

An Autopsy Study of Heart in Sudden Death Cases By Triphenyl Tetrazolium Chloride - At Tertiary Care Hospital

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Abstract

Myocardial infarction is the most common cause of sudden unexpected death. On autopsy the evidence of coronary artery narrowing and gross changes are important for the diagnosis. But gross changes took 24 to 48 hours to occur, so the histochemical staining on gross by TTC is more helpful, as it diagnoses infarction within 6 hours of death, and also the area from which the section should be taken also can be decided.

Key Words: Myocardial infarction; TTC stain; Gross Diagnosis.

Introduction

Establishment of clinical diagnosis in cases of sudden death due to myocardial infarction is usually difficult and post mortem examination remains the final step in confirming the diagnosis.

The features characteristic of infarction on H & E stained sections on microscopy are eosinophilia, swelling of muscle fibers, granularity of cytoplasm, blurring of cell membranes, corrugation of dead muscle fibers, increase in interstitial cells which occurs about 18 hours after death.^{1,2}

The changes of identification also shows personal variation, where changes are less. The time required for diagnosis also increases due to the processing time. Actual site of infarction may get missed during sectioning, so the histochemical methods are used that are most sensitive and specific which can detect ischemic areas within 6 hours of death and also differentiate between normal and ischemic area of heart. The actual site for sectioning of heart of infarction area may be missed in the absence of gross localizing techniques.^{1,2,3}

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Materials and Methods

The present study is carried out in the department of pathology Dr SCGMC Nanded, from the period of Jan 2019 to Dec 2019.

The heart was examined for the gross, before and after, TTC staining and simultaneously H&E section studied from that area.

Objective

1. To detect myocardial infarction by histochemical staining.
2. To compare the results with H & E stain with histology section.

Inclusion Criteria

Cases of sudden natural deaths suspected of cardiac origin.

Exclusion Criteria

Accidental deaths and deaths due to known cause.

Materials and Methods

The study is carried out on 33 cases of sudden and suspected deaths of myocardial infarction. Proper gross examination with inspection of all valve, aorta and coronaries done. Atherosclerotic changes of coronaries and aorta noted, and also ventricular wall thickness noted. During the medicolegal autopsy the heart is dissected out from the body

thoroughly washed under running water. After weighing, inspection of heart for any abnormalities and malformation done.

The valves are checked for stenosis after opening the atrium. Coronaries are inspected. Myocardium is examined for the gross changes of infarction like softening, hyperemia and white patches.

TTC Test

The heart is sliced from the apex to the atrioventricular junction of 1 to 1.5 cm thick. The slices are incubated in 1% solution of 2,3,5 triphenyl tetrazolium chloride for 20 to 30 mins. The slice should be cleaned in running tap water before incubating to remove excess of blood. The stain is prepared by dissolving 1 gm of TTC powder to 100 ml of phosphate buffer at pH 8.5.

A wide mouth container with screw cap is used to carry out the staining. We used acrylic jar for holding and staining during incubation process it should be screw cap and kept in dark place. As exposure to light and air will make the stain less potent.

TTC solution should be prepared freshly every time and used within half an hour. The slices should be 2 cm beneath the upper fluid level. The pH should be maintained at 8.5 otherwise the formazon pigment formation will be unsatisfactory.

The slices should be turned once or twice during the process for the better results. At the end of staining the slices are transferred to a jar containing 10% formal saline. This halts the reaction also and fix the tissue.

On completion of staining the infarcted part will show pale pink colored area and normal myocardium which takes brick red colour. Even old infarcts, scars can be identified. This staining method is recommended by knight B and LieJT et al.⁴

Results

Total 33 cases of sudden deaths were studied for myocardial infarction. Out of this 30 (90.90%) were males and 3 females(9.09%). The age range between 20 to 80 years.

TTC stain was positive in 26 cases with acute MI, one case with transmural infarct with hemopericardium, (i.e. total 27 cases positive for TTC & out of those 24 cases were positive for histopathological examination as shown in Table 2) three cases which were positive by TTC and not showing changes on histopathological examination, this may be due to early diagnosis by TTC stain

and 6 cases have not shown staining by TTC and histopathological examination due to autopsy done within one to two hours of death.

All unstained areas were again studied by histopathological examination, which shows histological changes of MI. The coronaries are also studied with aorta, showed atherosclerotic changes giving support to the diagnosis.

Out of 27 positive TTC cases 24 cases showed microscopic changes of infarction. Six cases of negative TTC do not show microscopic changes. Male cases were more as compared to females and is depicted in Table 1.

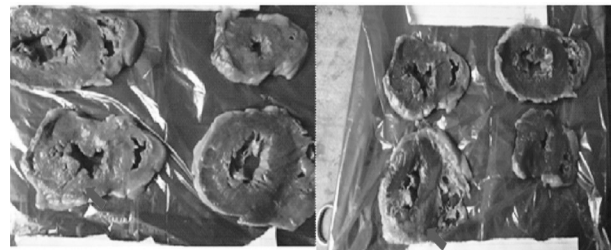


Fig. 1A

Fig. 1B

Fig. 1: A & 1B: Gross Sections of heart specimen before & after staining with TTC dye. Figure 1A Sections of heart before staining with TTC dye Arrow Show's infarcted Area. Figure 1B Sections of heart after staining with TTC dye Arrow Show's infarcted unstained area.

