ORIGINAL RESEARCH ARTICLE

NATIONAL JOURNAL NJMR MEDICAL RESEARCH

Histological and Morphometric Analysis with Grading of Atherosclerosis-Associated Myocardial Changes in Cases of Sudden Death at a Tertiary Care Hospital

Prathana Patel[1], Smita Jhaveri[2*], Parth Patel[3]

[1,2,3]Department of Pathology, SMIMER, Surat, India

DOI:
10.55489/njmr.150320251125

***Corresponding author**:
Dr. Smita Jhaveri
drsmitajhaveri@gmail.com

Date of Submission: 30/04/2025
Date of Acceptance: 19/06/2025
Date of Publication: 01/07/2025

Funding Support:
None Declare

Conflict of Interest:
The authors have declared that no conflict of interests exists.

How to cite this article:
Patel P, Jhaveri S, Patel P. Histological and Morphometric Analysis with Grading of Atherosclerosis-Associated Myocardial Changes in Cases of Sudden Death at a Tertiary Care Hospital. Natl J Med Res 2025;15(03):202-208. DOI: 10.55489/njmr.150320251125

ABSTRACT

Background: Sudden cardiac death (SCD) remains a significant public health concern worldwide, representing a considerable proportion of mortality linked to cardiovascular diseases. Atherosclerosis, a progressive disease characterized by the accumulation of lipids, fibrous elements, and inflammatory cells within the arterial walls, is a leading underlying cause of SCD.

Methodology: An observational study was conducted focusing on histology, morphometric analysis, and grading of atherosclerosis-associated myocardial findings in cases of sudden death in 194 cases over a period of 18 months in the Department of Pathology, SMIMER Medical College, Surat.

Results: The study involved 194 participants, with a notable gender imbalance favouring males, who represented 89.7% of the cohort. There was a clear trend of increasing coronary artery grade with advancing age. Ischemic Heart Disease (IHD) was the most prevalent myocardial finding, observed in 54.6% of the cases. 148 (76%) cases showed both the coronary involvement in patients with Ischaemic Heart Disease. For Ischemic Heart Disease (IHD), the AHA grades are predominantly VII (47.2%), V (21.7%), and VI (17.0%). As the coronary artery grade increases, there is a notable rise in both ITI and IMR values.

Conclusion: In conclusion, this study demonstrates that a comprehensive evaluation combining histological grading and morphometric analysis is crucial for a thorough understanding of atherosclerosis-related myocardial changes.

Keywords: Coronary Atherosclerosis, Sudden Death, Ischaemic Heart Disease, Intima Media Ratio, Intimal Thickness Index

INTRODUCTION

Sudden cardiac death (SCD) remains a significant public health concern worldwide, representing a considerable proportion of mortality linked to cardiovascular diseases. Atherosclerosis, a progressive disease characterized by the accumulation of lipids, fibrous elements, and inflammatory cells within the arterial walls, is a leading Under-lying cause of SCD.[1]

Histological examination allows for the detailed analysis of these structural alterations, revealing the extent and nature of myocardial damage. By employing morphometric techniques, which involve the quantitative assessment of tissue architecture, researchers can obtain precise measurements of myocardial features such as

Publisher: Medsci Publications [www.medscipublications.com] ISSN: 2249 4995 Official website: www.njmr.in

plaque size, fibrosis extent, and microvascular changes.[2]

Grading the severity of atherosclerosis and its myocardial implications is crucial for understanding the potential risks and prognostic factors associated with SCD.[3] Its etiology is multifactorial, involving a combination of genetic, environmental, and lifestyle factors. Key risk factors include hyperlipidaemia [especially elevated low-density lipoprotein (LDL)], hypertension, smoking, diabetes, obesity, and sedentary lifestyle. Additionally, genetic predispositions, such as familial hypercholesterolemia, can significantly increase the risk. Other contributing factors include age, male gender, and postmenopausal status in women.[4]

The purpose of this study is to evaluate the degree of severity of atherosclerosis based on its morphological grading and morphometric indices, to study myocardial morphology in cases of sudden death, and to correlate the morphological grades and morphometric parameters of atherosclerosis with the histological findings in the myocardium.

MATERIALS AND METHODS

Study Design: The study was observational in nature, focusing on histology, morphometric analysis, and grading of atherosclerosis-associated myocardial findings in cases of sudden death.

