulation, it persists for an extended time (2-3 weeks) after symptom onset. The half-life of cTnT in circulation is 120 minutes and this long diagnostic window is due to the continuous release of the marker from myocardial cells after necrosis, and slow clearance from the circulation. The clinical sensitivity for the diagnosis of AMI approaches 100% at about 12 hours after symptom onset and remains elevated at 100% for at least 4 days[5].

Salivary cTnT:

Mirzaii-Dizgah I et al conducted a study to identify whole saliva high-sensitivity cardiac troponin T (hs-cTnT) in patients with acute MI. The levels were analysed in serum and whole saliva within the first and second morning following the MI by ELISA method. The study conveyed that salivary hs-cTnT can be used for diagnosis and monitoring of myocardial infarction[8].

CREATINE PHOSPHOKINASE MB (CK-MB):

Creatine phosphokinase MB is an enzyme present primarily in cardiac muscle. The MB is one of the three creatine phosphokinase isoenzymes the other being the MM and BB. CK-MB is released rapidly after myocardial injury. CK-MB increases twice the normal within 6 hours and peaks in 12–24 hours on the onset of AMI. It have nearly 90% sensitivity in AMI three hours after a patient is first assessed in emergency department, which is approximately 6 hours after symptom onset. CK-MB plays a crucial role in defining the infarct size and risk of re-infarction. If cTn is not available, CK-MB is considered as alternative marker for AMI[4].

Limitations: Even though CK-MB is sensitive marker of AMI, this enzyme is not exclusively specific to myocardial damage, as it is elevated in conditions like muscle injury, surgical procedures. Furthermore it is present in the intestine, diaphragm, uterus, prostate and injury to these organs would result in release of CK-MB and thus impair its specificity. To increase the specificity and to discriminate the "true positive" elevations to "false positive" serum elevations due to other tissue injury, the measurement of creatine phosphokinase MB as a percentage of total CK has been used. There is no clear consent on whether absolute CK-MB or the CK-MB relative index is the preferred for patients with potential acute coronary syndromes, but the WHO international diagnostic criteria recommend use of absolute CK-MB[4].

SALIVARY CK-MB

Mirzaii-Dizgah I conducted a study to determine probable changes of CK-MB levels in saliva of patients with acute MI. This study proved there is strong link between levels of serum and salivary CK-MB, indicating that salivary CK-MB may serve as an easy-to-use diagnostic tool for point-of-care testing of acute MI[9].

MYOGLOBIN (MYO)

Myoglobin is a heme-containing, relatively small (17.8 kDa) globular protein found in myocyte cells of cardiac and skeletal muscle. It is a protein that has 154 amino acids residues. It consists of eight α-helices connected through the turns with an oxygen binding site. The key function is to transport oxygen within muscle cells, and it constitutes approximately 2% of muscle protein in both skeletal and cardiac muscles[4]. Myoglobin starts to increase in blood within 2 hours after symptom onset of AMI, peaks at 6-9 hours, and returns to normal within 24 hours[5]. The early release feature of MYO is attributed to its small size and localisation within the cytosol of the cell.

Of the biomarkers used to diagnose CVD, myoglobin is accepted as one of the earliest to appear during the development of the disease[4]. The overall diagnostic sensitivity and specificity ranged from 77-97% and 90-97.9% respectively. The sensitivity mostly depends on the time of presentation after symptom onset and it drops considerably with very early (< 2 hours) or late presentation (> 15 hours) of the patient[5]. It is recommend to measure myoglobin within 6 hours of chest-pain onset[4].

Limitations: Since myoglobin is only released as a result of tissue necrosis, it is a poor biomarker of acute cardiac ischemia. Furthermore, cardiac and skeletal muscle myoglobin share 100% homology, thus making it unspecific. Myoglobin is cleared by kidneys, and patients suffering from renal insufficiency have increased levels of myoglobin. There is difference in opinion to use myoglobin as a biomarker in the evaluation of patients with suspected acute coronary syndromes[4].

Salivary MYO

Myoglobin, which appears in both serum and salivary

bio-fluids, is used to detect AMI. Miller and his co-workers established that salivary myoglobin levels were increased within 48 h of the onset of angina in AMI patients[10].

BRAIN NATRIURETIC PEPTIDE (NT-PROBNP):

BNP is a 32-amino acid polypeptide cardiac neuro-hormone secreted from membrane granules in the cardiac ventricles, particularly the left ventricle, as a response to ventricular volume expansion and pressure overload. Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are of myocardial cell origin, while C-type natriuretic peptide (CNP) is of endothelial origin. BNP levels are found elevated in patients with various clinical conditions like heart failure, MI, left ventricular hypertrophy, cardiac inflammation, primary pulmonary hypertension, kidney failure, ascetic cirrhosis and is associated with advanced age. The levels correlate with severity of symptoms and prognosis, and so helps to detect the presence of heart failure, severity and prognosis. Due to its cost-effectiveness BNP is highly desirable in developing countries for detecting CVD. Originally, the US FDA approved the utilization of BNP or NT-proBNP (amino terminal pro-brain natriuretic peptide) to differentiate cardiac cause (congestive heart failure) from non-cardiac origin (chronic obstructive pulmonary disease). It was approved by FDA in assessing the prognosis of patients with congestive heart failure, acute coronary syndrome and risk stratification in acute coronary syndrome[4].

