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Assessment of PTAH and Troponin-I in Detecting Early Myocardial Infarction Postmortem

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Abstract

Introduction: Early detection of myocardial infarction (MI) in post-mortem heart samples is essential for understanding sudden cardiac deaths and evaluating the accuracy of diagnostic markers. Phosphotungstic Acid Hematoxylin (PTAH) staining and Cardiac Troponin I (cTnI) immunohistochemistry are promising techniques for identifying myocardial injury in post-mortem cases. This study aims to assess the effectiveness of PTAH and cTnI in detecting early myocardial infarction in post-mortem heart samples.

Materials and Methods: This cross-sectional study included 82 post-mortem heart samples collected from the Department of Pathology, Government Medical College, Thiruvananthapuram. Samples were categorized into three histological groups based on the stage of myocardial infarction: early (Group 1), intermediate (Group 2), and late (Group 3). PTAH staining and cTnI immunohistochemistry were performed to assess myocardial injury. The relationship between histological stages and diagnostic markers was analysed using Chi-square tests.

Results: The study found that 67.1% of the samples had coronary artery disease (CAD). The majority of samples were in the intermediate stage (Group 2, 46.3%), followed by early (Group 1, 29.3%) and late (Group 3, 24.4%) infarction stages. PTAH staining showed positive results in 63.4% of cases, with a higher frequency of positive findings in early and intermediate stages. cTnI expression demonstrated a significant loss in early stages, with 57.3% showing moderate loss, while late stages exhibited milder reductions. A significant association was found between the histological stage of MI and cTnI loss ($p = 0.01$).

Conclusion: PTAH staining and cTnI immunohistochemistry are valuable diagnostic tools for identifying early myocardial infarction in post-mortem heart samples. cTnI, in particular, correlates strongly with the stage of myocardial infarction, making it a reliable marker for early detection in post-mortem diagnostics.

Keywords: Early Myocardial Infarction, Postmortem Diagnosis, Phosphotungstic Acid Hematoxylin (PTAH), Cardiac Troponin I (cTnI)

Introduction

Sudden cardiac death (SCD), defined as an unexpected natural death of cardiac origin occurring within one hour of symptom onset or, in some cases, without any symptoms, is a significant global health problem, accounting for approximately 7 million deaths annually worldwide [1]. The most common underlying cause is myocardial infarction (MI), which is frequently encountered in both clinical settings and forensic autopsies.

The postmortem diagnosis of acute myocardial infarction poses a major challenge in forensic pathology, particularly when death occurs within a short period (minutes to a few hours) after the ischemic event [2].

During the early stages (within 6 hours), the myocardium may not exhibit any gross or routine histological changes on haematoxylin and eosin (H&E) stained sections, thereby complicating the confirmation of MI [3]. Although the earliest microscopic changes, such as waviness of myocardial fibres, myocyte hyper eosinophilia, and nuclear pyknosis, may appear between 30 minutes to 4 hours after the insult, these findings are often subtle and non-specific [4].

In forensic practice, many sudden cardiac deaths are diagnosed based on indirect evidence such as critical (>75%) coronary artery stenosis, which alone may not suffice for a definitive diagnosis of MI [5]. More pronounced histological features, such as neutrophilic infiltration, typically appear only after 6–12 hours of survival post-insult [6]. Moreover, factors like collateral circulation and variable cardiomyocyte resistance to ischemia can further delay or obscure these changes [7].

Recent advancements in immunohistochemical (IHC) techniques have provided new opportunities for the early detection of ischemic injury. Markers such as fibronectin, CD59, myoglobin, troponin T, troponin I, desmin, and cathepsin S have shown potential in identifying early myocardial damage, particularly when conventional histopathology is inconclusive [8–10]. Among these, cardiac-specific troponins (I and T) are considered superior serological markers, rising within 4–6 hours after myocardial injury [11].

Interestingly, studies have suggested that troponin release may occur even in the absence of overt necrosis, due to mechanisms like apoptosis, necroptosis, and cardiomyocyte wounding [12]. Therefore, integrating immunohistochemical staining with traditional histopathological methods may enhance the diagnostic accuracy of early myocardial infarction in forensic settings, especially in sudden death cases lacking definitive gross or microscopic findings.