Study Duration and Size: The sample size was determined based on the proportion of sudden deaths out of the total deaths during the 18-month study period in the Department of Pathology. Approximately 194 cases were included in the study.

Inclusion Criteria: The study included all autopsy cases of sudden death that occurred during the defined study period. Both male and female individuals were considered eligible for inclusion. Only cases involving individuals aged 20 years and above were included in the analysis, ensuring that the focus remained on adult populations.

Exclusion Criteria: Cases involving individuals below the age of 20 years were excluded from the study. Additionally, autopsies where the specimens were autolyzed were not considered due to compromised diagnostic value. The study also excluded deaths resulting from road traffic accidents, suicides, homicides, and burns, in order to specifically assess natural causes of sudden death.

Ethical Considerations: Ethical clearance was obtained from the Institutional Ethics Committee (Approval Letter no.- 94/2023, Date- 27/06/2023). As the study dealt only with pathological specimens received from department of Forensic Medicine, explicit consent was not required. Strict confidentiality of personal details and information related to the study was maintained at all levels.

Sample Collection and Preparation: Samples were obtained from the Forensic Medicine Department and transferred to the Pathology Department for autopsy analysis labelled with the full name, age, sex, and registration number, along with a fully completed autopsy requisition form mentioning the cause of death. All organs were fixed in 10% formalin to preserve tissue integrity.

The specimen was serially sectioned at approximately 0.5 cm intervals from the apex to the base of the heart, ensuring not to cut through. The specimen was fixed overnight in a container filled with formalin.

Heart Dissection: The heart was dissected the following day using the Modified Virchow's Method (Inflow-Outflow method). The right and left coronary arteries were dissected longitudinally until they entered the musculature. The coronaries were cut in cross-sections at 3-5 mm intervals and examined grossly. Representative samples from pathological areas were taken from both coronaries. The aorta was also examined, and representative sections were taken.

The collected sections were processed to create paraffin blocks. These blocks were then cut into thin slices for microscopic examination. The slices were stained using Haematoxylin and Eosin (H&E) to highlight general tissue structure and Verhoef-Van Gieson (VVG) to demonstrate the internal and external elastic lamina.

Histological Analysis: The atheromatous lesions in the coronary arteries were graded according to the American Heart Association (AHA) guidelines.

Histological changes in the myocardium including congestion, coagulative necrosis of fibers, inflammatory infiltrate and areas of fibrosis, were documented for comprehensive analysis.

Grading Systems for Atherosclerosis:

Modified American Heart Association criteria for grading atherosclerotic lesions [5]

1. Grade I: isolated intimal foamy cells (minimal change)
2. Grade II: numerous intimal foamy cells often in layers (fatty streaks)
3. Grade III: pools of extracellular lipid without a well-defined core (intermediate lesion of pre-atheroma)
4. Grade IV: well defined lipid core with luminal surface covered by normal intima (atheroma or fibro plaque)
5. Grade V: lipid core with a fibrous cap with or without calcification (fibro atheroma)
6. Grade VI: fibro-atheroma with cap defect such as haemorrhage and thrombosis
7. Grade VII: calcification prominent
8. Grade VIII: fibrous tissue changes prominent.

Morphometric Measurement: To take the morphometric measurements, microscopic images of the VVG stained slides were captured on a ZEISS AxioCam Erc5s.

In each section the Intimal Thickness Index (ITI) and Intima-to-Media Ratio (IMR) were measured.

a. Intimal Thickness Index (ITI) $= \frac{\text{Intimal area}}{\text{Medial area}}$

The intimal area is calculated by subtracting luminal area from the internal elastic luminal area (IELA).

The medial area is calculated by subtracting internal elastic luminal area from external elastic luminal area (EELA).

b. Intima to Media Ratio (IMR) $= \frac{\text{Width of intima at maximal intimal thickness}}{\text{Width of media at maximal intimal thickness}}$

RESULTS

The study cohort comprised a total of 194 participants, with a notable gender imbalance favouring males. Overall, the total gender distribution showed that males were significantly more represented (89.7%) compared to females (10.3%) (table 1).

