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A COMPARATIVE STUDY OF IMMUNOHISTOCHEMICAL MARKERS IN THE DETECTION OF EARLY MYOCARDIAL INFARCTION

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Abstract :
immunohistochemical techniques have proven effective in forensic pathology use as they can be applied to tissue samples fixed in formalin and embedded in paraffin. In this work, we selected a panel of antibodies, desmin, troponin T (cTnT), and myoglobin (MB), for the detection of early myocardial infarction in human autopsy hearts with suspected sudden cardiac death to compare the sensitivity and the reliability of these markers and to study the potential effect of autolysis on their sensitivity. Histologic sections from the myocardium from 20 autopsy cases were studied by immunohistochemistry with the three different antibodies, using a standard avidin–biotin–peroxidise system. Desmin, cTnT, and MB depletion occurred very
early after ischemic injury and preceded the histologic

Keywords : atherosclerosis , pathology , risk factors , markers

Introduction :

Myocardial infarction (MI) remains one of the most common causes of death, and so identification of MI as a contributing cause of death is fairly common in patients
at autopsy (Hansen and Rossen, 1999).
The recognition of early myocardial damage using routine histologic techniques such as hematoxylin and eosin (H&E) staining is possible only if death has occurred at least 6 h after the onset of the ischemic injury (Baroldi, 2001). A period of 1-h interval or less from the angina attack is not usually enough to produce the findings, commonly known as classic morphologic alterations of the acute myocardial infarction (AMI) (Bouchardy and
Majno, 1974; Thiene et al., 2001).
According to the Lodge-Patch classification, the earliest changes are represented by stretching and waviness of myocardial fibers, the eosinophilic cytoplasm of the
myocytes, and nuclear pyknosis (Lodge Patch, 1951; Ishikawa et al., 2003).
Early MI can be impossible to detect either by direct examination at necropsy or using conventional stains on histological sections (Doran et al., 1996), as features of an
AMI in an autopsy myocardium before neutrophilic infiltration are often very subtle on hematoxylin and eosin (H&E) staining (Jie et al., 2010). Therefore, immunohistochemical cardiac markers of myocardial damage are needed because an AMI can be detected only by conventional histologic procedures 4–6 h after the onset of ischemia (Ortmann et al., 2000).

Over the years, a number of different staining techniques have been used to assist in the identification of these early infarcts that are not yet recognizable on H&E (Campobasso et al., 2008).

Material and method :

A retrospective study of hearts from 20 autopsy cases of sudden unexpected death (SUD), with/without evidence of coronary artery disease examined at the Pathology Department, has been conducted. Ethical approval was obtained by EFMA. The distinction between sudden
death at rest and that during effort is very important because some lethal arrhythmias are triggered by catecholamines released during stressful activity (Yoshi-da, 2009). We selected a total of 20 sudden or unexpected deaths, and divided them into three main groups
according to the autopsy findings as follows:
(1) Group I: Sudden cardiac death (SCD) with no history
of trauma or stress (seven cases).
(2) Group II: SCD after trauma or stress (11 cases).
(3) Group III: SUD due to an unrelated cardiac cause
(two cases).
Tissue sections from coronaries and myocardia (about 250 sections in total) were obtained. Myocardial tissue samples were excised from the left ventricle, the ventricular septum, and the right ventricle, and samples from the corresponding coronaries were also taken; they
were fixed in 10% neutral-buffered formalin and e mbedded in paraffin. Thin sections were stained with hematoxylin-eosin.
Finally, 20 samples of myocardial tissue were selected according to the coronary findings, for immunohisto-chemical staining, including three sections with autolytic changes for the detection of the effect of autolysis on the loss and the expression of the three markers.
Three samples of normal ventricular tissue without any lesions were obtained as positive control slides for the three markers. The samples belong to a woman in the third decade, who was subjected to homicide.

Results

Eighteen of the 20 (90%) cases were males.
(1) Group I (seven cases) represented SCD with no history of trauma or stress. Five out of the seven cases of GI had acute coronary syndrome and one of them had a history of MI. Of the remaining two cases, one had a severe coronary occlusion, with a normal sized heart, mild mitral thickening, and unremarkable microscopic changes in the H&E-stained slides, and the other patient had cardiomegaly, MI, and infective endocarditis. (2) Group II (11 cases) represented SCD after trauma or stress. Eight cases showed complicated coronary
atheromas. Six had acute coronary syndrome, one showed a healing thrombus, and one had a healing hemorrhage inside the atheroma.
(3) Group III (two cases) represented SUD due to an unrelated cardiac cause. GIII cases belonged to two adult women with amniotic fluid and trophoblastic emboli.
Control slides showed diffuse brown myocyte staining for the three markers, denoting the integrity of the myocardium

Regarding Troponin, 18 out of the 20 cases (90%) showed variable degrees of depletion as follows :
(1) Six cases of the GI showed variable degrees of depletion; the remaining one case that showed no depletion belonged to a man in the third decade with SUD, and examination revealed a recent thrombus.
(2) Ten cases of GII showed variable degrees of cTnT depletion; the remaining one case showing no depletion belonged to a male soldier in the third decade who died during training. Heart dissection and examination revealed a recent thrombus.
(3) GIII showed depletion (scores – 2 and – 1).
Regarding myoglobin, 19 out of the 20 (95%) cases

showed variable degrees of depletion as follows :
(1) All GI cases showed variable degrees of MB deple-tion.
(2) Ten cases of GII showed variable degrees of MB depletion and one case showed no depletion, which was the same case with no cTnT depletion that belonged to the third-decade male soldier.
(3) GIII cases (two cases) showed the same results for cTnT: depletion (scores – 2 and – 1).

Discussion

Post-mortem diagnosis of early MIs is an ever recurrent problem in forensic pathology. Because of the lack of practical and satisfactory conventional techniques for the morphologic identification of early myocardial ischemia, only immunohistochemical methods and a reasonable combination of cell and plasma antigens can provide enough evidence of myocardial ischemia and/or necrosis, supporting the final diagnosis of SCD (Campobasso et al., 2008).
Previous studies at FMA indicated that immunohisto-chemical staining for cTnT and MB may be more sensitive than routine H&E staining for the recognition of myocardial necrosis in human hearts at autopsy (Amin et al., 2011). However, desmin seems to be more practical as it is more likely to be available than other markers.
Desmin is an intermediate filament expressed in both striated and smooth muscles (including the cardiac muscle) (Milner et al., 1999). In human hearts removed from heart-transplant recipients with myocardial ische-mia, desmin begins to disappear from the myocardial cytoplasm within 30 min of the ischemic insult, and is completely gone in 90–120 min (Hein et al., 1995).
Cardiac troponins I and T are integral proteins to the function of the cardiac muscle. They are very sensitive markers for the detection of myocardial damage, and the ability to assay their serum levels accurately and quickly have revolutionized the concepts of minor myocardial injury and infarction. Troponin T as a specific cardiac muscle protein has become a widely used marker for testing patients with acute chest pain (Sarko et al., 2002).
Myoglobin is a protein found in skeletal and cardiac muscles, which binds oxygen and is a very sensitive indicator of muscle injury. Moreover, the increase in myoglobin can help one to determine the size of an infarction (Gornall and Roth, 1996).

Conclusion

The above markers can be considered as reliable indicators of acute myocardial damage as they summarize the proper-ties of an ideal marker of myocardial injury. However, no single immunohistochemical reaction is ideal for diagnosing early myocardial ischemia. However, only a selected set of markers can improve the ability of the pathologists to detect ischemic areas when no macroscopic or microscopic evidence of necrosis is available.

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