Salivary NT-proBNP

Joharimoghadam A et al conducted to test if salivary BNP concentration might be a new biomarker in patients with chronic HF. Mean plasma NT-proBNP was found to be higher in HF patients compared to control groups. The study has concluded that the salivary BNP can be useful in the diagnosis and follow-up in patients with HF, especially in emergency [11].

C - REACTIVE PROTEIN (CRP)

C-reactive protein is a non-specific acute phase protein produced in the liver. It is associated with functions related to immune reactivity like complement activation, natural immunity and phagocyte stimulation. It has been widely used as an acute inflammation marker. It is found to be a reliable marker for atheromatic plaque vulnerability, atherosclerosis, coronary artery disease, coronary vasospasm, left ventricular dysfunction, angina pectoris and myocardial infarction. These are related to levels of cardiac enzymes and troponin I, and in some cases it was found to be a better marker of CVD than troponin T. C-reactive protein has been found to have a role in myocardial and cerebral infarct growth and has been consequently targeted by inhibitors to induce a cardio-protective effect. However this application has yet to be fully understood. Its reliability has several limitations as its levels greatly vary, depending on ethnicity, gender, genetics, obesity and weight loss. It is also an indicator for non-cardiac related pathologies such as anastomotic leakage, systemic lupus erythematosus (SLE), and dementia[4].

MATRIX METALLOPROTEINASES (MMP-8, 9) AND TISSUE INHIBITOR OF MMP-9

MMPs together with other proteases, like cathepsins and elastases, plays a key role in tissue remodelling in both physiology and pathologies like cancer, fibrosis and CVD. In normal physiologic conditions, there is balance between MMPs that degrade cardiac extracellular matrix components and tissue inhibitors of metalloproteinases (TIMPs). However, in pathology, events like decreased TIMP expression and increased MMP activation eventually leads to cardiac and vascular disease and ultimately death. MMP activity has been associated with cardiac and vascular pathologies like cardiomyopathies, atherosclerosis, aneurism, myocarditis, hypertension.

Matrix metalloproteinases effects and activity are related to the availability of substrates, some of which have been found to be specific. MMP specific for fibrillar collagens are MMP-8, -3 and -13; MMP-7 is against collagens I, III and proteoglycans; while MMP-2 and -9 were found to preferentially cleave proteoglycans in myocardial tissue. MMP interactions is reported in the ECM of both cardiac and vascular components. MMPs are also employed in non-cardiovascular related organs including liver, skin, and lung. This presents the sensible problem of how to pin-point the precise tissue source of the substrate MMP biomarker. Biomarkers that depend on matrix metalloproteinases and their action on specific substrates to form tissue-specific neoepitopes have been successfully employed for ECMR related pathologies. The utilisation of such technologies for cardiovascular pathologies should be further investigated. [4]

MYELOPEROXIDASE (MPO)

Myeloperoxidase is found to be relevant for congestive heart failure, acute coronary syndrome and atherosclerosis. MPO has been shown to be released early in the inflammatory process, and has been linked to both inflammation and oxidative stress[4]. MPO has been found to be associated with CVD due to its involvement in LDL and HDL oxidation which is closely related to plaque formation in arterial walls through increased cholesterol aggregation. MPO demonstrate a diagnostic value of CVD even in individuals showing negative results for troponin T. But MPO elevation may not be directly related to cardiac or vascular tissue remodelling and may be attributed to underlying inflammatory processes which ultimately lead to organ failure[12].

F2 isoprostanes are prostaglandin compounds derived from arachidonic acid peroxidation, have shown as promising potential markers of oxidant injury in atherosclerosis, hypertension and ACS. These levels also found in non-cardiovascular related pathologies like Alzheimer's disease, pulmonary disorders and renal failure, its presence has been strongly linked with well-known cardiovascular risk factors. F2 isoprostanes have not been used on a large scale as literature is limited[4].

Salivary CRP, MMP-9 & MPO

Foley JD et al conducted a study to determine if salivary

biomarkers demonstrate utility for identifying aspects of myocardial necrosis. Significant correlations were observed between serum and saliva for C-reactive protein, matrix metalloproteinase-9, and myeloperoxidase[13].

FUTURE PROSPECTS AND CONCLUSION

CVD is emerging as leading cause of mortality in many countries. The problem caused in terms of suffering and health care costs, is escalating. Saliva can be used as a diagnostic media for biomarkers as it is economical, easy to collect, painless and also allows multiple sampling. As research advances in salivary biomarkers, the evidence will emerge establishing clear, definitive roles in the presentation of various disease processes. Point-of-care testing and screening devices such as Programmable bio-nano-chip (P-BNC) system, Cardio Micro-Electro-Mechanical Systems (MEMS) contributes to revolution in the salivary diagnostic technology towards the CVD detection. Future developments of saliva as diagnostic aid will lead to further advancement that can revise the approach of screening cardiovascular diseases.

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