Materials & Methods

This cross-sectional study will be conducted in the Department of Pathology, Pushpagiri Institute of Medical Sciences & Research Centre, Kerala, India, over a period of 18 months from the date of approval by the Institutional Ethics Committee. The study population will include postmortem heart samples obtained from cases of sudden death, with or without evidence of coronary artery disease, received at the Department of Pathology, Government Medical College, Thiruvananthapuram.

Inclusion criteria will include all such heart samples meeting the above description, while exclusion criteria will consist of cases where myocardial infarction is the only identifiable cause of death and samples that are completely autolysed.

The minimum sample size was calculated using the formula for estimating sensitivity. The sensitivity of Troponin I immunohistochemistry (p) was assumed to be 90% (0.90), with an absolute precision (δ) of 15% (0.15), a prevalence of coronary artery disease of 18.7% (0.187), and a Z value of 1.96 corresponding to a 95% confidence level. Substituting these values into the formula yielded a minimum required sample size of approximately 82 postmortem heart specimens.

Ethical Clearance: Ethical clearance for the present study was obtained from the Institutional Ethics Committee of Pushpagiri Institute of Medical Sciences & Research Centre, Kerala (Ref No: PIMSRC/IEC/2024/3185). A detailed Participant Information Sheet was provided to all participants, and written informed consent was obtained prior to their inclusion in the study.

Statistical Analysis: Data will be entered into Microsoft Excel and analyzed using Statistical Package for the Social Sciences (SPSS) software version 22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics will be used to summarize demographic and clinical characteristics. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of Troponin I immunohistochemistry for

detecting myocardial injury will be calculated using 2×2 contingency tables. Chi-square test or Fisher's exact test will be applied to compare categorical variables, and a p-value of <0.05 will be considered statistically significant.

Results

Table 1: Age distribution among Study Participants

Age group in years	Frequency (n)	Percentage (%)
<20	1	1.2
21 – 30	5	6.1
31 – 40	11	13.4
41 - 50	22	26.8
51 – 60	18	22.0
61 – 70	15	18.3
71 - 80	6	7.3
>81	4	4.9
Total	82	100.0

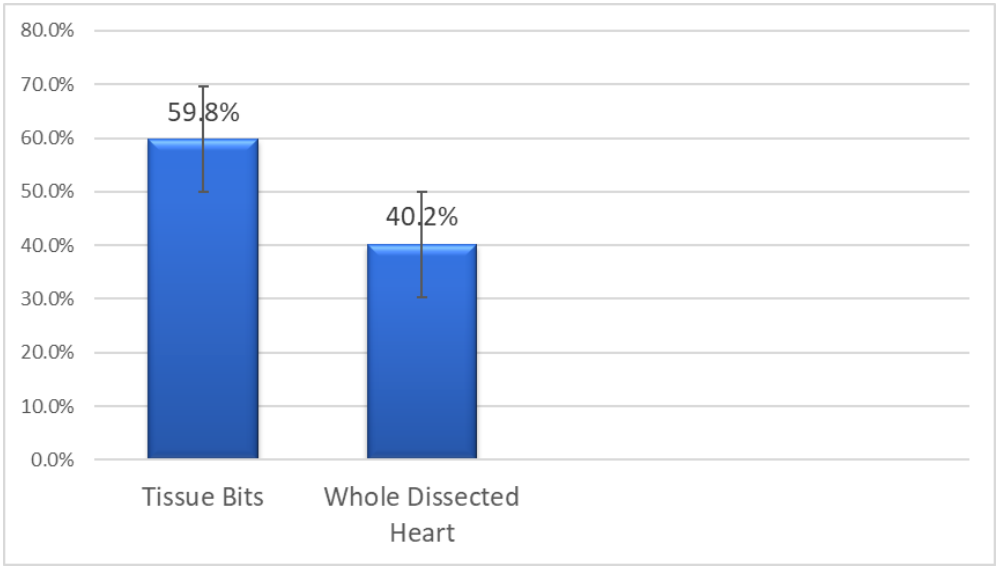
The study analyzed 82 post-mortem heart samples with a mean age of 52.3 years (range: 16–89). The majority were middle-aged adults (41–70 years), accounting for 67.1% of cases. The most common age group was 41–50 years (26.8%), followed by 51–60 (22%) and 61–70 (18.3%). Younger individuals (<40 years) comprised only 7.3%, while those over 70 made up 12.2%. This distribution reflects the age range typically associated with ischemic heart disease and myocardial infarction.

