EARLY DIAGNOSIS OF MYOCARDIAL INFARCTION DURING AUTOPSY

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ABSTRACT

Objective: To determine the usefulness of Acridine Orange Fluorescence Test and Macro test for detection of infract in its early stages during autopsy.

Material and Method: The study was conducted in histopathology section of Forensic Medicine department Khyber Medical College Peshawar. 18 cases of myocardial infarction were selected. For standardization of negative control 10 cases of sudden unexpected death were also included. Triphenyl Tetrazolium Chloride (TTC) Macro Test and Acridine Orange Fluorescence (AO-F) test used for staining of tissue for microscopic examination of infarcts. 10 histologically diagnosed cases of Myocardial infract were used as a positive control. The study was done for a period of three months that is Jan-Mar 2019.

Results: In this study it was found that the AO-F stain and TTC Macro test can detect any infract (both old and fresh). In case of AO-F, infracted area shows green florescent color under florescent microscope. Dark color indicated old infract. AO-F stain can detect infract as early as less than 2 hours old. Whereas TTC can detect infract as early as 4hours old. Both these test are simple, cost effective and give accurate results. These can help to determine exact cause and time since death especially in cases of sudden unexpected death.

Key Words: Acridine Orange Fluorescence, Age of infarct, Chloride macro test, Myocardial infarct, Triphenyl Tetrazolium chloride macro test

INTRODUCTION

In most of the sudden unexpected deaths myocardial infarction is considered as a primary diagnosis on gross examination of coronary artery embolism and ischemic changes in the sliced heart tissue[1]. This diagnosis is not based on histopathology of cardiac tissue. In most of the cases diagnosis is based on naked eye examination of the infracted area in the heart and presence of thrombus in the coronary vessels. However this is not true for all the cases. In all such undiagnosed cases where naked eye examination fails to reveled MI the tissue is subjected to microscopic examination. The coronary vessels and myocardial tissues are examined under microscope after processing through Hematoxylin and Eosin (H&E) Staining.[2] The limitation of H&E staining is that, it can detect infract with survival time more than 6 hours. It means that H&E staining gives positive results only if the patient survived more than six hour after ischemic heart attack. In cases of death with less than six hour we need some other test for the detection of Ischemic changes.[3] The aim of the current study was to explore the usefulness of Acridine Orange Fluorescence test and Triphenyl Tetrazolium chloride (TTC)(macro test) for detection of infract of lesser duration (4-6 hours). Triphenyl Tetrazolium chloride test (TTC) can detect MI of 4-5 hours of age and staining (AO-F) can detect MI where survival time is less than 2 hours[2,3].

Material and Methods: The gross examination of heart was carried on 77 cases during routine postmortem examination. 18 cases of myocardial infarction were selected for the study. Inclusion criteria: All cases with a history of sudden unexpected death were included. Ten diagnosed cases of Myocardial infarction were also included in the study as control (with H&E staining positive for MI) The study was carried out at Khyber Medical College histopathology section of Forensic Medicine department. The samples of Cardiac tissue were stained by all the three methods that include hemetoxyline and eosin (H&E), staining, Triphenly Tetrazolium Chloride (TTC) (macro test) and Acridine Orange Florescence (AO-F) stains.

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TTC Macro Test

Triphenyltetrazolium chloride (TTC) is one of the most conventional stains to detect infracted area of the heart in animal experiments. Preparation Method: one centimeter cubic thick slice of cardiac tissue was dissected from a ventricular part of heart the slice was incubated in 1% solution of Triphenyl Tetrazolium Chloride (TTC) for 20-30 mins at 37-40 °C[4] After staining the sliced specimen was dipped in 10% formalin that causes fixing of the tissue. On completion of the staining process the gross examination of the tissue showed pale pink colored area for infracted myocardium and bright red color for normal myocardium. There is uniform bright red coloration of the sliced tissue in the absence of infarct[5]. An infract can be detected up to 12 hours after death Apoptosis starts and dehydrogenese enzyme activity disappeared after 10-12 hours[5, 11] .

