

Detection of C-9 as a Marker of Early Myocardial Infarction in Sudden Death Cases by Immunohistochemistry

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Abstract

Background: Autopsy changes in sudden cardiac death due to Acute Myocardial Infarction (MI), is a real challenge for Forensic Pathologist. Immuno-histochemistry has proved significant role in identifying myocardial necrosis.

Aim: To find the incidence of deaths due to myocardial ischemia, its changes among the postmortem cases and the role of C9 in identifying deaths due to myocardial infarction by immuno-histochemical staining methods among autopsy cases.

Materials and Methods: Study was conducted on 44 autopsy cases. Multiple tissue samples were collected from the heart and other organs, tissue processing and paraffin blocks were made and Hemotoxylin and Eosin (H&E) staining and immuno-histochemical expression was performed for C 9 marker to find out the myocardial necrosis. Data were analyzed using Statistical Package for Social Sciences (SPSS-20) as descriptive statistics.

Result: Common age group was 51-60 years. 75% of them were found to have co morbid conditions. Histopathology for C9 showed 66% to be positive.

Conclusion: Complement C9 factor was used as a reliable marker for identification of early MI in cases which do not show significant histopathological changes with haemotoxylin and Eosin stains.

Key words: C9, Immunohistochemistry, autopsy, myocardial infarction

Introduction

Diagnosis of sudden death is always a challenge for the forensic expert. The most common cause

for sudden death, according to World Health Organization is cardiovascular diseases. Among the adult population the single important cause

of sudden cardiac death (SCD) is atherosclerotic coronary artery disease. Over time, the origin of sudden cardiac death evolves as a consequence of structural remodeling of the left ventricle [6]. In acute myocardial infarction, specifically when death occurs within minutes to a few hours of ischemic insult which makes the diagnosis difficult without pathological or microbiological intervention.

Histologically atheromatous plaques obtained at autopsy have demonstrated the presence of inflammatory mononuclear cells with foci of monocytes, macrophages and T-lymphocytes in the arterial wall. Anatomically, the most common site of plaque rupture in ACS appears to occur in the shoulder region, where inflammatory cells are most prominent and might serve to compromise the integrity of the surrounding connective tissue.

Immuno-histochemical complement factor C9 has very high reactivity and specificity for necrotic myocytes which is a protein involved in the complement system as a part of the innate immunity. It provides protection for the host and reduces susceptibility to foreign micro-organisms. When Myocardial Infarction (MI) occurs, cardiac cell necrosis triggers the activation of the complement system, causing neutrophilic infiltration in the myocardium. Viable myocardium does not activate the complement system because of the lack of cell necrosis. C9 antibodies react strongly and diffusely with necrotic myocytes in all types of infarctions for up to 2 days, whereas normal myocytes are nonreactive, resulting in a clear delineation between damaged and viable. [5]

Myocardial cells from the infarcted heart start expressing foreign antigens for the immune system of the body. This results in strong activation of the complement system and accumulation of extensive deposits of components of the final pathway C5b-9, MAC. Studies demonstrated co-localization of the complement components and inhibitors in the myocardial tissue. [42]

C9 showed greater advantages in detecting MI than the haematoxylin and eosin (H&E) stains. The MAC complex had been used to localize areas of MI, and these complexes can occur at the cytoplasmic membranous surface of ischemic cells [1].

The usefulness of immune-histochemical markers to the diagnosis of early myocardial damage has been recently suggested, Schafer et al found C5b-9 deposits in myocardial cells located within the zones of infarction in the autopsy material derived from patients with acute MI. However, there has been no convincing evidence that associates the degree of activation of the complement system with the myocardial necrosis or cardiac function in vivo in patients with AMI, nor is there compelling evidence that the complement system is activated in the patients with angina pectoris. [8]

Henceforth, this study aims to find the incidence of deaths due to myocardial ischemia its changes among the postmortem cases and the role of C9 in identifying deaths due to myocardial infarction by immuno-histochemical staining methods among autopsy cases in the Department of Forensic Medicine, Sri Ramachandra Medical College and Research Institute.

Materials and Methods

The study duration was from August 2014 - August 2017 in the Department of Forensic Medicine, Sri Ramachandra Medical College and Research Institute. This study was done as a retrospective study during the period of August 2014 to August 2016 and as a prospective study during the period of September 2016 to August 2017 in the Department of Forensic Medicine, Sri Ramachandra Medical College and Research Institute. All sudden death cases which were brought for medico legal autopsy to the Department of Forensic Medicine were included as study subjects. Cases with signs of decomposition and consent were not obtained from the relatives were excluded from the study. After applying exclusion criteria, a total sample of 44 autopsy cases were taken for the study. Institutional Ethics Committee approval was obtained and informed consent was gathered from the blood relatives of the Deceased.

