Original Article

Role of Triphenyl Tetrazolium Chloride to detect early myocardial ischemia in sudden death cases

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ABSTRACT

Background: Coronary artery disease is the most frequent cause of sudden and unexpected deaths which constitute a significant portion of the autopsies that are conducted. In most of them we do not find any positive evidence of early infraction. So we cannot give cause of death in those cases.

Aims: To diagnose ischemic changes in heart of such cases of sudden deaths by TTC.

Materials & Methods: The present study was carried at the Department of Forensic Medicine Shri M. P. Shah Govt. Medical College, Jamnagar during the period of December 2010 to August 2012. In present study 130 sudden deaths cases were included. The heart was examined in these cases for gross [before and after Triphenyl Tetrazolium Chloride (TTC) test] and microscopic [Hematoxylin & Eosin (H&E)] staining for any changes of ischemia in relation to survival time.

Results: TTC test was positive in 26(20%) case out of which 12(46.6%) cases were only TTC positive and remaining 14 (53.8%) cases were positive for both TTC and H&E staining. Total H&E positive were 17(13%) cases out of which 3(17.6%) cases were showed H&E positive but TTC negative. 21cases (16.1%) showed ischemic changes by TTC those had postmortem interval <6hours, and no any case showed TTC positive which had postmortem interval >12 hrs.

Conclusion: An attempt should be made to establish TTC testing at all the hospitals where the postmortem examinations are conducted. The technique of TTC is quite simple and easy to perform and reasonably cheap.

Key words: TTC, H&E, Myocardial ischemia

INTRODUCTION

Coronary artery disease is the most frequent cause of sudden and unexpected deaths which constitute a significant portion of the autopsies that are conducted by Forensic Medicine Department in our country [1-5].

Macroscopically atheromaous area looks like raised yellowish plaque. Initially the lesion is focal then become confluent, mostly eccentric thickening of wall then whole lumen is involve [4, 6]. The following features are characteristic of infarction in sections stained by haematoxylin and eosin, Eosinophilia, swelling of muscle-fibers, granularity of cytoplasm, blurring of cell membranes, corrugation of dead muscle fibers, increase in interstitial cells, which occurs at about 18 hours [4,6]. The significance of these changes is difficult to interpret due to uncertainity about the time of onset. Increased eosinophilia can be seen at 4-6 hours but even in the most favorable circumstances, a firm diagnosis of infarction less than 6 hours old cannot be made from sections stained with haematoxyline and eosin [4,6].

The first diagnosis may be obtained in the 8 to 12 hour range, though again this limit may be considerably extended. At the lower end of this time limit the changes are too vague to be reliable, sometimes the experienced observer may strongly suspect infarction, but the changes are so slight and subjected to postmortem autolysis that they are unreliable. Apart from this unreliability, the histological methods are long-drawn by the fact that they take a long time for their preparation, fixation and processing, as a result of which it may be difficult to apply these methods in routine postmortem work,

where the report is expected immediately after conclusion of the postmortem examination. Moreover the actual site of the infarction may be missed while taking sections from the heart musculature for histological examination in the absence of gross localizing techniques [4, 6, 7].

With these considerations, it was thought worthwhile to study the myocardial ischemic changes, by gross histochemical staining methods which were chosen because they can detect early ischemic changes. The histochemical method for studying the enzyme reaction in the myocardium, to differentiate between the healthy myocardium and the infarcted myocardium is by far the most sensitive method for diagnosing early myocardial ischemia, is comparatively simple as compared to other methods used and can be performed with a minimum of apparatus in the postmortem room [4, 8-15].

MATERIALS AND METHODS

The present study was carried at the Department of Forensic Medicine Shri M. P. Shah Govt. Medical College, Jamnagar during the period of December 2010 to August 2012. Post Mortem study was carried out in cases of sudden death. Total 130 cases were studied. Cases of decomposition were excluded from study.

Naked eye examination:
Dissected hearts were examined by naked eye examination for atheromatous changes, stenosis, thrombosis and calcification of coronary arteries, stenosed coronary arteries graded 1, 2, 3 or 4 depending on % of blockage coronary arteries [16]. Myocardium was examined for gross changes of infraction like softening, hyperemia and white patches.

TTC Test:
We did slicing of heart of approximately 1-1.5 cm size from apex of heart to atrio-ventricular junction.

