

Role of TTC in Early Diagnosis of Suspected Acute Myocardial Infarction Autopsy Cases: Our Experience in Central India

Sunil K Jaiswal¹, Ashok Yadav², Pramendra S Thakur³, Amrita Tripathi⁴

Abstract

Introduction: Acute myocardial infarction is a most common cause of sudden death. Microscopic evidence of infarction is seen in H & E stained section only if person survived for a minimum period of 6 hours after fatal ischemic attack. So for visualisation of infarct of lesser age TTC test can be used. It is a gross staining procedure which can reveal infarct of within 5–6 hrs. age Aim of this study to incorporate this test in study protocol of sudden cardiac death with aim of visualising infarct rather than giving indirect evidence. **Objective:** To study usefulness of TTC stain as indicator of early myocardial infarction in Autopsy cases. **Materials and Methods:** The present study was conducted in MGM Medical College Indore on 210 Post-mortem autopsy cases of death due to suspected myocardial infarction. Heart obtained from autopsy cases subjected to gross examination of all three coronary vessels. After cleaning heart, complete transverse slices of ventricular myocardium subjected to TTC macro test. Knife cut slice is dipped in 1% solution of TTC for 20–30 minute. **Result:** Out of 210 cases brought for post-mortem examination with history of suspected cardiac attack on which TTC stain applied over heart 60 cases were found to show TTC staining reaction. In these cases, it was infarcted area show pale to pink and normal areas as bright red. **Discussion:** Due to the absence of gross and microscopic changes before 6–8 hrs. of post MI survival time, there was a major hurdle in establishing the cause of death by light microscopy in cases of early death due to myocardial infarction. Since biochemical alteration occur following injury to any tissue form pathological changes. After myocardial infarction due to leakage of multiple dehydrogenases enzymes infarcted area did not react with TTC and remain pale and normal myocardial enzyme react with TTC and form Formazone which was bold colour.

Keywords: Sudden cardiac death; Myocardial infarction; TTC Macro test.

How to cite this article:

Sunil K Jaiswal, Ashok Yadav, Pramendra S Thakur *et al.* Role of TTC in Early Diagnosis of Suspected Acute Myocardial Infarction Autopsy Cases: Our Experience in Central India. Indian J Forensic Med Pathol. 2019;12(3):185-188.

Introduction

Myocardial infarction is a major cause of death in recent era throughout the world. In most of the cases it is due to ischemic heart disease. Narrowing of coronary arteries is major cause of myocardial infarction. If patient survive for more than 6 hrs.

then only gross and microscopic finding can be detected and if death occur within 6 hrs. then there is lack of finding by gross and microscopic examination.¹

In such cases where death occurred in 6 hrs. post infarction, Triphenyl tetrazolium chloride test able to predict infarcted area. It is a gross staining procedure. By this test Forensic pathologist able to visualise infarcted area during autopsy examination.²

The heart should be fresh 36 hrs. old for TTC and should not be fixed in any fixative. If body kept in cold storage then this test can be carried even after 68 hrs. after death but colour intensity may be variable.

The dyes form coloured farmazon over the normal myocardium when this dye (TTC) react with various dehydrogenases and the infarcted

Authors Affiliation: ¹Resident ²Professor ⁴Assistant Professor, Department of Pathology, ³Professor and Head, Department of Forensic Medicine & toxicology, Mahatma Gandhi Memorial Medical College, Indore, Madhya Pradesh 452001, India.

Corresponding Author: Ashok Yadav, Professor, Department of Pathology, Mahatma Gandhi Memorial Medical College, Indore, Madhya Pradesh 452001, India.

E-mail: dr.ashokmyh@gmail.com

Received on 07.05.2019, **Accepted on** 08.06.2019

area is seen as light yellow colour due to loss of cytochemical dehydrogenases. Clinical reports and experiment show that after myocardial infarction enzymes levels increases in blood but their activities decreases at infarcted area.⁶⁻¹² After infarction enzymes get destroyed from infarcted.⁴

Aims and Objectives

To evaluate usefulness of TTC stain in early diagnosis of myocardial infarction in cases where death occur due to clinically suspected myocardial infarction or there is history of symptoms of myocardial infarction and death occur within six hrs. of symptoms or sustained myocardial infarction.

Materials and Methods

In MGM Medical college indore more than 2500 autopsy done per year. After ethical approval by local ethical committee, a prospective study was carried out 210 cases on in MGM Medical college & MY Hospital, Indore during 2018-2019 on autopsy cases received in forensic department of MGM Medical College, Indore.

Heart of case on which test has to perform is taken out and clean the heart with cold water. Weight of heart is taken. Gross examination of heart is done, surface of heart is observed carefully to see presence of any gray white area is seen over surface of heart. All coronary arteries were examined by serial transverse section and narrowing is noted.

Now a complete transverse slice of heart was taken from ventricular myocardium including both ventricular myocardium and interventricular septum or from those area show infarct by gross appearance. Multiple slices of nearly 1 cm. were taken for study.

Slice remain unfixed (without formalin) and fresh. Now prepared solution is of TTC dye taken in glass container. This solution is prepared by taking 100 ml. phosphate buffer solution and 1g. of TTC salt. After mixing well a light yellow colour solution is prepared. It is Important to know that Ph of this solution should be between 8.5 for better result otherwise formation of formazan pigment remain unsatisfactory.³⁻⁴

Slice of myocardium were kept in prepared 1% solution of TTC and phosphate buffer, dipped well for nearly 30 minute. Slices should be completely dipped in solution. This procedure was carried out at room temperature. After keeping slices in incubator solution, now container is closed and kept in dark for 30 minutes as light and air reduces potency of solution.

After completion of staining procedure slice is taken out and kept in 10% formalin solution which halt staining procedure and fix the tissue.