Table 1:Age group and gender-wise distribution of study participants

Age Group	Female (%)	Male (%)	Total (%)
<=30	3 (14.3)	18 (85.7)	21 (100.0)
31-40	6 (12.5)	42 (87.5)	48 (100.0)
41-50	1 (1.9)	52 (98.1)	53 (100.0)
51-60	4 (9.1)	40 (90.9)	44 (100.0)
61-70	6 (24.0)	19 (76.0)	25 (100.0)
71-80	0 (0.0)	3 (100.0)	3 (100.0)
Total	20 (10.3)	174 (89.7)	194 (100.0)

Table 2: AHA grading of Coronary artery- age wise distribution of study participants

AHA grade	<=30 (%)	31-40 (%)	41-50 (%)	51-60 (%)	61-70 (%)	71-80 (%)	Total (%)
0	11 (35.5)	12 (38.7)	5 (16.1)	3 (9.7)	0 (0.0)	0 (0.0)	31 (100.0)
2	3 (50.0)	1 (16.7)	0 (0.0)	1 (16.7)	1 (16.7)	0 (0.0)	6 (100.0)
3	1 (9.1)	2 (18.2)	4 (36.4)	3 (27.3)	1 (9.1)	0 (0.0)	11 (100.0)
4	0 (0.0)	8 (42.1)	9 (47.4)	2 (10.5)	0 (0.0)	0 (0.0)	19 (100.0)
5	2 (6.9)	6 (20.7)	8 (27.6)	10 (34.5)	2 (6.9)	1 (3.4)	29 (100.0)
6	2 (8.7)	8 (34.8)	5 (21.7)	4 (17.4)	4 (17.4)	0 (0.0)	23 (100.0)
7	2 (2.9)	9 (12.9)	20 (28.6)	21 (30.0)	16 (22.9)	2 (2.9)	70 (100.0)
8	0 (0.0)	2 (40.0)	2 (40.0)	0 (0.0)	1 (20.0)	0 (0.0)	5 (100.0)
Total	21 (10.8)	48 (24.7)	53 (27.3)	44 (22.7)	25 (12.9)	3 (1.5)	194 (100.0)

Table 3: Myocardial finding seen in cases

Myocardial findings	Cases (%)
IHD	106 (54.6)
Hypertrophy	15 (7.7)
Valvular Heart Disease	2 (1)
Cardiomyopathies	8 (4.1)
Pericardial Disease	7 (3.6)
Granulomatous Inflammation	1 (0.5)
Histologically Unremarkable	71 (36.6)

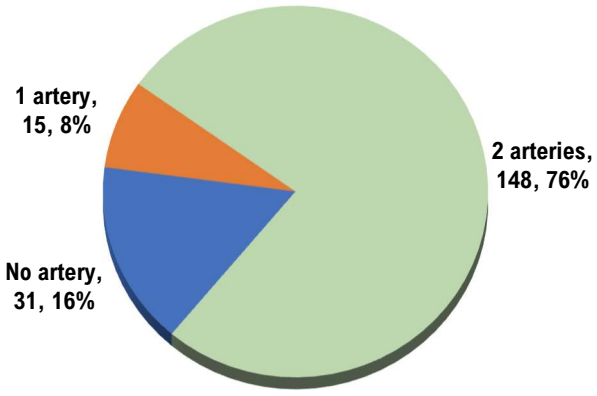


Figure 1: No. of Coronaries involved in cases of IHD

The distribution of participants by the AHA coronary artery grade across different age groups reveals a distinct pattern with increasing severity of coronary artery involvement. The total distribution highlights that the severity of coronary artery involvement increases with age, as evidenced by the higher grades being more prevalent in the middle-aged and older age groups (table 2).

The distribution of myocardial findings in the study reveals a diverse range of conditions with varying frequencies. Ischemic Heart Disease (IHD) is the most common finding, observed in 106 cases, accounting for 54.6% of the total (table 3).

148 (76%) cases showed both the coronary involvement in patients with Ischaemic Heart Disease. 15 (8%) cases showed 1 coronary involvement and 31(16%) showed no coronary involvement in patients with Ischaemic Heart Disease (Fig 1).

The distribution of the coronary arteries involved in atherosclerosis shows significant involvement of the LCA 65 (33.5%) more than the RCA 30 (15.4%). 68 (35%) of the cases show both the coronary involvement showing the same grade of atherosclerosis. 31 (15.9%) cases had no coronary involvement (table 4).

For Ischemic Heart Disease (IHD), the AHA grades are predominantly **VII** (47.2%), **V** (21.7%), and **VI** (17.0%), indicating extensive coronary artery involvement in this condition. This pattern suggests severe and widespread coronary pathology among IHD patients (table 5).

