Post-mortem Gross Detection of Early Myocardial Infarction using Triphenyl Tetrazolium Chloride Test

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Abstract

Background: Ischemic heart disease is the leading cause of mortality in the world. More than 17 million people die each year as a result of myocardial infarctions. Post-mortem histochemical detection of early myocardial infarction using 2,3,5-Triphenyl Tetrazolium Chloride (TTC) technique is based on the fact that ischemic myocardial cells lose their membrane integrity and release dehydrogenase enzyme into the blood stream, resulting in decrease or total depletion of these enzymes within the necrotic areas of the myocardium, and so, after immersion in TTC solution, enzyme depleted necrotic myocardium will not retain TTC dye and therefore appears as pale/yellow areas. **Materials and Methods:** This prospective postmortem study was conducted at Medicolegal Directorate in Baghdad city during the period from March 2020 to December 2020. Myocardial specimens were isolated from 75 cases of sudden natural death and were subjected to 1% TTC solution staining for gross assessment, and subsequently, tissue samples were taken from suspected areas, processed and stained with H&E for histopathological evaluation and detection of a possible acute myocardial infarction. **Results:** The mean age of studied cases was 50.3 ±12.57 years, ranging from 18 to 78 years, median age was 53 years. Males constituted the majority of cases (80%). Cases were predominantly within 40-59 years age group (58%). The postmortem interval was ranging from 2 to 17 hours (mean= 8.6 ±3.6 hours). After immersion in 1% TTC solution, 45 myocardial specimens showed macroscopic pale/yellow areas which were indicative of acute myocardial infarcts within the ventricular wall, while histopathological examination of specimens revealed features of acute myocardial infarction (AMI) in 62 specimens. TTC stain was found to have a diagnostic sensitivity of 69.4% and specificity of 76.9% in postmortem detection of AMI. **Conclusion:** The TTC technique allows identification of early myocardial necrosis. It's practical, reliable and valid method that promises to be of considerable value. It can be used together with histopathology for postmortem detection of visually unapparent acute myocardial infarcts.

Keywords: *TTC, myocardial infarction, sudden death, postmortem diagnosis*

Introduction

Ischemic heart disease (IHD) is considered as the main cause of natural deaths in both men and women. [(1)] Sudden natural deaths involve important challenges in forensic pathology, especially when death takes place shortly after coronary occlusion. Following myocardial ischemic damage, it usually requires 24 to 48 hours for the damaged area to be visually apparent, area of hyperemic borders with central tan softening maybe observed after 3-7 days. Wavy fibers, in association with interstitial edema are often described as the earliest light microscopic changes of necrosis as it can be detected in the infarcted region within 3 hours after coronary occlusion.[(2)] Triphenyl tetrazolium chloride (TTC) is commonly used in histochemical staining for detection of necrotic myocardial tissue, it's based on the fact that ischemic myocardial cells lose their membrane integrity and release their enzyme contents into the blood stream, resulting in a marked decrease or total depletion of these enzymes in the ischemic areas of the myocardium. The postmortem histochemical detection of myocardial

infarction is relied on immersion of myocardial specimens in TTC solution, intact myocardial tissue will be visualized as brick red while dehydrogenase deficient infarcted zones will not retain TTC stain and therefore appear grossly as pale/yellow areas[3]. The current study aim to explore the reliability of Triphenyl Tetrazolium Chloride staining in gross delineation of necrotic myocardium. Furthermore, to facilitate postmortem macroscopic recognition of early myocardial necrosis for subsequent histopathological evaluation.

Materials and Methods

This prospective postmortem study was conducted at Medicolegal Directorate in Baghdad city during the period from March 2020 to December 2020. Seventy five cases of sudden natural death were investigated for a possible underlying acute myocardial infarction (AMI) as a cause of death. On a hard dissecting platform, serial 10mm-thick sections of the heart was obtained using a dissection knife, each section was thoroughly examined in order to identify the ischemic risk zones, the territories of coronaries perfusion were examined, with careful appreciation to zones at risk. Slices were photographed using digital camera with magnification. Specimens with no visually apparent infarcted zones were taken from each heart, washed with distilled water, the slices were placed in a sealed container with 1% TTC solution and incubated in 38°C degrees; slices were turned upside down after 15 minutes to obtain a uniform staining. After incubation for 30 minutes, heart slices were photographed and examined for unstained zones within myocardium, normal myocardium stained brick red whereas infarct zones appear pale/yellow or show very much reduced staining. Pale unstained zones were regarded as indicative for AMI, whereas slices completely stained brick red regarded as negative for AMI. After macrostaining with TTC solution, both TTC positive and negative specimens were incubated in 10% formalin. Sections were mounted on labelled microscope slides for routine histopathological examination. Microscopic observations of ischemic changes including myofibrillar waviness, hypereosinophilia, coagulative necrosis, neutrophilic infiltration were considered as a diagnostic feature of AMI.

