

Diagnosis of early myocardial infarction by histochemical staining of heart on autopsy table

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Abstract

Background: Determination of cause of death is the main objective in every Medico-Legal Autopsy. Most often, the identification of early change of Myocardial infarction becomes difficult during postmortem examination, as a gross change of infarct will not be apparent for 24 to 48 hours following myocardial ischemic damage. Identification of the earliest Stage of myocardial ischemia remains a pressing challenge. **Aim:** To determine the diagnostic validity of the Histochemical Staining (TTC) of heart in gross detection of early Myocardial Infarction. **Materials and Methods:** The Present Study Was Conducted In 2018 The Institute of Forensic Medicine Government Mohan Kumaramangalam Medical College Hospital, Salem The Study Sample Consists of 18 Hearts Taken From Cases Of Sudden Deaths With Either History Or The Morphological Features Of Heart Suggestive Or Suspected The Cause Of Death To Be Of Cardiac Origin. **Results:** Males occupy a predominant number of cases, accounts for about 94.4% of the sample, whereas female constitute only 5.61% of the sample. It signifies the incidence of myocardial infarction / sudden cardiac deaths is greater among males. Out of 18 hearts stained using 1% Triphenyl Tetrazolium Chloride solution, 6 hearts with no apparent gross change showed areas of unstaining were considered TTC positive for early myocardial, Histopathological examination of the 6 TTC positive hearts showed features of early myocardial infarction in 5 hearts, One TTC positive for early infarction heart showed features of normal myocardium i.e. Considered as false TTC positive. 5 hearts showed areas of old infarct, one heart showed area of old infarct and area of hyperemia with softening on gross examination. TTC staining of these 6 hearts showed the area of unstaining in addition to the area of unstaining due old infarct (which is evident on gross examination) and was considered TTC positive for early myocardial infarction and old infarction. Histopathological examination of the 6 TTC positive hearts showed features of both early myocardial infarction and features of old infarction. **Conclusions:** The present study concludes that Histochemical staining of the heart using Triphenyl Tetrazolium Chloride is a reliable method in the detection of early myocardial infarction for Forensic pathologist during postmortem examination, as the method in the present study, has got the diagnostic validity of 88.8%. **Key Word:** Histo Pathological Examination, Left Anterior Descending Artery, Left Ventricle, Myocardial Infarction

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INTRODUCTION

The incidence of Sudden Unexpected Death is increasing globally. Cardiovascular disease is the most common cause of sudden death. Eighty percent of sudden cardiac death is due to coronary arterial disease.¹ In 25% of cases death occurs abruptly and unexpectedly within the first hour of onset of clinical symptom. Myocardial infarction has major psychological and legal implications for the individual and to society. Establishment of clinical diagnosis of Myocardial Infarction usually becomes difficult as most often the death is sudden.² Final chance to establish the cause of death is, therefore, the Post mortem Examination. Determination of cause of death is

the main objective in every Medico-Legal Autopsy. Most often, the identification of early change of Myocardial infarction becomes difficult during postmortem examination, as the gross change of infarct will not be apparent for 24 to 48 hours following myocardial ischemic damage. "Identification of the earliest Stage of myocardial ischemia remains a pressing challenge"³ Histochemical staining techniques are based on the fact that ischemic myocardial cells lose their membrane integrity and release their enzyme contents into the blood, resulting in a marked decrease or total depletion of these enzymes in the ischemic areas of the myocardium. Enzyme depleted infarct myocardium remain unstained, which is the principle of this study.⁴

MATERIALS AND METHODS

The Present Study Was Conducted In 2018 The Institute Of Forensic Medicine Government Mohan Kumaramangalam Medical College Hospital, Salem The Study Sample Consists Of 18 Hearts Taken From Cases Of Sudden Deaths With Either History Or The Morphological Features Of Heart Suggestive Or Suspected The Cause Of Death To Be Of Cardiac Origin. During Postmortem examination heart was removed, washed thoroughly under running water and weighed. Gross examination of the entire heart was done to look for any presence of scar due to old infarct, areas of softening surrounded by hyperemia or any other morbid condition. Serial sections of the coronary artery were made at the distance of 3mm to look for any presence of occlusion by plaques or thrombus. The consistency of the coronaries was appreciated. Serial transverse section involving the full thickness of heart was made at the distance of 1 cm each from the apex to the AV groove. Slices are examined for old fibrotic scar and softening. The heart is dissected along the line of flow of blood, observed for raised atheromatous plaques on the luminal

surface of the root of the aorta, narrowing of coronary Ostia, and narrowing of the lumen of coronaries by atheromatous plaque or thrombus. Two slices are selected at random between the mid-ventricle and the apex is taken for histochemical staining.