We prepared a solution by adding 10 gm of Triphenyl Tetrazolium Chloride (TTC) solution in 100ml of phosphate buffer at ph 8.4 in to the screw caped wide mouth bottle in which slices of heart were kept. It was ensured that all the slices should be dipped in to the solution properly. Then the bottle was kept at 30-40^{o}C in incubator for 25-30mins, in between the time slices would be turned once or twice.

Interpretation of TTC test:

After staining of myocardium those areas were pale (gross ischemic changes detected) that labeled as ischemic myocardial areas (TTC test positive) and those staining bright red they were labeled as normal myocardium (TTC test negative). If white color seen it was labeled as old infarct or scars [4, 6].

Microscopic examination [conventional H&E staining]:
Myocardium was examined for any changes of ischemia by conventional histopathological examination using H&E stain. Any changes of ischemic myocardium seen microscopically were labeled as H&E positive. The pale and white areas of myocardium were selected for making sections [4, 6].

RESULTS

Out of the 130 cases studied 99 (76.2%) were males and 31(23.8%) were females. Out of 130 cases 26(20%) showed TTC test positive, from which 23(17.7%) were male and 3(2.3%) were female. Maximum numbers of TTC test positive cases (7.6%) were seen in age group 41-50 years. Cases aged < 50 years were 90 (69.3%) out of which only 12 (9.3%) TTC test positive and cases were aged >50 were 40(30.7%), out of which 14(10.7%) cases showed TTC test positive (Table 1).

Table 1: Age and sex wise distribution of cases

Age group (yrs)	Males (%)	Females (%)	No. of cases (%)	TTC test +Ve cases (%)
<10	1(0.8%)	0(0%)	1(0.77%)	0(0%)
11-20	3(2.3%)	5(3.8%)	8(6.1%)	0(0%)
21-30	11(8.5%)	11(8.4%)	22(16.9%)	0(0%)
31-40	23(17.7%)	5(3.8%)	28(21.5%)	4(3.1%)
41-50	27(20.8%)	3(2.3%)	30(23.07%)	10(7.6%)
51-60	16(12.3%)	6(4.6%)	22(16.9%)	6(4.6%)
61-70	13(10%)	0(0%)	13(10%)	3(2.3%)
71-80	4(3.1%)	0(0%)	4(3.07%)	3(2.3%)
81-90	1(0.8%)	0(0%)	1(0.77%)	0(0%)
Total	99(76.2%)	31(23.8%)	130(100%)	26(20%)

Most of the cases presented with survival time >4 hours. Only 2 cases showed TTC test positive which had survival time <4hours. Out of 26(20%) cases showed early infarction by TTC test 22(16.9%) cases had survival time of 4 to 8 hours. The period of survival was not known in 34(26.2%) cases. Earliest survival time was 2hour that showed TTC test

positive. 21cases (16.1%) showed gross ischemic changes those had postmortem interval <6hours and no any case showed TTC positive which had postmortem interval >12 hrs. Highest postmortem interval was 12hours which showed TTC test positive (Table 2 & 3).

Table 2: Correlation of period of survival and gross ischemic changes by staining

Period of survival	TTC test +Ve (%)	No. of cases (%)
<4hrs	2(1.5%)	52(40%)
4-8 hrs	22(16.9%)	33(25.3%)
>8 hrs	2(1.5%)	11(8.5%)
Not known	0(0%)	34(26.2%)
Total	26 (20%)	130(100%)

Table 3: Correlation of postmortem interval and gross ischemic changes by staining

Post mortem interval	TTC test +Ve (%)	No. of cases (%)
<6hrs	21(16.1%)	72(55.3%)
6-12hrs	5(3.9%)	30(23.1%)
>12hrs	0(0)	28(21.6%)
Total	26(20%)	130(100%)

Table 4: Correlation of maximum grade of stenosis and gross ischemic changes detected by staining (n=130)

Artery	Maximum grade of stenosis (Grade:3&4) No. of cases*		TTC test +Ve* (%)	No. of cases* (%)
	Grade:3	Grade:4		
R1 (RM)	5(3.9%)	4(2.3%)	8(6.1%)	9(6.9%)
R2 (RPD)	3(2.3%)	0(0%)	2(1.5%)	3(2.3%)
L1 (LM)	6(4.6%)	10(7.6%)	10(7.7%)	16(12.3%)
L2 (LAD)	5(3.9%)	27(20.8%)	23(17.7%)	32(24.6%)
L3 (LC)	5(3.9%)	4(2.3%)	7(5.4%)	9(6.9%)