Results

This prospective study included 75 cases of sudden death autopsies conducted at Baghdad Medicolegal Directorate; their mean age was 50.3 ±12.5 years, ranging from 18 to 78 years, the median age was 50 years. Males constitute the majority of cases (n=60). Table 1 illustrates the frequency of cases according to age groups, 44 subjects were within 40-59 years age group and within this group, 35 subjects were males. Considering past history prior to death, 70.7% (n=53) of cases had at least a single pre-existing risk factor for developing IHD.

Table 1: Number of cases according to age groups.

Gender	Age Group 18-39	40-59	60-80	Total	Mean	SD
Male	10	35	15	60	50.87	13.2
Female	4	9	2	15	48.27	9.78
Total	14	44	17	75	-	

Approximate time of death was obtained from information provided by police autopsy inquest, in addition to the observed postmortem changes. The postmortem interval (PMI) was ranging from 2 to 17 hours (mean= 8.67 ±3.69).

Following immersion in 1% TTC solution, slices from 4 cases failed to retain the dye, and within the remaining 71 cases, 45 showed macroscopic pale/yellow areas which were indicative of acute myocardial infarcts within the ventricular wall (Figure 1). Microscopical histopathological examination of specimens revealed features of AMI in 62 specimens (Figure 2).

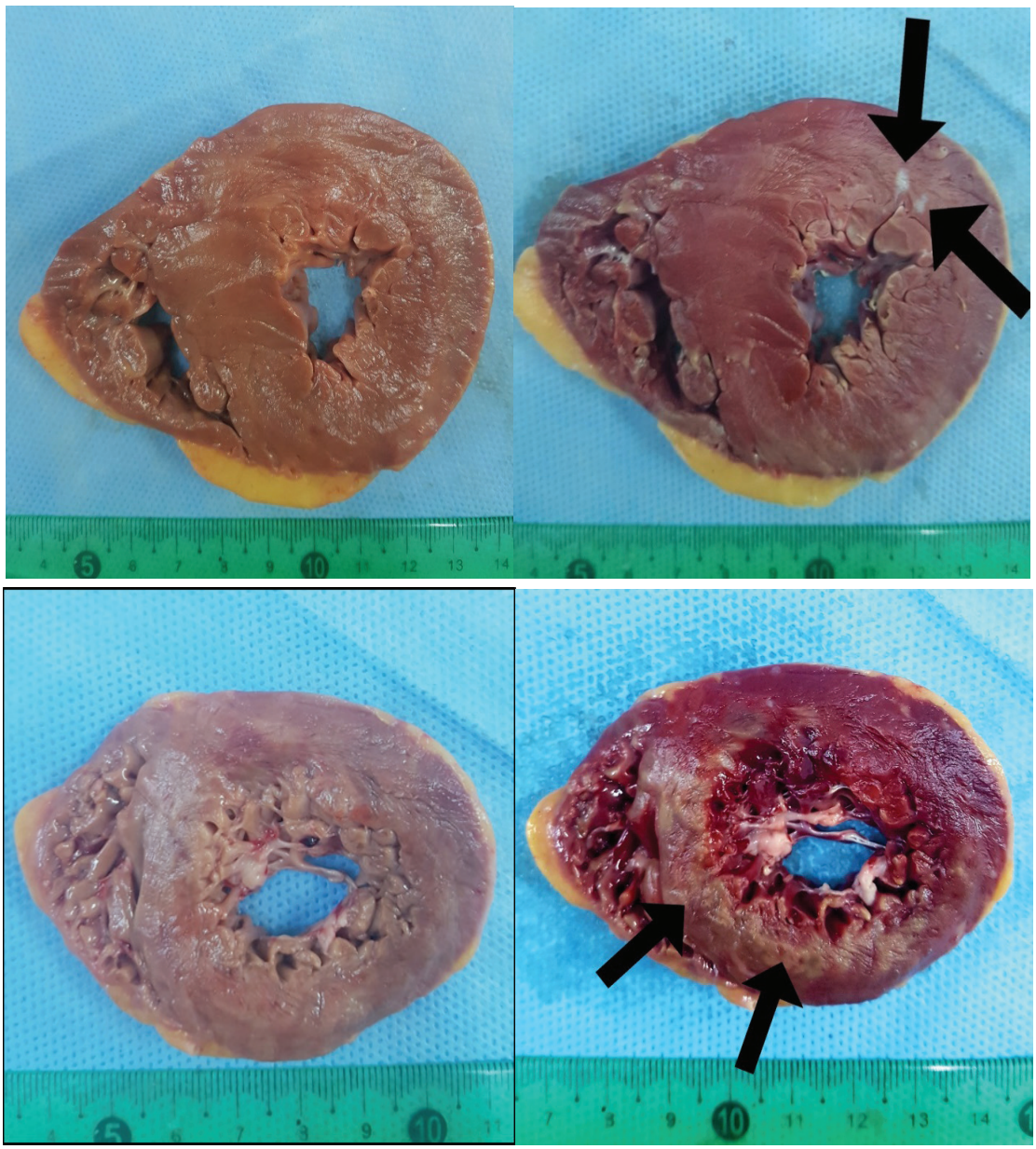

Figure 1: Triphenyl tetrazolium chloride (TTC) staining of transverse heart slices. Photographs on the left are myocardial sections before TTC staining. Same slices on the right after immersion in TTC solution showing areas of staining defect which are indicative of acute myocardial infarcts (arrows).