As the coronary artery grade increases, there is a notable rise in both ITI and IMR values, indicating a correlation between the extent of coronary pathology and these indices.

Ischaemic Heart Disease - comparison with ITI and IMR: For cases of **ischemic heart disease (IHD)**, both ITI and IMR grades are significantly elevated, with ITI averaging **2.3 ± 0.8** and IMR averaging **4.3 ± 2.2**. This indicates a substantial increase in intima thickness and myocardial involvement in IHD cases compared to other conditions (table 6).

Table 4: Distribution of the coronaries involved in atherosclerosis

Coronaries involved	Cases (%)
LCA	65 (33.5)
RCA	30 (15.4)
Both coronaries involved	68 (35)
No coronaries involved	31(15.9)
Total	194 (100)

Table 5: AHA grading of coronary artery comparison with Ischaemic Heart Disease

AHA coronary grade	Ischemic Heart Disease (IHD)
0	2 (1.9)
2	0 (0.0)
3	2 (1.9)
4	7 (6.6)
5	23 (21.7)
6	18 (17.0)
7	50 (47.2)
8	4 (3.8)

Table 6: AHA grade of Coronary artery - comparison with ITI and IMR

AHA grade	ITI	IMR
0	0.57 ± 0.04	1.08 ± 0.64
2	0.73 ± 0.03	2.02 ± 0.82
3	0.95 ± 0.07	2.60 ± 0.68
4	1.89 ± 0.31	3.22 ± 0.85
5	2.33 ± 0.14	5.25 ± 1.31
6	3.57 ± 0.38	6.13 ± 1.16
7	1.97 ± 0.37	2.94 ± 0.33
8	2.93 ± 0.76	11.75 ± 1.37
Total	1.91 ± 0.93	3.57 ± 2.21

DISCUSSION

Socio- Demographic distribution: In our study, males were significantly more prevalent, comprising 89.7% of the total 194 participants, while females made up just 10.3%. Similarly, in the study by Bhanvadia VM et al.,64% of the cases were male (168 cases) and 36% were female (98 cases).[6] These findings align with those of Bhargava MK et al., who reported 74.8% males and 24.2% females, and Tandon OP et al., who found 66.5% males and 33.5% females in their study.[7,8]

AHA grading of Coronary artery- Age group wise distribution of study participants: In our study, higher grades were more frequent in older participants. Grade VII, for instance, was prevalent in the 41-50 (28.6%) and 51-60

(30.0%) age groups.

In Bhanvadia VM et al.'s study, the Modified AHA classification of atherosclerosis revealed that the highest number of cases were in the 40-49 years age group. This suggests that this age range is particularly vulnerable to atherosclerosis, based on their findings.[6]

Myocardial finding: In our study, ischemic heart disease (IHD) is the most prevalent condition, identified in 106 cases, making up 54.6% of the total. These results indicate the predominance of IHD among myocardial conditions, with other cardiac abnormalities occurring less frequently.

In Biare SD et al.'s study on histopathological changes in the heart from post-mortem cases, Myocardial Infarction (MI) was present in 13.80% of cases.[9] In the study by Marwah N et al., Myocardial Infarction (MI) was a significant finding, affecting 36% of the cases.[10]

AHA grading of coronary artery comparison with Myocardial findings: In our study, ischemic heart disease (IHD) is most commonly associated with AHA grades 7 (47.2%), 5 (21.7%), and 6 (17.0%), indicating severe coronary artery involvement.

In Singh P et al.'s study, significant cardiac lesions were most frequently associated with atherosclerotic type VIII lesions (75%), followed by type VII (66.66%) and type VI (33.33%). The majority of significant myocardial lesions were linked to grade IV coronary luminal narrowing (66.66%), followed by grade III (45.71%) and grade II (38.46%). The study also found a significant correlation between higher grades of coronary atherosclerotic lesions and ischemic heart disease.[11]

AHA grade of Coronary artery - comparison with ITI and IMR: In our study, higher coronary artery grades correlate with increased ITI and IMR values, highlighting the impact of coronary severity on myocardial parameter.