* multiple response

Table 5: Correlation of gross changes and staining methods (n=130)

Sign of fresh Infarction	No. of cases*	TTC Test Positive*	H&E Positive*
Hyperemia	4(2.3%)	4(2.3%)	4(2.3%)
Softening	9(6.9%)	9(6.9%)	4(2.3%)
Total	13(10%)	13(10%)	8(6.1%)

* multiple response

Incidence of stenosis was more in left anterior descending artery (LAD) (32.3%) followed by left main (LM) (27.7%) and right main (RM) (22.3%). Maximum grade of stenosis were seen in total 69 (53.1%) in which 24(18.5%) cases showed grade: 3 stenosis and 45(34.6%) cases showed grade:4 stenosis. 32(24.6%) cases were showed stenosis in L2 and 23(17.7%) of them showed TTC test positive. Only 3(2.3%) cases were showed maximum grade of stenosis in R2 out of which 2 showed TTC test positive. Complete occlusion of the lumen with pin point lumen was encountered in LAD (L2) in 2 cases (Table 4).

Total 13 cases showed gross areas of fresh infarction before TTC test by naked eye examination, in which 4cases showed hyperemia, 9 cases showed softening. Staining demarcates the infarcted areas and they were found to be more extensive than supposed by gross examination. In this study total 26(20%) cases showed TTC test positive, so other 13 cases did not reveal any evidence of fresh infarction before TTC test, which were revealed only after TTC staining. Fibrotic areas of old infarct were seen in 3 hearts and they showed white patch at fibrotic areas (Table 5).

Table 6: Correlation of tests (n=130)

Test	No. of cases* (%)
Only TTC +Ve	12(9.2%)
Only H&E +Ve	3(2.3%)
Both TTC and H&E	14(10.8%)

* multiple response

Out of 26 cases (20%) of TTC test positive, 12(46.2%) cases were only TTC positive and 14 (53.8%) cases were both TTC and H&E both staining positive. Total H&E stain positive were 17(13%) cases out of which 3(17.6%) cases were showed only H&E positive but TTC negative (Table 6).

DISCUSSION

In this study maximum number of coronary lesion cases were found in age group 5th and 6th decade, this findings corroborate with findings of Gupta et al [2], Sharma et al[7], Revathi et al [14], Gohel et al [15], Ahmad et al. [17] While Chen and Huang [9] said that it was common in 4th and 5th decade. This age difference may be due to different geographical region and environmental factors like dietary habits.

In this study we had found male cases more who showed ischemic changes they were 23(17.7%) and only 3(11.5%) were females. Which is consisting with the findings of Sharma et al [7], Revathi et al [14] and

Gohel et al [15]. This is due to male cases are more at autopsy and males have more stress, smoking habits.

Another issue to be considered was the detection of myocardial ischemia in relation to the period of survival and also whether these techniques which outline myocardial infarctions, before the gross or microscopic changes become manifest, can be used to assist pathologists in the recognition of early or small infarctions in the human subject. In our study we found earliest infraction at survival period of 2 hours by TTC method, which was more or less consistent with work of Andersen and Hansen [13]. They said Nitro B.T. detected localized subendocardial infarctions at 1 hour duration of survival. Most of other studies did not coincide with our study. Kotabagi et al [10] said that TTC test was useful when survival time was $5^1/_2$ hours. Revathi et al [14] found that TTC test may be useful when survival period was >8hours. Vivaldi et al [19] found that 48hours after coronary artery ligation (in rats), infracted margins were macroscopically sharp, and the enclosed area were homogeneous, as determined both by TTC and histological examination. Ramkisoon [11] employed Nitro BT method and found earliest infarct showing loss of dehydrogenase activity in < 8 hours old. McVie [12] used Nitro B.T. method and found that earliest infarction revealed by staining was 3½ hours duration. Nachlas and Shnitka [20] used Nitro BT, which showed that 30% of their cases developed homogeneous staining reactions, but failed to show gross or microscopic evidence of recent muscle necrosis. The earliest infarction in humans by this method was of 8 hours duration. Wachstein and Meisel [21] found that changes of infarction did not become apparent for some 24 to 48 hours following the occlusion of a major coronary artery in man or 8 to 24 hours in experimental animals.