In our study those cases were included those were brought with clinical history of heart attack or suspected myocardial infarction. Other 10 cases were included as control. Autolysed cases and cases in which death occurred due to any other reason are not included (except control cases).



Image 1: Showing infarcted pale area and cherry red normal myocardium on use of TTC dye.

Results

In our study total 210 cases were taken out of which 180 cases were male and 40 cases were female. Only 22 cases were below 40 yrs. of age and all others were above 40 yrs. of age. In our study out of 210 cases, 60 cases were showed infarcted area after staining with TTC dye.

On finishing of staining procedure, infarcted part of myocardium become light pale to pink while normal part become bright red in colour. If there is no infarction then both surface of slice show uniform bright red in colour.

Discussion

In case of myocardial infarction atleast 6 hours of survival is to be needed for gross and microscopic changes to occur. If there was early death, then it was very difficult to predict cause of death as there was usually, there was no visible gross or microscopic changes (neutrophilic infiltration) in myocardium.¹

Experimental and clinical reports indicate that there is alteration in enzyme level in myocardial infarction.²⁻³ Watchstein and Meisel were found that activity of enzyme succinic dehydrogenase is lost from necrotic muscle with in six to 8 hrs. So possibility of detection of myocardial infarction by detection of alteration of these enzymes is highly significant. Activity of enzyme dehydrogenases and cytochrome oxidase is altered in infarcted myocardium.⁵

TTC reaction depends upon decreased activity of multiple dehydrogenase enzyme. Loss of activity of enzyme dehydrogenase occur due to leakage of enzyme or loss of glycogen storage leads to non deposition of farmazan compound over myocardium.¹ Sandritter and Jestadt became employed triphenyl tetrazolium for detection of myocardial infarction. Oxidation and reduction reaction leads to formation of farmazane compound formation at place where normal myocardium present. They had performed this test on 112 human heart and in eleven cases were found activity of TTC in absence of gross and microscopic activity.¹ Caine and Assmen had been already worked on 14 human heart and out of this 6 cases were clinical diagnosed case of myocardial infarction of 1 or 2 days.

In study by Marvin Nachals Theodor K Shnitka on 23 human heart were included in study and they belongs patients died suddenly or due to CHF or

due to hypothermia without coronary perfusion. All cases treated with TTC dye and staining were found positive.¹ Our study had done on 210 cases and TTC staining were found in 60 cases where infarcted area were visible distinctly.

Conclusion

In our study we conclude and recommend that TTC (Triphenyl tetrazolium chloride) can be used for early diagnosis of Myocardial infarction in all sudden death cases. This method is very suitable for forensic doctor also for medicolegal autopsy and they can put this method in their practice. In such cases where survival time is veryless and gross and microscopic changes are not visible, in such cases TTC staining is a rapid, easy and spot techenique to confirm myocardial infarction.

Source(s) of support: Nil

Presentation at a meeting: Nil

Conflicting Interest: Nil

References

1. Marvin M, Nachlas and Theodor K Shnitka, *et al.* Macroscopic Identification of Early Myocardial Infarcts by Alterations in Dehydrogenase Activity. *Am J Pathol.* 1963Apr;42(4):379-405.
2. Lt Col RB Kotabagi, Dr (Mrs) VV Apte, Col PR Pathak, *et al.* Post-mortem diagnosis of early myocardial infarction-MJAFI. 2000 Apr;56(2):99-102.
3. Ramesh, K Kundal, DS Bhullar, *et al.* The autopsy diagnosis of early myocardial infarction (mi) by triphenyltetrazolium chloride (ttc) or nitrobluetetrazolium (nbt) dye.test. *J Punjab Acad Forensic Med Toxicol.* 2012;12(1):60-1.
4. Knight B. Early myocardial infarction. Practical methods for its post mortem demonstration. *J Forensic Med.* 1967Jul-Sep;14(3):1017.
5. Lie JT, Pairolero PC, Holley KE, *et al.* Macroscopic enzyme-mapping verification of large, homogeneous, experimental myocardial Infarcts of predictable size and location in dogs. *J Thorac Cardiovasc Surg.* 1975 Apr;69(4):599-605.
6. Nydick I, Wr6blewski F, and Ladue JS, *et al.* Evidence for increased serum glutamic oxalacetic transaminase (SGO-T) activity following graded myocardial infarcts in dogs. *Circulation.* 1955;12:161-8.

7. Redetzki M, Ruskin A, Nowinski W, *et al.* Changes in enzyme activity (glutamic oxaloacetic transaminase, lactic dehydrogenase, cytochrome C and cytochrome oxidase) in serum and heart muscle after experimental myocardial infarction in the dog. *Texas Rep Biol & Med.* 1958;16:101-115.
8. Rueggsegger P, Nydick I, Freiman A *et al.* Serum activity patterns of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and lactic dehydrogenase following graded myocardial infarction in dogs. *Circulation res.* 1959;7:400-410.
9. Jennings RB, Kaltenbach JP and Smetters GW, *et al.* Enzymatic changes in acute myocardial ischemic injury. Glutamic oxaloacetic transaminase, lactic dehydrogenase, and succinic dehydrogenase. *Arch Path.* 1957;64:10-16.
10. Hamolsky MW and Kaplan NO. Measurements of enzymes in the diagnosis of acute myocardial infarction. *Circulation.* 1961;23:102-10.
11. Strandjord PE, Thomas KE and White IP, *et al.* Studies on isocitric and lactic dehydrogenases in experimental myocardial infarction. *J Clin Invest.* 1959;38:2111-118.
12. Graves MD, Koepke JA, Lafond DJ *et al.* Myocardial enzymes in myocardial infarction. *Am J Clin Path.* 1962;37(16):282-88.