Details regarding the identification, socio-economic class, occupation, time and date of incident and other relevant history, co-morbid conditions of the Deceased person were obtained from the relatives through a questionnaire by interview schedule. In autopsy weight of the heart, clots and patency

of valves were noted and multiple samples were collected from the heart and other organs during autopsy. Tissue processing and paraffin blocks were made from the collected samples. Hemotoxylin and Eosin staining and immuno-histochemical expression of cardiac tissue was performed for C 9 marker to find out the myocardial necrosis. Data entry was done in Microsoft Excel and the results were analyzed using Statistical Package for Social Sciences (SPSS-20) as descriptive statistics. Data are expressed in frequency and percentage.

Results

Among the forty four sudden death cases 19% of them were between the age group of 51-60 years with mean age of 49.3 years. The next common age group was between 41- 50 years. Lower incidence of sudden death was noted in extreme age groups. Sudden death was more common among males 81.82% the ratio was for every five males one female sudden death occurs. Among the cases 25% of them have completed middle schools and 23% of them have finished their high schools whereas only 5% of the deceased have done professional degree. 43.18% of the cases belonged to upper lower class in the socio economic scale, as shown in Table 1.

Of all the sudden death cases 75% were recorded with co-morbidities like alcoholism, diabetes and hypertension. During autopsy 68% of the cases had atheromatous plaque of the aorta, as shown in Table 2.

Histopathological examination of myocardial tissue showed acute MI findings in 34% of autopsy cases and 23% old MI findings, given in Figure 1.

Out of all 44 autopsies, 66% were positive for complement C9 stain and 20 tissue blocks subjected to 2,3,5 Triphenyl Tertrazolium Chloride (TTC) stain were 80% positive, shown in Table 3.

Table 1: Sociodemographic profile of autopsy cases

Characteristics	Frequency (n=44)	Percentage (%)
Age group (in years)		
21-30 years	5	8
31-40 years	8	13

41-50 years	10	16
51-60 years	12	19
61-70 years	7	11
71-80 years	2	3
Sex		
Female	8	18.2
Male	36	81.8
Educational status		
Illiterate	5	11
Primary school	3	7
Middle school	11	25
High school	10	23
Post high school / diploma	7	16
Graduate	6	14
Profession/ honour	2	5
Socio economic status		
Upper (Class I)	3	6.8
Upper Miiddle (Class II)	8	18.2
Middle/Lower Middle (Class III)	11	25
Lower / Upper lower (Class IV)	19	43.2
Lower (Class V)	3	6.8

Table 2: Co-morbities and Atheromatous changes in aorta

Characteristics	Frequency (n=44)	Percentage (%)
Co-morbid condition		
Present	33	75
Absent	11	25
Atheromatous plaque of aorta		
Present	30	68
Absent	14	32

Table 3: Details of C9 stain and TTC staining

Report	C9 (N=44) Frequency (%)	TTC (N=20) Frequency (%)
Positive	29 (66)	16 (80)
Negative	15 (34)	4 (20)

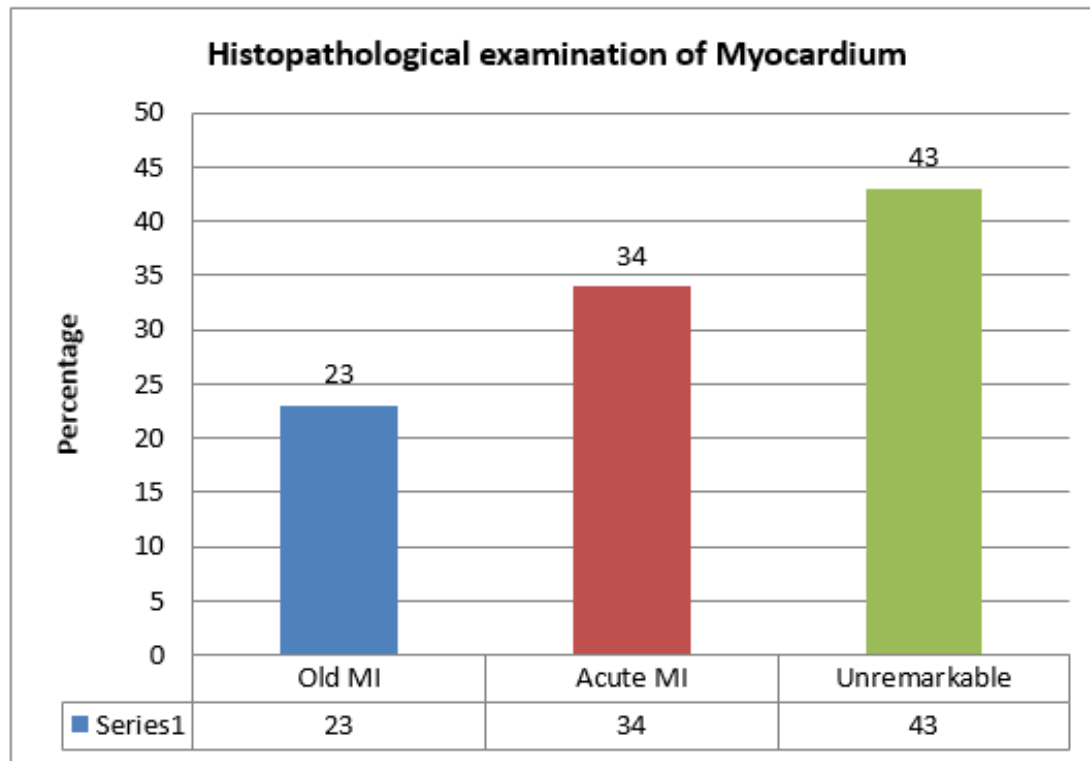


Figure 1: Histopathological Examination of Myocardial tissue

Discussion

In the present study maximum sudden death has occurred in the age group of 51- 60 years which is in accordance with 2011 census of India. Various studies have shown sudden death to be very common in male gender as 65.3%, 82% and 74.8% done by Jenkins et al, Murthy et al and Bhargav et al respectively [1,7].