Table 2: Gender distribution among Study Participants

Gender	Frequency (n)	Percentage (%)
Female	25	30.5
Male	57	69.5
Total	82	100.0

Among the 82 post-mortem heart samples, 69.5% were from males and 30.5% from females, showing a clear male predominance. This aligns with the established trend of higher cardiovascular risk and incidence of early myocardial infarction in men, especially in middle-aged and older age groups.

Figure 1: Type of Specimen



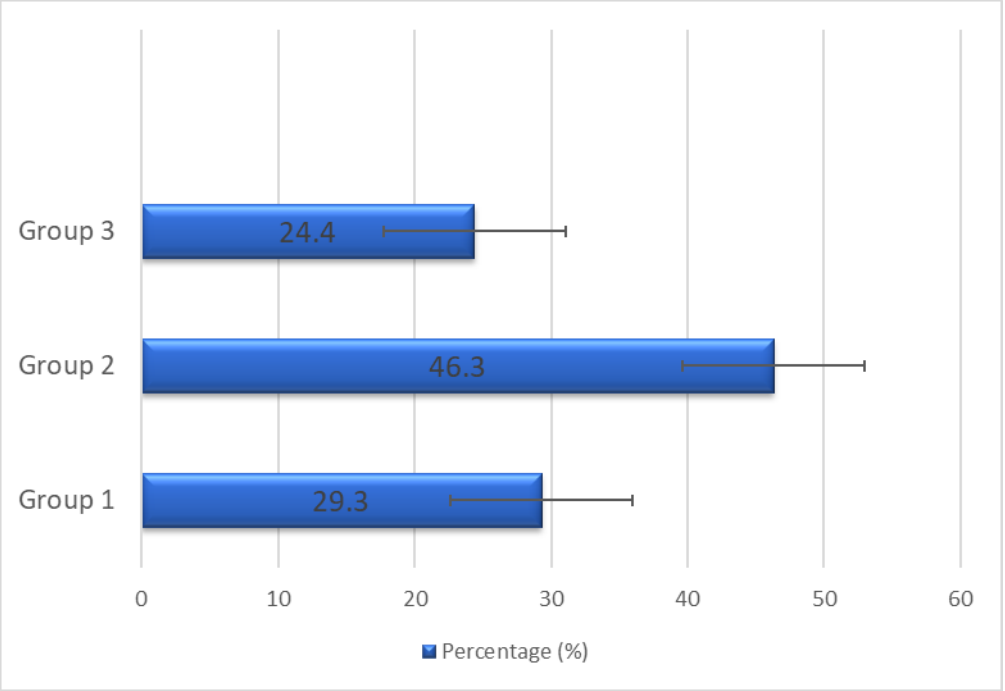
Of the 82 heart samples analysed, 59.8% were tissue bits and 40.2% were whole, dissected hearts. Tissue bits were more commonly available for post-mortem histopathological examination, allowing a comprehensive evaluation of early myocardial infarction using various staining techniques.

Table 3: Evidence of Coronary Artery Disease

Coronary Artery Disease	Frequency (n)	Percentage (%)
Absent	27	32.9
Present	55	67.1
Total	82	100.0

Among the 82 post-mortem heart samples, 67.1% showed coronary artery disease (CAD), highlighting its major role in myocardial infarction and its strong association with ischemic injury in sudden cardiac deaths.

