

An Autopsy Study on Coronary Atherosclerosis with Morphological and Morphometric Analysis

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Abstract: Objectives: Atherosclerosis is the pathology that underlies Coronary artery disease which can be best studied on autopsy. The study aims to evaluate coronary atherosclerosis by means of morphological and morphometric analysis. Study also intends to assess the correlation between morphometric parameters and morphological grades of coronary atherosclerotic lesions. Methodology: Study was carried out on 150 autopsy cases of both sexes in the 30-60 years age group. Representative bit of each coronary artery was taken and processed conventionally. Morphological assessment of atherosclerosis was done according to AHA (American Heart Association) criteria while two morphometric parameters namely percentage of luminal narrowing and intima-to-media ratio (IMR) were used to evaluate degree of atherosclerosis. Results: Study included 114 males and 36 females. Coronary atherosclerosis was present in 122 cases (81.3%). Values of both morphometric parameters showed a linear increase with each higher grade of atherosclerosis in all three coronaries. Conclusion: The study results indicate that the morphometric parameters correlate with the morphological grades of atherosclerosis in all three coronaries. Hence these parameters can serve as reliable indices for assessing the severity of atherosclerotic lesions supplementing the histomorphological analysis.

Keywords: Atherosclerosis; Autopsy; American Heart Association; Coronary Artery Disease; Coronary Vessels.

1. Introduction

Atherosclerosis is characterized by intimal lesions called atheromas that protrude into vessel lumens and weaken the underlying media [1]. Coronary Artery Disease (CAD) is a leading cause of morbidity and mortality worldwide, commonly caused due to obstruction of coronary arteries by atheromatous plaque [2]. The World Health Organisation (WHO) has drawn attention to the fact that CAD is our modern "epidemic", a disease which affects populations and is not an unavoidable attribute of aging [3]. An autopsy study gives a good measure of prevalence, grading and distribution pattern of atherosclerotic lesions. American Heart Association (AHA) classification of grading atherosclerosis has been preferred for the morphological assessment of coronary atherosclerosis on microscopy. Percentage of luminal narrowing and intima-to-media ratio (IMR) are the morphometric parameters used to analyse the severity of atherosclerotic lesions [4,5].

This autopsy study was undertaken to evaluate coronary atherosclerosis by means of morphological assessment and morphometric analysis. The study also aims to assess the correlation between the morphometric parameters and morphological grades of coronary atherosclerotic lesions.

2. Methodology

This study was carried out at Department of Pathology, Bangalore Medical College and Research Institute (BMCRI), Bangalore on 150 autopsy cases of both sexes with the 30-60 years age group, having history of deaths due to natural as well as unnatural causes (accidental, suicidal, homicidal, etc.). Collection of cases was done from Department of Forensic Medicine and associated Mortuary in Victoria

hospital of BMCRI.

The heart was dissected by inflow-outflow method [6] at autopsy. For each side of the heart, the atrium is opened first, and then the ventricle is opened along its inflow and outflow tracts, following the direction of blood flow. All three major coronary arteries namely Left anterior descending (LAD), Left circumflex (LCX) and Right coronary artery (RCA) were dissected longitudinally until they entered the musculature. The coronary arteries were cut in cross sections at 3-5 mm intervals and examined grossly for presence of any atherosclerosis, thrombus and narrowing. If found, a representative bit was taken and in absence of any lesion, a random section was taken from each coronary artery. Heavily calcified vessels were removed and decalcified prior to sectioning.

The collected specimens (Coronaries) were fixed in 10% formalin solution for 2-5 days and later were processed conventionally by embedding in paraffin blocks. After blocking the specimens in paraffin, 4 to 5 micron slide sections were prepared, stained with Haematoxylin and Eosin (H & E) method according to standard procedures.

2.1 Morphological Assessment

Histological evaluation of each slide was performed using a light microscope (Olympus BX51) equipped with polarization filters. All three major coronaries were examined for presence of any atherosclerotic lesion. If present, the assessment of atherosclerosis was done by grading the atherosclerotic lesion according to the criteria [7, 8] suggested by the American Heart Association (AHA) which is based on morphological descriptions.