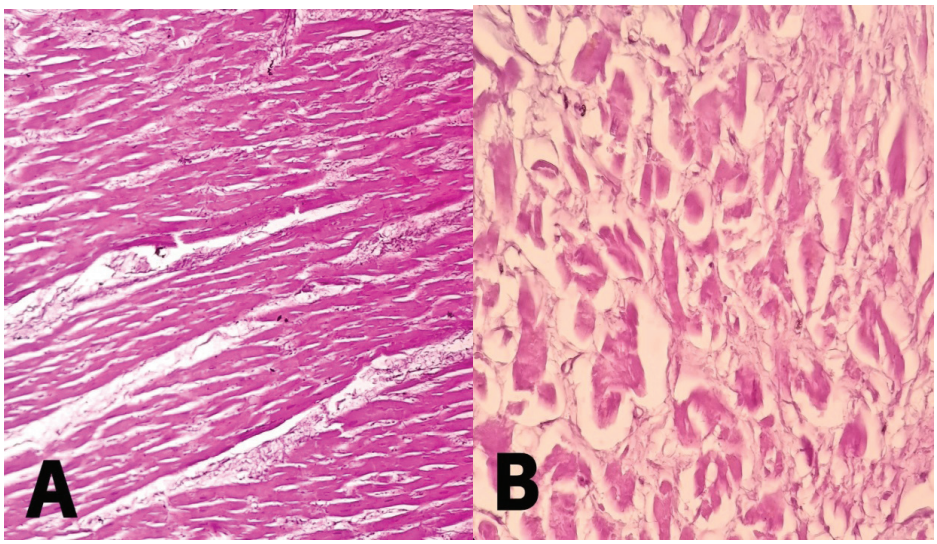

Figure 2: Photomicrograph showing acute myocardial infarction by H&E A. Interstitial edema with myofibrillar waviness (10x). B. Subendocardial MI showing vacuolated myocytes with coagulative myocytolysis (40x).

Table 2 summarizes the statistical values considering TTC and H&E staining of specimens. There was significant statistical differences (p value= 0.001). TTC stain was found to have a diagnostic sensitivity of 69.4% and specificity of 76.9%.

Considering the four specimens which fail to retain TTC stain, no significant correlations were evident when the stainability of TTC was compared with age of deceased's or the PMI. The final postmortem diagnosis was ruled as AMI in 62 cases (82%), other cardiac diseases in 6 cases and respiratory infections in 4 cases. AMI was more prevalent in male and more profound within 40-59 years age group.

Table 2: Comparative analysis between TTC and H&E staining methods.

Staining			Histopathology		Total
			Negative for AMI	Positive for AMI	
TTC Staining	No staining	Count	1	3	4
		100% within TTC	25%	75%	100%
		100% within Histopathology	7.7%	4.8%	5.3%
	Negative for AMI	Count	10	16	26
		100% within TTC	38.5%†	61.5%	100%
		100% within Histopathology	76.9%§	25.8%	34.7%
	Positive for AMI	Count	2	43	45
		100% within TTC	4.4%	95.6%‡	100%
		100% within Histopathology	15,4%	69.4%*	60%
Total		Count	13	62	75
		100% within TTC	17.3%	82.7%	100%
		100% within Histopathology	100	100%	100%

† Negative predictive value ‡ Positive predictive value * Sensitivity § Specificity