Figure 2: Histologic Type



All 82 heart samples were histologically categorized into three groups based on the stage of myocardial infarction. Group 2 (intermediate stage) was most common (46.3%), followed by Group 1 (early stage) at 29.3%, and Group 3 (late stage) at 24.4%. This indicates that many cases were identified in the early and evolving stages, emphasizing the value of special stains and immunohistochemistry for early post-mortem detection.

Table 4: PTAH Findings

PTAH Findings	Frequency (n)	Percentage (%)
Negative findings	30	36.6
Positive findings	52	63.4
Total	82	100.0

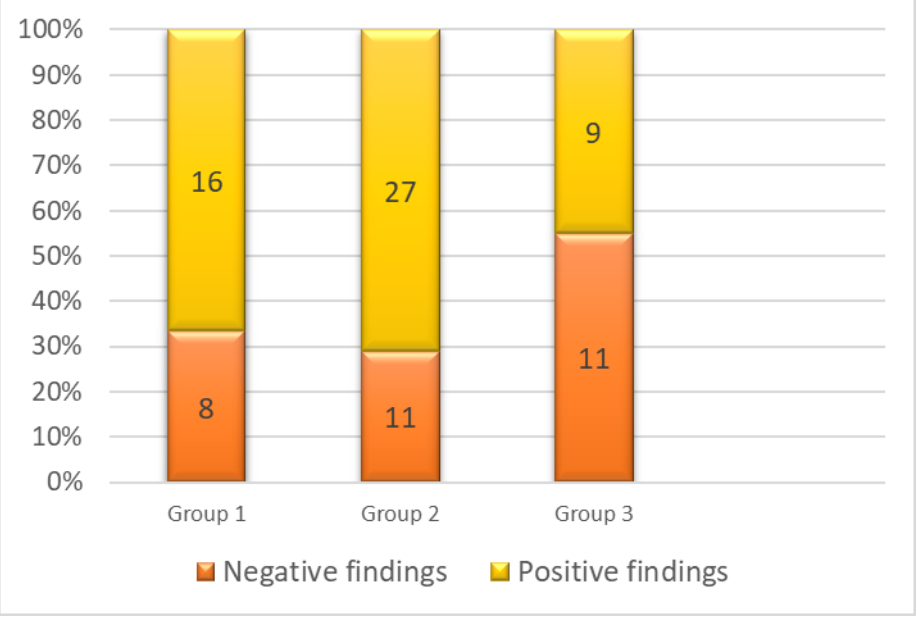
Out of the 82 heart tissue samples examined, 52 cases (63.4%) showed positive findings with Phosphotungstic Acid Hematoxylin (PTAH) staining, while 30 cases (36.6%) demonstrated negative findings. The relatively high proportion of positive results indicates that PTAH staining is a valuable histochemical method for identifying early myocardial changes, particularly in cases of suspected myocardial infarction. This staining technique effectively highlights muscle fiber damage, helping to differentiate between viable and necrotic cardiac tissue in post-mortem analysis.

Table 5: cTnI Expression

cTnI Expression	Frequency (n)	Percentage (%)
-3.0	26	31.7
-2.0	47	57.3
-1.0	9	11.0
Total	82	100.0

Among the 82 post-mortem heart samples, 47 cases (57.3%) exhibited a moderate loss of cTnI expression (-2.0), followed by 26 cases (31.7%) with a complete loss (-3.0), and 9 cases (11.0%) showing a mild loss (-1.0). These findings indicate that a progressive reduction in cTnI immunoreactivity correlates with the severity and stage of myocardial injury. The substantial number of cases with moderate to complete loss supports the utility of cTnI as a sensitive marker in identifying early myocardial infarction in post-mortem tissue, even before significant morphological changes are evident on routine H&E staining.

Figure 3: Association of Histology type with PTAH Findings



Groups 1 and 2 showed more positive PTAH findings, indicating better detectability in early and intermediate stages. Group 3 had a balanced distribution, suggesting reduced effectiveness in late infarction. The p-value of 0.07 suggests a potential trend, warranting further investigation with a larger sample size.