Staining method:^{5,6,7} Heart slices were washed with running water and wiped with tissue paper and placed in a plastic container of a size larger than the heart slice (Fig.10). One container was used for each slice. TTC solution was poured into the container containing heart slice so that the solution level in the container was about 2 cm above the heart slice to prevent atmospheric oxygen penetration. Then the system is placed in a cardboard board box to avoid light exposure. The incubation is carried out at room temperature for 20 minutes. Heart slices should not touch the container surface as it may result in artifactual non-staining. Ten minutes after incubation, the heart slice was turned upside down to prevent artifactual nonstaining. After incubation for 20 minutes, heart slices were removed and examined for unstained areas of the myocardium. Normal myocardium stained brick red whereas infarct area remained unstained or shows very much reduced staining. The positive result was inferred by suspected infarcted. The area remaining unstained with the TTC. Both the stained and unstained areas were subjected to paraffin-embedded histological examination. In TTC positive cases, sections were taken from the unstained area, whereas in negative cases two random sections were made from the left ventricle. Histopathological diagnosis of acute myocardial infarcts is made from the following criteria, which include wavy myofibers, coagulative necrosis, ischemic contraction band necrosis, myonecrosis, and polymorphs infiltrations of the interstitium.¹ Then the results of TTC staining were correlated with the Histopathological examination findings.

RESULTS

Table 1: Sex Distributions Among The Study Sample.

Sex	Frequency	Percentage
Male	17	94.4%
Female	01	5.6%
Total	18	100%

Table 1 Male occupy a predominant number of cases, accounts for about 94.4% of the sample, whereas female constitute only 5.61% of the sample. It signifies the incidence of myocardial infarction/sudden cardiac deaths is greater among males.

Table 2: histopathological examination results

Sr. no	Observations	No. Of cases
1	Evidence of early myocardial infarction	6
2	Evidence of early myocardial infarction and old infarction	6
3	Evidence of old infarction	2
4	Normal myocardial histology. No areas of infarction	4

Table 2: Histopathological examinations of the TTC stained heart slices for early myocardial infarction showed features of myonecrosis, waviness of fibers, nucleomegaly, Pyknotic nuclei, and polymorphs infiltrations. In areas of old infarction, Histopathological examination revealed evidence of dense fibrous tissue replacing normal myocardial tissue. In HPE positive for early infarction heart, not all the above said features of early myocardial infarction were present. Presence of anyone of the features of the early myocardial infarction was taken into consideration for positive HPE for early myocardial infarction.

Table 3: Combined results of TTC Staining Method And HPE

Positive/ negative	HPE Positive for early mi	HPE Positive for early mi and old mi	HPE Negative for early mi and positive for Old mi	HPE Negative for both mi
TTC Positive for Early MI	5	0	0	1
TTC Positive for Early MI and Old MI	0	6	0	0
TTC Negative for Early MI and Positive for Old MI	0	0	2	0
TTC Negative MI	1	0	0	3