Discussion

Triphenyl tetrazolium chloride (TTC) staining is a convenient procedure for detection and demarcation of myocardial infarct zones. It's dependent on loss of dehydrogenases from the irreversibly damaged myocytes. In this study, MI was the leading post-mortem diagnosis of natural death autopsies and was more prevalent in 40-59 age group which is consistent with recent studies conducted in Iraq by Al-Qazzaz *et al*. (2012)[4] and Abdulrahman (2016).[5]

More than 70% of the examined individuals in this postmortem study had at least single risk factor for developing IHD. A review study by Aljefree and Ahmed (2015) showed high prevalence of risk factors for developing cardiovascular diseases in the countries of The Gulf Cooperation Council, which was attributed to urbanization, cultural, lifestyle, and environmental factors in these countries.[6] In Iraq, however, bad social and dietary habits, lack of awareness, poor preventive measures might be crucial contributing factors.

The main concept of this study is the visual macroscopic detection of AMI using TTC regardless the size and extent of necrotic area, a study by Kakimoto *et al*. (2013) relied on computerized software planimetry to detect minute foci of infarction as well as to quantify the size and ratio of infarct zones in comparison to the remaining viable ventricular mass.[7]

As summarized in Table 2, the comparative analysis of histopathological examination with TTC staining technique in the present study showed that out of the total 75 cases, AMI was evident in 62 specimens by H&E, and out of these cases, it was observed that specimens of 43 cases showed macroscopic demonstration of AMI by TTC staining technique and 16 specimens were TTC-stained negative for AMI. The overall TTC sensitivity and specificity were 69.4% and 76.9% respectively.

Adegboyega *et al*. (1997) reported that histochemical staining using TTC solution for gross detection of early infarction has diagnostic sensitivity of 77%.[8] A review article by Kundal *et al*. (2012) showed that the overall efficacy of TTC test in postmortem diagnosis of AMI is 88%.[9]

Another study by Shrigiriwar *et al*. (2019) had noticed that TTC staining technique is more superior to the conventional H&E staining in detection of AMI, the study included known cases of MI and IHD. TTC staining technique was positive for MI in 80% of cases while H&E method was positive in 33.3% of cases, the study further recommended the TTC staining technique to be included in natural deaths autopsies as it was considered more sensitive and efficient method than the H&E microscopic examination.[10] These results support the previous findings by Gupta *et al*. (2013) who suggested that TTC stain has a higher efficacy than microscopical evaluation by H&E in detection of AMI.[11]

Unfortunately till date, there is no local published data considering the use of TTC technique in postmortem practice, and it's well known that postmortem diagnosis of AMI is essentially relied on histopathological examination by H&E to define necrotic zones, but unapparent infarcted zones are often missed during random sectioning.

There were 26 cases that showed no evidence of AMI by TTC staining even though H&E examination identified AMI in 16 cases in this group. Some studies have questioned whether staining of viable cells can mask necrotic ones in the highly irregular border zone. It is known that the formazan compound of TTC is lipid soluble and readily diffuses to adjoining cells/tissues, especially those rich in lipids, therefore necrotic tissue in the TTC stained slices might be estimated as being smaller than their actual size.[12]

Some observations suggest that TTC staining may be subjected to artefacts, it appears that superoxide dismutase has the potential to enable necrotic myocardium to retain its ability to stain as viable tissue for at least 24 h after the onset of the ischemic insult followed by reperfusion.[13-15] It's possible that myocardial cells, which have become irreversibly injured but have not yet lost a sufficient amount of NADH will be stained,

therefore, infarct size will be underestimated.

Following immersion in TTC solution, only 4 specimens fail to retain the TTC dye, this could be attributed to PH of the solution, a precise PH range of 7.4 is required for proper tissue staining. Photosensitivity of the Tetrazolium dye, prolonged exposure of TTC salts or TTC solution to light leads to inactivation of the tetrazolium salt. Another factor that may compromise the efficacy of the test is the prolonged storage of TTC salts.[16] Kakimoto *et al*. (2013) found that TTC stainability decrease logarithmically when PMI is more than 1.5 days. Another limitation was the decrease in TTC staining quality for aged patients (more than 80 years).[7] In the current study, however, no correlations were evident between TTC staining results and age or PMI in this study.

Conclusions

The overall Triphenyl Tetrazolium Chloride (TTC) sensitivity and specificity in this study were 69.4% and 76.9% respectively. TTC technique allows identification of early myocardial necrosis. It's practical, reliable valid method that promises to be of considerable value. Random sectioning of heart for histopathological examination often miss the necrotic zones if the section does not include the unapparent infarct area, in these instances, TTC can be used together with histopathology for detection of early and unapparent myocardial infarctions.

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