Acridine Orange Fluorescence (AO-F) Study

Significance of this test was in detecting early myocardial ischemia and in demarcating zonal differences in ischemia. Method of preparation: Thin sections of Paraffin embedded myocardial tissue from areas containing infarcts were soaked in 0.01% acridine orange solution for 3 mins. The selected section was then washed with Phosphate Buffer Solution (PBS), and dipped in calcium chloride solution for 3 mins. Rewash the slide with Phosphate Buffer Solution (PBS). Now the slides were ready to be mounted on fluorescence microscope[6]. This study gave positive results for infract even with lesser duration i.e less than 2 hours. The visible green florescent indicates infract. The bright green florescent indicate older infract with visible damage; while light green color indicates infract of lesser duration. The results were positive for early infract where survival time of the patient after sustaining heart attack was less than two hours. The normal tissue gave golden brown florescence[7].

RESULTS

In sudden unexpected deaths (n=10) especially in younger age males 07 (70%) the diagnosis of myocardial infarction was very difficult especially when the myocardial infarction was in early stages. Out of 18 autopsies 10 (55.5%) cases of sudden unexpected death (SUD) had myocardial infarction on gross examination due to narrowing of one or more of the coronary arteries during autopsy. On histopathology the H&E stain was pseudo negative for infract while AO-F and TTC gave positive result. There was limitation of *haematoxylin* and *eosin* staining. It can stain older infract with extensive damage only. Myocardial infraction can be visible with H&E only if the patient survived at least six hours after the heart attack. Whereas H&E stain was pseudo negative in cases which were clinically diagnosed for acute MI with duration less than 06 hours. Out of 77 postmortem, eighteen cases (23.3%) with a history of heart attack were selected for the study. The survival time was between 1.5 to 12 hours were analyzed.

Of these 13 were males and five were females. Eleven persons belong to >60 years age group. Four persons were aged between 41 to 59 years. The remaining 3 persons age was less than 40 years.

The hearts were dissected by Four chamber cut method The cardiac tissue slices were stained with three methods (1) Hematoxiline & Eosin staining (2) Triphenyl Tetrazolium Chloride (TTC) and (3) Acridine Orange Fluorescence staining. The result obtained were as under Table -3

In three cases the survival time after onset of symptoms was between 2-4 hours. In two cases survival time was five and half hours after onset of symptoms. Out of these ten cases of SUD, two cases of lesser duration (survive less than 02 hours) showed acute MI when stained with acridine orange (AO-F) and showed

Age	Number of patients (n=18)
More than 60 years	11
41-59	04
Less than 40	3

Survival time	N=18
6 to 12 hours	8
5 to 6 hours	2
4-5 hour	3
2-4 hours	3
Less than 2 hours	2

Table 3: Shows visibility of infract after staining of cardiac tissue

Staining Color	Hematoxiline and Eosin Pink & blue	Acridine Orange Florescent Green colored	Triphenye Tetrazolium Chloride Light pink color
6 to 12 hours	+++	+++	+++
5 to 6 hours	Negative	+++	+
4-5 hour	Negative	+++	Negative
2-4 hours	Negative	++	Negative
Less than 2 hours	Negative	+	Negative

