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Review Article

GENE EXPRESSION PATTERN ANALYSIS FOR FORENSIC DIAGNOSIS OF SUDDEN CARDIAC DEATH Saikat Das¹, Ritwik Ghosh², Dr. Soumeek Chowdhuri³

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Abstract:

Diagnosis of Sudden cardiac death (SCD) is challenging for forensic experts. In the current scenario, genetic analysis is rapidly gaining interest in forensic science. Previous studies are not only limited to using protein markers. In this regard, analysis of mRNA is also a key feature. Analysing mRNA offers insight into the diseases and mechanisms leading to death. It can also be used for forensic diagnostic purposes. This review article takes the help of this methodology to discuss about those mRNA species that can aid in the process of sudden cardiac death (SCD) diagnosis. These are mRNA encoding Heat Shock Protein (HSP), mRNA encoding Hemoglobin A1/2 & B, mRNA encoding Pyruvate Dehydrogenase (PDK4), mRNAs encoding Connexin 43 (Cx43) & Zonula occludens-1 (ZO1), mRNA encoding TNNI3, MYL3, TGFB1, MMP9, VEGFA and mRNA expressing Brain Natriuretic Peptide (BNP). Owing to the difficulty of diagnosing SCD, molecular markers are often developed that can aid in the process. Nowadays, mRNA is showing promising usefulness to supplement the process.

Keywords: Sudden Cardiac Death, forensic, gene expression

Introduction:

Sudden cardiac death (SCD) is caused by a plethora of varied cardiac diseases. So, it remains a challenge for forensic experts to discover the aetiology and correctly diagnose the cause. The definition of sudden death has been given by WHO as death within 24 hours form the inset of symptoms. The description "sudden" or "unexpected" is not always accurate as unexplained is an equally common reason for medico legal investigation. The clinician is often unable to offer a cause of death though the patient was under medical care and this problem of obscure autopsy is seen. With the emergence of new biochemical techniques, protein markers (1) have been complementarily used with diagnosis of SCD. But the use of histochemical finding can never be avoided. In case of using traditional methods of histochemistry, it has not been possible to diagnose an acute myocardial infarction or ischemia with little time of survival; due to lack of positive pathological findings (2).

However, studies are not only limited to using protein markers. They also involve analysis of gene expression of the individuals. Although RNA is known to be instable; it can be extracted in adequate amount from tissue samples during medico-legal autopsy (3). Unlike RNA with stability ranging from few minutes to days; researchers have confirmed the utility of mRNA, even in samples more than 20 years old (4).

In this article, we have focused on the qualities of mRNA which decide the usefulness of these molecules in

diagnosis of SCD during autopsies. We have systematically reviewed different articles for this purpose from databases of PubMed, Google Scholar, etc. Keywords related to the study aim and those included in the search string were: sudden cardiac death, mRNA, forensic diagnosis.

Heat Shock Protein

Cellular stress response is demonstrated at the time of death and it can induce alterations in mRNA profiles. Heat Shock Proteins (HSP) play vital role in mediating these cell stress responses to extreme conditions of increased temperature, oxidative stress, radiation, exposure to toxic substances and other stimuli (5-7). Demonstrations show that postmortem gene transcripts in brain tissues are relatively more stable compared to others. They remain intact with longer PMI (Postmortem Interval), and are thus suitable for examination of RNA profiles of HSP from cerebral cortex (8-10).

Ischemia (11-13) can cause increase of mRNA levels of HSPA1. They are also reported to be increased in brain after drowning in greater intensity; when compared with other cases of ASP (14). HSPA1 mRNA poses as a potential marker for SCD in acute cases, but have not been expressed significantly (15) during cellular stress (usefulness not concluded).

Attempts were made to analyse HSP mRNA transcripts in postmortem occipital lobes to differentiate causes of death, such as traumatic injury (TI), mechanical asphyxiation (ASP) and sudden cardiac death (SCD) (15).

HSPA2 mRNA levels were higher in ASP (significant) and SCD subjects (not significant) in comparison to TI. In contrast to this, HSPA7 and A13 mRNA levels were significantly lower in case of both ASP and SCD samples. Interestingly, HSP90AA1 mRNA, which is known as the housekeeping gene, showed significant lower levels in the SCD group than those of ASP. Also, indices for HSPA2:A7/A13 ratios were higher in ASP and SCD subjects in comparison to TI subjects.

It is noteworthy to mention that HSP90AA1 mRNA significantly differentiates between SCD and ASP. Other mRNA profiles although are useful to differentiate ASP & SCD with TI. They are not able to differentiate between SCD and ASP. This gap is filled by HSP90AA1 mRNA if found appropriate as forensic marker by further studies.

Hemoglobin A1/2 & B

High levels of reactive oxygen species (ROS) induce the cellular expression of hemoglobin subunits. The hemoglobin subunits help to protect the cells from oxidative stress (16-18). Since oxidative stress is associated with various modes of heart failure (19-21), prolonged ROS can result in a variety of cardiac dysfunctions leading to cardiac death. These are indicative that mRNA encoding hemoglobin subunits (A1/2 & B) are suitable markers for SCD diagnosis.

Analysis showed that Hba1/2 and Hbb mRNA from left ventricular free wall tended to be higher in all SCD-related groups (ischemic, non-ischemic & acute myocardial infraction); than in traumatic death (TD) group by 4 folds (22). In contrast to the findings in myocardial tissues; significant difference in levels of Hba1/2 and Hbb mRNA from occipital cortexes of brain was not found between SCD and TD cases.