In our study considering the validity of the enzymatic staining reaction in relation to the increasing postmortem interval, where the bodies were stored between 2^oC to 4^oC, the 130 hearts subjected to the staining gave equal deposition of the dye, with same intensity and sensitivity from 1 hours and 20minutes to 12 hours of post-mortem interval. In this study we had no such case which had postmortem interval >12hour and showed TTC test positive. Ramkissoon [13] and Nachlas and Shnitka [20], who were able to get positive cases by applying the methods till 48 hours after death and bodies being kept at 4^oC. while Andersen and Hansen[13], got positive result up to 72 hours after death on bodies kept at 4^oC, and Revathi et al[14] found that TTC test may be useful in cases up to 36 hours if not refrigerated and 60 hours if refrigerated. This is probably due to fact that enzymes inactivation is not affected by decomposition changes till 36-60 hours of post mortem interval.

In this study most common artery involved for stenosis was LAD in 42 (32.3%) cases which were consistent with findings of Sharma et al [7], Chen and Huang[9], Davies[22] and Ahmad et al [17].

The cases which had ischemia could be divided into 2 groups, one group with high (maximum) grades of arterial stenosis and the other with lower grades. We had found that maximum grade of stenosis seen in LAD in 32(24.6%). This is consistent with most of studies [7,13]. Out of 32 cases, 23(70%) showed TTC test positive this was consistent with finding of Chen and Huang [9] and Revathi et al [14]. This was explained by the fact that if there was increases grade of stenosis there would be decrease in the blood to myocardium and so less perfusion to that area and therefore ischemia can occur easily.

Another objective of the study was to correlate myocardial ischemia detected with the grade of stenosis in the coronaries. This study also indicates a strong correlation between the arterial lesions of coronary atherosclerosis and ischemic heart disease in that both the incidence and severity of atherosclerotic lesions were greater in the hearts that showed ischemic changes, which was consistent with work of Sharma et al [7] , Chen and Huang[9]. Strong and McGill [16].

Most of the authors [9-12, 15, 20, 21] did not give the percentage of cases of the fresh infraction. In our study we found fresh infraction in 10% cases which was corroborative with Kotabagi et al [10] While Chen and Huang [9] found recent infraction in 2.2%cases, Ahmad et al [17] found recent infraction in 20.8%. None of the studies [9-12, 15, 20, 21] had mention about softening of myocardium by naked eye examination before TTC staining. While we had found that in 9(6.9%) cases. Hyperemia found in 4 (2.3%) cases (which all showed TTC test positive) while Sharma et al [7] found only in 1 (3.8%) case. This difference in percentage may be due to number of cases studied and survival period.

In our study fibrosed myocardium was found in 11.5% cases (which all showed TTC test positive), Sharma et al [7] found it in 23% cases. While Ahmad et al[10] found old infraction in 35.1% of their cases. That

difference in percentage may be due to number of cases studied and survival period. Hyperemia and fibrosis seen in 5th decade that was corroborative with findings of Sharma et al [7].This is due to other risk factors like age.

Present study reveals that we can detect more number of fresh infarction by using TTC that cannot be detected by naked eye or microscopically which is consistent with findings of Revathi et al [14].

Microscopically by H&E stain changes in myocardium seen earliest at 6hours of survival in our study which were not consistent with findings in most of studies [15, 16]. In our study we found 13% cases which showed microscopic (H&E stain) positive ischemic changes which is consistence with work of Sharma et al [7] and not corroborative with findings of Chen and Huang[9]and Gohel et al [15]. That may be due to variation in number of cases studied and difference in the survival time of cases. So this method of staining is not important for early myocardial ischemia detection, for that we can use TTC macro test which is gross and easy method.

Out of the TTC and H&E methods, TTC can detect infraction in those cases had survival time >4hour [10,12,19]. While for microscopic detection of infarction by H&E stain survival time of at least 6 hours was necessary. TTC is simple method while H&E method is difficult. So we can use TTC test at local level for detection of early myocardial ischemia. [7,12].

CONCLUSION

Gross staining by TTC revealed fresh myocardial infarction which had not been suspected at the ordinary macroscopic or microscopical evaluation (H&E). This indicates the use of TTC test in the detection of early myocardial ischemia in sudden death cases. We found that when we apply TTC test in sudden death cases the chances to detect early myocardial ischemia are increased. So we can give cause of death in those cases more positively. If we take section from the pale (ischemic) area of myocardium after TTC test chances to get positive H&E results would also increase. An attempt should be made to establish TTC testing at all the hospitals where the postmortems are conducted. The technique of TTC procedure is quite simple and easy to perform and reasonably cheap.

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