Table 6: Association of Histology type with cTnI Expression

		cTnI Expression			Total	P value
		-3.0	-2.0	-1.0		
Histologic Type	Group 1	12	1	1	24	**0.01**
	Group 2	23	3	1	38	
	Group 3	12	5	3	20	

This analysis showed a significant association between the stage of myocardial infarction and cTnI expression loss. Group 1 (early MI) had the highest cTnI loss, followed by Group 2. Group 3 (late MI) showed milder loss. The p-value of 0.01 confirms a significant relationship, supporting cTnI immunohistochemistry as a reliable tool for detecting early myocardial infarction in post-mortem heart.

Discussion

The Early and accurate detection of myocardial infarction in post-mortem cardiac samples remains fundamental in the investigation of sudden cardiac death. This study evaluated the diagnostic utility of Phosphotungstic Acid Hematoxylin staining and cardiac Troponin I immunohistochemistry in identifying myocardial injury across different histological stages. The findings confirm that both techniques are valuable, with cardiac Troponin I immunohistochemistry demonstrating superior diagnostic relevance, particularly in the early stages of infarction.

Among the 82 samples analyzed, Phosphotungstic Acid Hematoxylin staining was positive in 63.4% of cases, mainly in the early and intermediate stages of myocardial infarction. This observation is consistent with earlier studies reported by Roberts et al., who described the effectiveness of Phosphotungstic Acid Hematoxylin in demonstrating muscle fiber damage and sarcomeric disruption during the early phases of myocardial injury [6]. However, the reduced positivity in late-stage infarcts indicates limited sensitivity beyond the acute phase. Similar conclusions were drawn by Burke et al., who reported that conventional histochemical techniques lose diagnostic sensitivity as myocardial necrosis progresses and is replaced by granulation tissue [4].

Cardiac Troponin I immunohistochemistry demonstrated greater diagnostic accuracy, with complete or moderate loss of immunoreactivity observed in 88.9% of cases. The strongest association was noted in early myocardial infarction, supporting the role of cardiac troponins as highly specific markers of myocardial injury. These findings align with the observations of Apple et al., who emphasized that troponin depletion can be detected before overt structural changes become evident on routine hematoxylin and eosin staining [11]. Comparable results were also reported by Kanchan et al., who highlighted the superior diagnostic value of troponin-based immunohistochemistry in forensic cases, particularly when survival time following infarction is short [10].

Furthermore, the results of the present study are in agreement with findings reported by Maiese et al., who demonstrated a significant correlation between the degree of cardiac Troponin I loss and the histological stage of myocardial infarction, underscoring its utility not only in early detection but also in staging myocardial damage [9]. The progressive pattern of troponin depletion observed in this study, ranging from moderate loss in early infarction to milder reductions in later stages, further supports its role as a dynamic marker of myocardial viability.

Although Phosphotungstic Acid Hematoxylin staining remains a useful adjunct technique, particularly in settings where immunohistochemistry is unavailable, the higher sensitivity and specificity of cardiac Troponin I immunohistochemistry make it the preferred modality for post-mortem detection of early myocardial infarction. Nevertheless, the combined use of both techniques provides a more comprehensive and reliable diagnostic approach, especially in complex or equivocal cases.

Conclusion

In conclusion, this study underscores the utility of Phosphotungstic Acid Hematoxylin (PTAH) staining and cardiac troponin I (cTnI) immunohistochemistry in the post-mortem detection of early myocardial infarction. The findings reveal a higher detectability of myocardial changes in the early and intermediate stages of infarction, particularly with cTnI loss, which correlated significantly with the stage of infarction. The significant association between cTnI expression and the histologic stage of myocardial infarction highlights the value of immunohistochemistry as a reliable diagnostic tool in post-mortem heart samples.

These techniques not only enhance the accuracy of identifying early myocardial injury but also offer valuable insights into the pathophysiology of ischemic heart disease, particularly in cases of sudden cardiac death. Further studies with larger sample sizes may strengthen these findings and refine post-mortem diagnostic protocols.

Conflict of Interest: Nil

Reference

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