Pyruvate Dehydrogenase (PDK4)

PDK4, a mitochondrial protein plays a major role in maintaining metabolic flexibility; loss of which leads to onset of cardiovascular complications (23-28). Thus, down-regulation of cardiac PKD4 mRNA expression might be related to susceptibility to cardiac dysfunction. Unlike brain samples, analysis of myocardial samples proved that Pkd4 mRNA levels were significantly lower in SCD than TD (22). Also, relative ratio of Hba1/2 or Hbb-to-Pdk4 is seen to be higher in all groups of SCD.

Connexin 43 (Cx43) & Zonula occludens-1 (ZO1)

Connexin 43 is a gap junction protein that mediates cell-to-cell coupling and is thought to be responsible for propagation of action potential (29). Deterioration in quality of Cx43, phosphorylation and distribution can cause electrical conductive disorders; ultimately leading to cardiac complications (30).

Zonula occludens-1 (ZO1) is a membrane-associated guanylate kinase that stabilises Cx43 at gap junction plaque through cytoskeletal anchoring. Disruption of ZO1 leads to alteration in stabilization of the plaque equilibrium (31). Thus, both Cx43 and ZO1 are expected to be indicative of SCD.

Genetic analysis (32) showed that levels of mRNA encoding Cx43 and ZO1 were significantly were lower in ventricles in comparison to the atria of heart. Because of the interaction between Cx43 and ZO1; loss of ZO1 staining in the failing hearts coincide with reduction of Cx43 in the individual (33,34), and the genetic analysis also reveals the same.

TNNI3, MYL3, TGFB1, MMP9, VEGFA

These five proteins (TNNI3, MYL3, TGFB1, MMP9, VEGFA) are related with ischemic myocardial injury and its repair. Cardiac muscle structural protein TNNI3 is quantitively measured clinically for AMI (Acute Myocardial Infraction) diagnosis. MYL3 is also a structural protein in cardiac muscle, level of which changes after ischemic cardiac disease (35-37). TGFFB1, a cytokine protein is strongly activated in infracted myocardium (38-40). MMP9 plays important role in remodelling the vessel matrix after ischemia (41-43). VEGFA is an angiogenic factor that is also activated in ischemic myocardium (44).

Specifically amongst the SCD cases, increased MYL3, VEGFA and MMP9 mRNA values in the anterior wall of the right ventricle were found in genetic analysis (45) when AMI was the cause of death. TNNI3 mRNA expression was found to be unspecific for any particular cause of death. Higher TGFB1 mRNA expression was seen in interventricular septum in SCD cases without AMI, which supports previous studies (46,47); where decreased levels of TGFB1 was found in AMI cases. These indicated that TGFB1 is activated during short survival periods in infracted myocardium (48).

Brain Natriuretic Peptide (BNP)

BNP and NT-proBNP are considered as reliable biomarkers for diagnosis of cardiac dysfunction. But their usefulness for diagnosis of SCD is arguable (1). The synthesis of BNP is induced by mechanical stress, ischemia and hypoxia; which result in BNP rise in the myocardium (49). In support of this, animal studies (50) demonstrated that BNP mRNA in left ventricle of rats is influenced by Angiotensin II, stimulated by mechanical stress.

In forensic cases such as hemopericardium and pulmonary thromboembolism, mRNA levels in myocardium increased more, in comparison with SCD cases (51). This indicates that BNP mRNA can be used for differential diagnosis.

Discussion

In sudden death the immediate cause is almost to be found in the cardiovascular system even though topographically the lesion is not in the heart or great vessels. Ischemic heart disease is the most common cause of death in western countries, but the term has been employed loosely rather inaccurately as it encompasses a number of diseases namely coronary atherosclerosis, hypertensive heart disease, aortic valve disease, anomalies of coronary circulation and other coronary artery disease like polyarteritis (52). All these cases have different pathological manifestations which are indistinguishable from each other when examined by naked eye. This leads to the problem of negative autopsy in such conditions especially as the patient is often health and young. Histopathological and microscopic examination of the cardiac tissue can be of limited use in these conditions. There it is step forward in the diagnosis of such sudden cardiac deaths if a detailed gene expression pattern is known.

Currently, gene expression investigations are gaining interest in forensic medicine. Study of RNA is a key component of such gene expression investigations. However, stability of RNA depends on the type of tissue it originates (53,54). Correlation of RNA degradation with postmortem interval (PMI) is debatable. Best results for postmortem RNA extraction can be obtained from brain, muscle and heart tissue (55). Additionally, other factors such as gender, age at death, temperature, hypoxia and dehydration (56) needs to be discussed specifically for SCD.

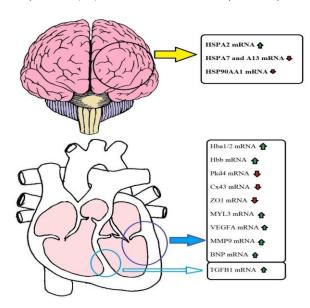


Figure 1: Forensic Diagnosis of SCD: mRNA species extracted from cerebral cortex of brain and ventricular wall & interventricular septum of heart (up arrow indicates increased level; down arrow indicates decreased level)

The mRNA profiles discussed here (Fig 1) are indicative of SCD. Their concentration levels are influenced by the cause of death. Even in cases of SCD due to early infraction, mRNA has shown great potential as biomarker which can be used for forensic diagnostic purposes. Further studies have the scope to explore the quality of SCD diagnosis with increased PMI.

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