Among 44 cases, 75% cases were with co-morbid conditions such as alcoholism, smoking, diabetes mellitus and hypertension, which was similar to studies done by Prasad VN et al [1,8]. Atherosclerosis of aorta was a reliable indicator, was noted in 68.18% of autopsy cases in the present study. M Maruet al [1,9] observed 47% of the autopsy cases with atherosclerotic changes.

In the present study histopathological examination by Haematoxylin and Eosin staining showed 34.09% of autopsy cases with acute myocardial infarction changes and in 43.18% cases myocardium changes was unremarkable. Out of the 44 cases, subjected to complement C9 marker immuno-histochemical analysis, 65.91% stained positive for complement C9 marker.

C9 as a marker of early acute myocardial infarction prove to be a reliable immuno-histochemical marker. Its utility is enhanced in cases which do not show obvious histopathological feature of myocardial infarction.

Cases which were unremarkable in Histopathological examination (H&E stain) when subjected to immuno-histochemical analysis for C9 marker were positive for C9 complement marker which indicates early acute MI.

Immuno-histochemical techniques have proven better suited to forensic pathology as they can be applied to tissue samples fixed in formalin and embedded in paraffin but mainly because they can detect ischemic areas when assessing acute myocardial damage.

The usefulness of immuno-histochemical markers to the diagnosis of early myocardial injury has been recently promoted because most of them can be visible as early as few minutes after the beginning of the symptoms (chest pain and angina attacks). [10]

Myocardial cells from the infarcted heart start expressing foreign antigens for the immune system of the body. This results in strong activation of the complement system and accumulation of extensive deposits of components of the final pathway C5b - 9, MAC. Studies demonstrated co-localization of the complement components and inhibitors in the myocardial tissue. [11]

Immuno-histochemical reaction for the complement components and inhibitors examined under the light microscope, demonstrated widespread abundance of these proteins in the tissues. Positive immuno-histochemical reaction was found in the myocardial cells, intercellular matrix as well as in the vessel lumens. [12]

Among the various antigens, C5b - 9 is most commonly used one because it can reveal small areas of myocardial necrosis, even limited to a single cell (Myocytolysis). [13]

The property of C9 is due to its direct involvement in the complement cascade, which directly cause cell damage by opening pores of the cellular membrane surface (hence its name, membrane attack complex), through which the cell contents leak out (cytolysis). As plasma antigen, C5b - 9 typically accumulates in ischemic areas of the myocardium and can detect early myocardial injury 40 minutes after the beginning of hypoxia [14]

Immunostains for complement component C9 have been shown by a number of investigators to be a reliable and sensitive method for detecting early myocardial hypoxia. Normal (non -hypoxic) cardiac muscle lacks staining with C9, but hypoxic myocardium demonstrates positive immunostaining for this marker. [15]

Doran et al studied 25 autopsy cases of suspected MI, and 25 cases without appreciable morphological evidence of MI. 24 of the 25 cases of known MI showed positive C9 immunostaining. [16] Piercecchi -Martini et al studied 121 heart specimens, including 33 cases with histologically evident ischemic change, with 20 patients who died with EKG evidence of ischemia but no H&E changes of infarct, 35 cases with severe coronary disease but unknown cause of death. [17]

Cases where MI is suspected but typical histological changes are not apparent, immuno stains for complement component C9 can be very useful in documenting the presence of myocardial ischemia in specimens that are obtained too early after the hypoxic event to show appreciable morphological changes on H&E. [11]

Conclusion

Sudden death is defined as the death without any apparent clinical signs and symptoms. One of the major causes of sudden death is myocardial infarction that results due to decrease in blood supply to the heart. In this study it was found among 44 autopsy cases which was considered as sudden death cases, the number of cases who had atherosclerosis of aorta was 30, indicating there is an associated factor for myocardial infarction. Complement C9 factor was used as a reliable marker for identification of early MI in cases which do not show significant histopathological changes with haematoxylin and Eosin stains. Also it was found male genders are more prone for sudden cardiac death and most MI are associated with co-morbid conditions which requires health education among the general population regarding the risk factors for myocardial infarction and life style modifications like healthy diet, avoiding alcohol, smoking and physical activities to avoid ischemia and silent MI.

On the part of the forensic pathologist it is necessary to subject every sudden death cases for TTC staining and C9 immuno-histopathology which identifies most of the acute MI in case of sudden death.

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Conflict of Interest: No conflicts of interest

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