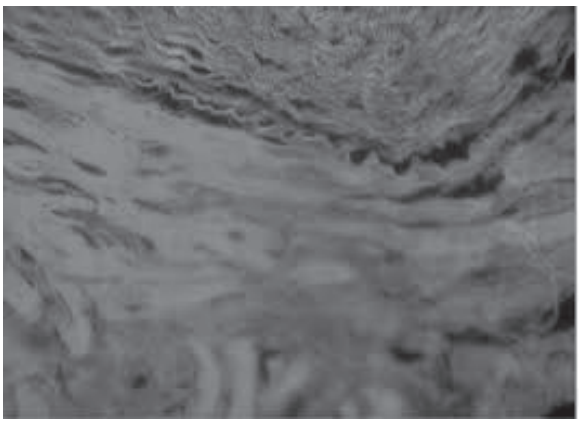

Figure 1: AO-F stained, Acute MI L.Green color

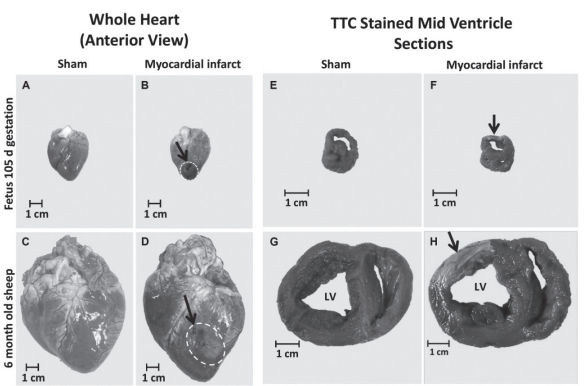

Figure 2: TTC stained MI, Pink color

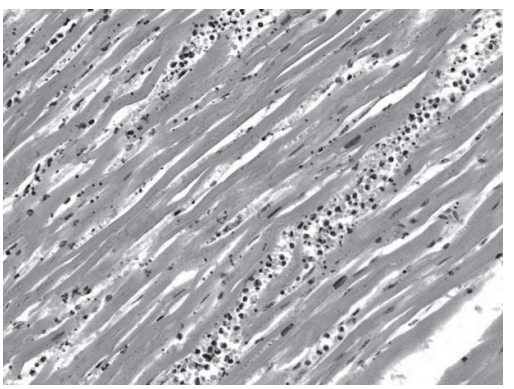

Figure 3: H&E stained, MI

no changes when stained with in H & E. Thus acridine orange staining helped to detect infarcts of less than two hours of age. Other 8 cases with older infarcts showed positive results with TTC, AO-F and H&E staining.

In cases of AO-F staining the normal tissue stained golden brown florescence while the color of infracts vary with the degree of injury and duration between onset and infraction. Early infarcts were of lighter green in color and older infarcts were bright green in color. The intensity of color brightness was directly proportional to the extent of damage.

The results of our study were significant and showed usefulness of TTC and acridine orange fluorescence, staining in detecting infarcts which are 'invisible' by H & E staining. Furthermore these tests were also showed positive results for older infracts.

DISCUSSION

One of the major objectives of medico-legal post-mortem is to determine the cause of death and estimate time since death. The diagnosis of the cause of death in sudden unexpected death cannot be determined by using hematoxylin and eosin[8]staining. Different studies have been conducted to study the biochemical changes at the site of injury. In case of ischaemia that last for at least 20 mins the cardiac tissue may undergo irreversible ischemic changes. These changes include microscopic and metabolic changes in cardiac tissue; this may lead to death of myocardial cells. The dead cells releases, K^+, Mg ions, enzymes and co-factors that diffuses out into the interstitial space and in to the main blood stream. This leakage of enzymes and ions can be detected by histochemical techniques[9].

The TTC reaction depends upon the activity of multiple dehydrogenase enzymes. Absence of the dehydrogenase enzyme activity leads to non-deposition of formalin pigments over the area of infarction. The dehydrogenas enzymes disappear from the infracted tissue as early as 10 to 12 hours after death. Presence of this enzyme indicates estimated time since death was between 4 to 10 hours. But in case of preservation its disappearance delayed upto36 to 60 hour after death[10]

The study has been able to establish the usefulness of the two procedures in diagnosing the infarcts of around 2 to 6 hours of age. The techniques of both the procedures were quite simple and easy to perform. The results in TTC can be visualized with naked eye; pale pink color indicates infracted area and bright red color indicate normal tissue. Whereas in AO-F staining the interpretation needs some experience to 'visualize' especially early infarcts[11]. The non-availability of U-V fluorescent microscope might become a limiting factor. But such equipment is readily available in all teaching institutions[12].

CONCLUSION

It was concluded from this study that the Triphenyl Tetrazolium Chloride Test and Acridine Orange Fluorescence study must be included in the study protocol of all sudden death cases. These tests have significance in diagnosis and estimation of time since death during autopsy. Their processing is easy and the results are rapid which can be visualized under florescent microscope. It is recommended that further studies are required to explore the other outcomes and utilities of these tests especially in our setup.

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