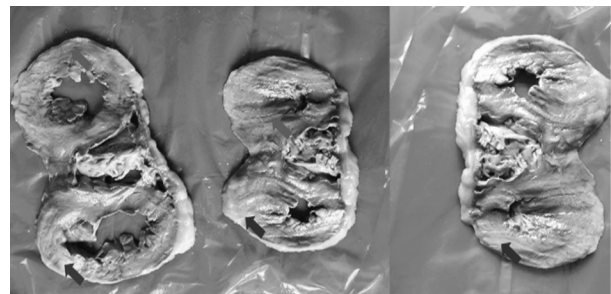


Fig. 2: Gross changes in heart after staining with TTC dye: Red Arrow Shows Normal Myocardial Area Blue Arrow Shows Infarcted Area.

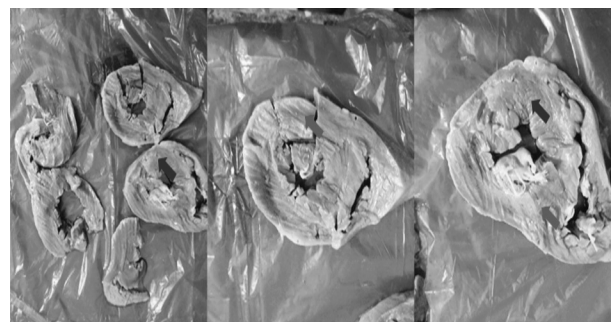


Fig. 3: Gross changes in heart after staining with TTC dye. Red Arrow Shows Normal Myocardial Area Blue Arrow Shows Infarcted Area.

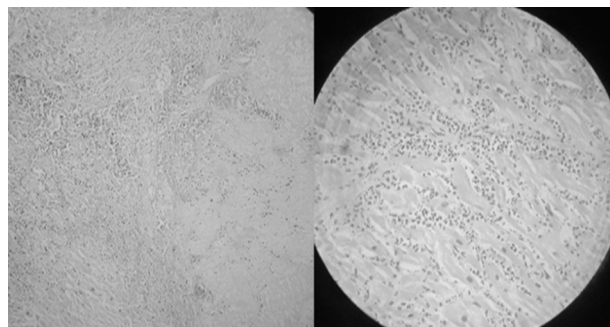


Fig. 4A

Fig. 4B

Fig. 4A & 4B : H & E stained section of infarcted area. Figure 4A : Low Power (10x) View Shows Areas of Hemorrhages, Necrosis, inflammation. Figure 4 B: High Power (40x) View Shows hypertrophy Necrosis of myocardial fibers with acute inflammatory cells.

Table 1: Age and Gender Wise Distribution of Cases Included in Study:

Age in Years	Males	Females
Upto 20	-	-
20 To 40	6	1
40 To 60	20	-
60 To 80	4	2
Total	30 (90.90%)	3 (9.09%)

Table 2: TTC and Histopathologically Confirmed Cases.

	TTC	Histopathology	Both
Positive	27	24	24
Negative	6	6	6

Discussion

This study is carried on the basis to set the staining method of TTC by pathologist as they have to give final impression of the diagnosis. So in suspected sudden death cases this gross method of diagnosis is really very helpful. This helps to diagnose the infarction of heart.

Diagnosis at autopsy of patients with myocardial infarction occurring within hours of death is often difficult when time lag between irreversible ischemic insult in the myocardium is insufficient for the development of gross and histological changes, indicative of necrosis is less than 6 hours^{5,6}. These changes occur between 0 to 12 hours of ischemia.

TTC is salt, which is water soluble and by reaction with normal tissue enzymes forms the light sensitive compound (formazon) which turns normal tissue purple red and abnormal unstained.

There are many biochemical changes in a normal tissue after injury. Similarly the myocardial injury causes leakage of electrolytes like potassium,

magnesium, phosphate etc. and various enzymes like lactic dehydrogenase(LDH) Maleic dehydrogenase(MDH), Succinic Dehydrogenase (SDH).

So the histological changes may or may not be there and atherosclerotic changes may or may not be seen. The diagnosis can be given on macroscopic staining method.

The TTC reaction depends upon the activity of these lactic dehydrogenase(LDH) Maleic dehydrogenase(MDH), Succinic Dehydrogenase (SDH) enzymes The inactivation of dehydrogenase enzymes due to leakage out of cell or exhaustion of glycogen stores from within the dead cells, leads to non deposition of formazan pigment over infarcted area.

Inactivation of these enzymes is time bound but variable. It can be noticed as early as five hours of infarction⁷. The enzyme activity can't be affected up to 36 hours and if refrigerated up to 60 hours.⁸

Conclusion

The forensic pathologist has the task of diagnosing Acute MI many times. So before histological changes do appear the pathologist can diagnose the Acute MI by TTC method. This will help in giving the cause of death as well as for benefits of any health policy claim of the diseased.

So routine set up for sudden death cases of infarction TTC is more useful and easy technique, not requiring any costly equipment and can be performed in any set up.

Even 36 hours after death, diagnosis can be given by this TTC method.

So every cases of sudden death and suspected of MI should have this staining strategy to confirm the diagnosis of MI and localizing the area of infarction and the section from the area not stained by TTC can give more positive results by H & E stain.

As our study comprises less samples, more and more studies need to be conducted to establish the definitive data regarding the utility of TTC.

There is no Conflicts of Interest

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