Table :3 Out of 18 hearts stained using 1% Triphenyl Tetrazolium Chloride solution, 6 hearts with no apparent gross change showed areas of unstaining were considered TTC positive for early myocardial, Histopathological examination of the 6 TTC positive hearts showed features of early myocardial infarction in 5 hearts, One TTC positive for early infarction heart showed features of normal myocardium i.e. Considered as false TTC positive. 5 hearts showed areas of old infarct, one heart showed area of old infarct and area of hyperemia with softening on gross examination. TTC staining of these 6 hearts showed the area of unstaining in addition to the area of unstaining due old infarct (which is evident on gross examination) and was considered TTC positive for early myocardial infarction and old infarction. Histopathological examination of the 6 TTC positive hearts showed features of both early myocardial infarction and features of old infarction. In two cases there is evidence of old infarct scar on the gross examination which remains unstained and the other area was stained completely. Histopathological examination of this unstained old infarct revealed features of old infarction and stained area of the same slice revealed normal histology. In 4 cases the entire heart slice stained (no unstaining area) and considered TTC negative for early and old infarction. Histopathological examination revealed features of normal myocardial histology in 3 cases. In one TTC negative heart, Histopathological examination revealed features of early myocardial infarction and considered as false TTC negative.

Table 4: TTC Staining And Histopathological Examination Confirmed Cases

Positive / Negative	Positive for Early Myocardial Infarction by Histopathological examination	Negative for Early Myocardial Infarction by Histopathological examination
TTC Positive for early MI	11	1
TTC Negative for early MI	1	5

Table 4: The sensitivity of the test is 91.66% The specificity of the test is 83.33%. The positive predictive value of the test is 91.66% The negative predictive value of the test is 83.33%. Overall Diagnostic validity of the test is 88.88% Likelihood ratio for the positive test:5.49. The likelihood ratio for the negative result:10

DISCUSSION

Sudden cardiac death due to acute myocardial infarction is common among both men and women. WHO projects, by the year 2020, ischemic heart disease ranks one among the disease burden.⁸ In many cases death occurs rapidly and evidence of acute MI is not detected during postmortem examination. Apparent gross morphological changes of acute myocardial infarction take 24 to 48 hours for its appearance. Usually, diagnosis of myocardial infarction is made by random sectioning of the heart for Histopathological examination.⁹ Random sectioning of heart for Histopathological examination is inefficient, as often the method may miss myocardial infarction if the section does not include the inapparent infarct area.¹⁰To

overcome the difficulty Forensic pathologists have adopted various methods to establish the diagnosis of acute myocardial infarction. Triphenyl Tetrazolium chloride staining of the heart for early myocardial infarction is a simpler, easier, rapid technique to carry in autopsy hall and it is possible to provide a fair result at the end of the autopsy.¹¹ Many animal studies conducted using TTC for detection of acute myocardial infarction, may not be applied directly to humans due to species difference.¹² The reasons for a false positive result are heart slice remaining in contact with the container during incubation in the TTC solution, prolonged exposure of TTC solution to light, old TTC solution, contamination of the solution and greater postmortem

interval resulting in autolysis.¹³ H.H. Klein *et al* in reported that Histochemical staining using TTC for gross detection of early infarction has diagnostic sensitivity of 77.4% and specificity of 92.6%. The positive predictive value of the test was 80.5% and the negative predictive value was 91.2%. The overall efficiency of the test was 88%¹⁴ Hougen HP *et al* in, Histochemical staining using TTC for detection of acute myocardial infarction was conducted on 40 hearts removed from cases of sudden death. The study showed that sensitivity and specificity of the TTC staining method is 100%. In the study, hydrochloric acid is used for adjustment of pH of the solution instead of phosphatebuffer.¹⁵

CONCLUSIONS

The present study concludes that Histochemical staining of the heart using Triphenyl Tetrazolium Chloride is a reliable method in the detection of early myocardial infarction for Forensic pathologist during postmortem examination, as the method in the present study, has got the diagnostic validity of 88.8%. The preparation of 1% TTC solution, adjustment of pH using low pH and high pH phosphate buffer is quite easy, staining method is cost-effective, simple to carry out in autopsy hall and does not require any complex types of equipment. However, if death occurs due to arrhythmias during the ischemic period before the development of infarction, the method has limitation in the identification of the cause of death due to arrhythmias. In such a situation, present and past history of the patient, circumstances, the ultrastructural examination of myocardial tissue and conducting fibers help in identification of the cause of death. As there is an occurrence of a false positive and false negative result in Histochemical staining using TTC for early myocardial infarction, a combination of both Histochemical technique and Histopathological examination helps in diagnosing or ruling out inapparent early myocardial infarction as a cause of death in a fair number of cases.

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