According to the study by Khiste JA et al., the severity of the Intima-to-Media Thickness Ratio (IMTR) increases with the severity of atherosclerotic grades up to Grade V. However, there is a slight decrease at Grade VI, which is attributed to medial muscular hypertrophy, a response to injury.[12]

Our study found a strong correlation between higher AHA grades and increased ITI and IMR values. The rising ITI and IMR with higher AHA grades highlight the progressive impact of coronary artery disease on myocardial tissue and function, offering insights into the extent of myocardial ischemia and dysfunction.

Similarly, Jain et al. (2013) found a direct correlation between the increasing grades of atherosclerosis and the percentage of luminal narrowing, as well as the Intima-to-Media Thickness Ratio. These findings are consistent with our study data.[13]

Figure 2: Grade 5 coronary atherosclerosis (H&E, 4X)

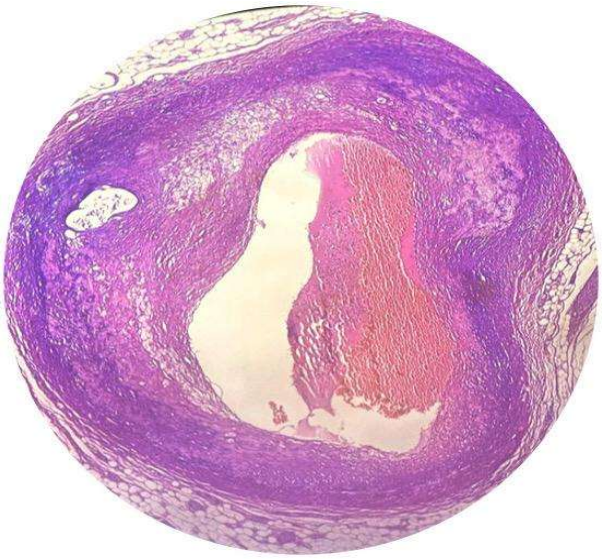


Figure 3: Grade 6 coronary atherosclerosis (H&E, 4X)

Figure 4: Grade 7 coronary atherosclerosis (H&E, 4X)

Figure 5: Grade 8 coronary atherosclerosis (H&E, 4X)

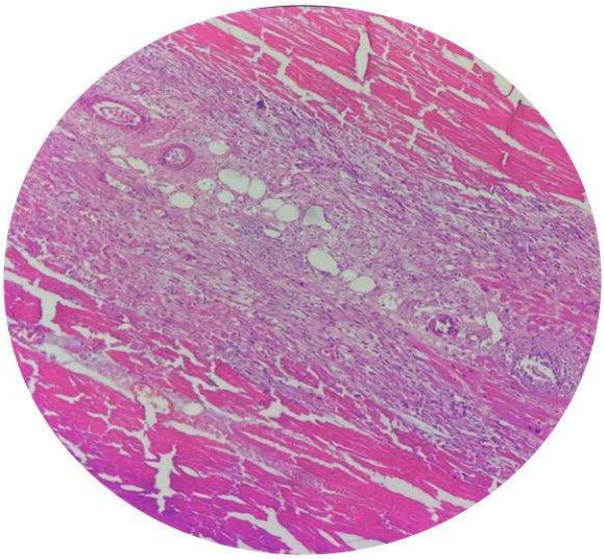


Figure 6: Changes of Acute MI (H&E, 4X)

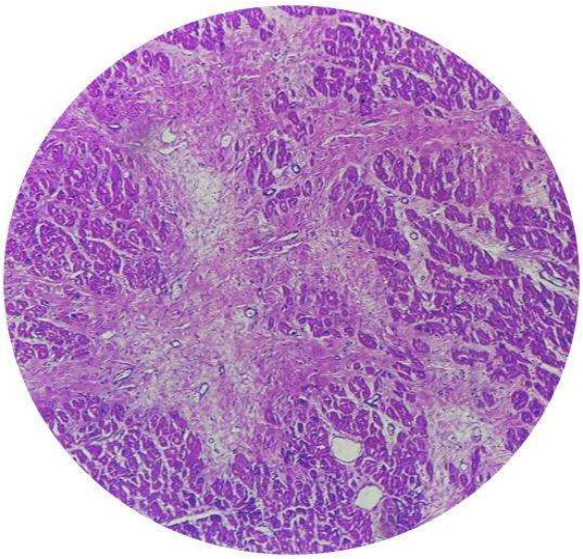
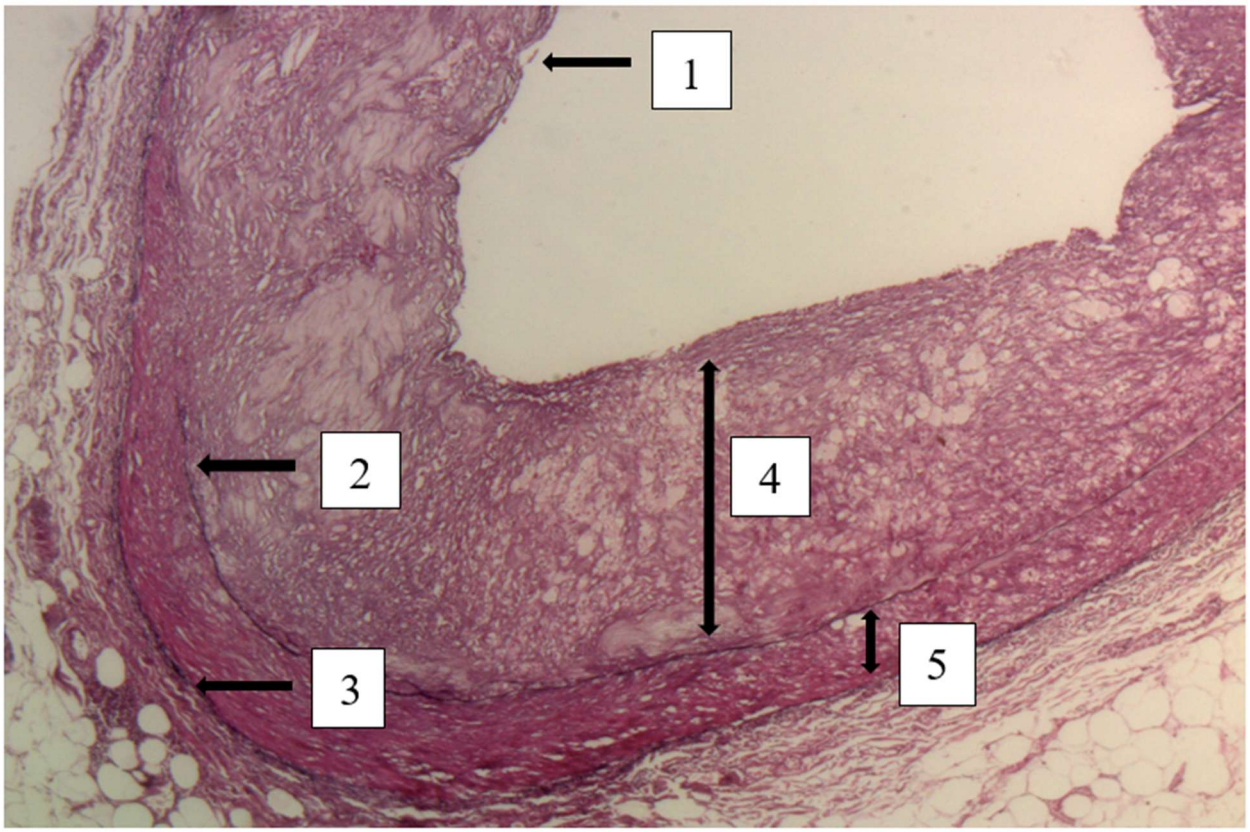


Figure 7: Changes of Healed MI (H&E, 4X)



(1) Luminal Border, (2) Internal elastic lamina, (3) External elastic lamina, (4) Intimal thickness, (5) Medial thickness

Figure 8: Cross section of Grade 4 Coronary atherosclerosis (Verhoef stain, 5X)

Distribution of the coronaries involved in atherosclerosis: In our study, the coronary arteries involved in atherosclerosis accounts for 63(33.5%) in the LCA and 30(15.4%) in the RCA. This shows a significant involvement of the LCA.

Our study correlates with the study of Chatzizisis YS et al., coronary atherosclerosis was more prevalent in the left coronary artery compared to the right.[14]

CONCLUSION

In conclusion, this study demonstrates that a comprehensive evaluation combining histological grading and morphometric analysis is crucial for a thorough understanding of atherosclerosis-related myocardial changes. The findings suggest a strong correlation between the severity of atherosclerosis and the associated myocardial pathology, reinforcing the need for integrated histological and morphometric approaches in autopsy studies of sudden death.

Author Contribution: PP contributed to all aspects of the study, including the conception, design, data collection, data analysis and interpretation, and manuscript preparation. **SJ** was involved in the study's conception and design, as well as in the analysis and interpretation of data. **PP** contributed to the study conception, participated in data collection, and assisted with data analysis and interpretation.

REFERENCES

1. Yow AG, Rajasurya V, Ahmed I, et al. Sudden Cardiac Death. [Updated 2024 Mar 16]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK507854/
2. Gusev E, Sarapultsev A. Atherosclerosis and Inflammation: Insights from the Theory of General Pathological Processes. Int J Mol Sci. 2023 Apr 26;24(9):7910. DOI: https://doi.org/10.3390/ijms24097910 PMid:37175617 PMCid:PMC10178362
3. Sachdev V, Rosing DR, Thein SL. Cardiovascular complications of sickle cell disease. Trends Cardiovasc Med. 2021 Apr;31(3):187-193. DOI: https://doi.org/10.1016/j.tcm.2020.02.002
4. Luca AC, David SG, David AG, Țarcă V, Pădureț IA, Mîndru DE, Roșu ST, Roșu EV, Adumitrăchioaiei H, Bernic J, Cojocaru E, Țarcă E. Atherosclerosis from Newborn to Adult-Epidemiology, Pathological Aspects, and Risk Factors. Life (Basel). 2023 Oct 14;13(10):2056. DOI: https://doi.org/10.3390/life13102056 PMid:37895437 PMCid:PMC10608492
5. Harveen B, Vijaya C, Smita M, Atherosclerosis in coronary arteries and aorta by modified American heart association classification: An autopsy study. IP Arch Cytol Histopathol Res 2022;7(2):100-104. DOI: https://doi.org/10.18231/j.achr.2022.021
6. Bhanvadia VM, Desai NJ, Agarwal NM. Study of Coronary Atherosclerosis by Modified American Heart Association Classification of Atherosclerosis-An Autopsy Study. J Clin Diagn Res 2013;7(11):2494-7. DOI: https://doi.org/10.7860/JCDR/2013/6828.3588 PMid:24392381 PMCid:PMC3879834
7. Bhargava MK, Bhargava SK. Coronary atherosclerosis in North Karnatka. Indian J Pathol Microbiol. 1975 Apr;18(2):65-79. PMID: 1213818.

8. Tandon OP, Agrawal VC, Katiyar BC. Coronary and aortic atherosclerosis. (A study on 300 medicolegal postmortems). Indian Heart J. 1969 Jan;21(1):5-10. PMID: 5783200.

9. Birare SD, Swami DV, Gaikwad SS, Malpani RR. Study of the Histopathological Changes in the Heart in Postmortem/Autopsy Cases in Tertiary Care Center. Heart Views. 2024 Jan-Mar;25(1):9-12. DOI: https://doi.org/10.4103/heartviews.heartviews_45_23

10. Marwah N, Bhawna S, Sumiti G, Amrita D, Sunita S, Rajeev S. Histomorphological Spectrum of Various Cardiac Changes in Sudden Death: An Autopsy Study. Iranian Journal of Pathology, 2011; 6(4): 179-186.

11. Singh P, Satarkar RN, Kalhan S, Hasija S, Bhawna, Sangwaiya A, Chawla S. Correlation of coronary artery atherosclerosis typing and luminal narrowing with ischemic myocardial lesions in post mortem heart specimens: a four year retrospective study. Trop J Pathol Microbiol. 2018 Jul.3;4(3):248-57. DOI: https://doi.org/10.17511/jopm.2018.i03.03

12. Khiste JA, Kurdukar MD, Pandit GA, Kokate PS. Morphologic and morphometric analysis of atherosclerotic coronary arteries in Medicolegal Autopsies. International Journal of Current Research. 2018;10(04):67671-67674.

13. Jain S, Biligi DS. An Autopsy Study on Coronary Atherosclerosis with Morphological and Morphometric Analysis. Intl J Sci Res 2013;4:2319-7064.

14. Chatzizisis YS, Giannoglou GD, Parcharidis GE, Louridas GE. Is left coronary system more susceptible to atherosclerosis than right? A pathophysiological insight. Int J Cardiol. 2007 Mar 2;116(1):7-13. DOI: https://doi.org/10.1016/j.ijcard.2006.03.029 PMid:16908081