The AHA classification consists of 6 different numeric categories to include:

Grade 0: Sections showing normal histology or adaptive thickening without macrophages or foam cells.
 Grade 1: Presence of isolated macrophage foam cells.
 Grade 2: Intracellular lipid accumulation with formation of multiple foam cell layers.
 Grade 3: Grade 2 lesions along with small extracellular lipid pools.
 Grade 4: Grade 2 changes along with a core of extracellular lipid.
 Grade 5: Lipid core and fibrotic layer or multiple lipid cores and fibrotic lipid layers.
 Grade 6: Complicated plaques with surface defects, and/or hematoma-hemorrhage, and/or thrombosis.

2.2 Morphometric Analysis

The histomorphometric analysis was performed using the ProgResC3 CapturePro (Jenoptik AG, GmbH; Jena, Germany) imaging software. Morphometric measurements were performed after calibration of the system. Direct analysis of the arterial lumen was achieved by magnification of 40x, while analysis of the intimal layer thickness/changes and tunica media was attained at a magnification of 200x.

Morphometric measurements and calculated measurements [5] mentioned below were done on all three major coronary arteries (LAD, LCX and RCA).

Morphometric measurements done included:

- width of intima (distance between intima and IEL),
 - width of media (area between IEL and EEL)
 - diameter internal to the media (Diameter of lumen plus intima; DLI).
- (IEL = internal elastic lamina; EEL = external elastic lamina)

Calculated measurements done included:

- Lumen area (in mm^2) = πr^2 (where r = diameter of lumen/2).
- IEL area (in mm^2) = πR^2 (where R = DLI/2).
- Intimal area (in mm^2) = IEL area – Lumen area.

Based on the above measurements, following two morphometric parameters were derived to evaluate the degree of coronary atherosclerosis. Also termed as severity indices of atherosclerosis [5]:

- (a) Percentage of luminal narrowing,
- (b) Intima-to-Media Ratio (IMR)

These parameters were derived using the following formulas:

- Percentage of luminal narrowing = $100 \times \text{intimal area} / \text{IEL area}$;
- IMR = width of intima at maximal intimal thickness / width of media at maximal intimal thickness.

Percentage of luminal narrowing in all the atherosclerotic coronary arteries was calculated and grouped as under those with <25%, 25-50%, 50-75% and >75% luminal narrowing.

However, for the assessment of correlation between the morphometric parameters and morphological grades, mean Percentage of luminal narrowing and mean IMR was calculated for each AHA grade of atherosclerosis in all the

three atherosclerotic coronaries. Values thus obtained were tabulated and comparison of results was carried out.

3. Results

The present study included 114 males and 36 females between the age group of 30-60 years. Majority were in the age group of 30-40 years followed by 41-50 years and 51-60 years respectively (Table 1).

Table 1: Showing Age-wise Sex Distribution of cases (n=150)

Sl. No.	Age (years)	Males		Females		Total
		No. of cases	Percentage	No. of cases	Percentage	
1	30-40	58	38.6 %	23	15.3 %	81
2	41-50	38	25.3 %	10	6.6 %	48
3	51-60	18	12.1 %	03	2.1 %	21
	Total	114	76 %	36	24 %	150

3.1 Prevalence of coronary atherosclerosis

Out of 150 cases studied, 28 cases including 16 males and 12 females showed normal histology in all three major coronaries. Coronary atherosclerosis was present in 122 cases (81.3%) with mean age of 43 years. 85.9% (98/114) males and 66.6% (24/36) females had coronary atherosclerosis.

3.2 Distribution of atherosclerotic lesions

LAD was the most commonly involved coronary artery (74%) followed by LCX (68%) and RCA (60%) respectively. 63% (77/122) cases showed atherosclerotic lesions in all three major coronaries (triple-vessel disease), while 22% (27/122) in any two coronaries (double- vessel disease) and only 15% (18/122) showed involvement of any one coronary artery (single-vessel disease). Thus majority of the cases in our study had triple vessel disease. Among the cases of single artery involvement, LAD was involved in 9 cases, LCX in 6 cases and RCA in 3 cases.

3.3 Morphological Assessment (AHA Grading)

AHA Grade 2 (Figure 1) atherosclerotic change was the most common type of atherosclerotic lesion seen in both LAD and LCX while in RCA; AHA Grade 1 atherosclerotic change was most common. AHA Grade 4 (Figure 2) and 5 atherosclerotic changes were highest in LAD followed by LCX and RCA (Table 2). None of the coronaries showed AHA Grade 6 changes.

Table 2: Showing AHA grade distribution for all three coronaries

Coronary Artery	AHA Grade					Total (%)
	1	2	3	4	5	
LAD	18	34	22	14	23	111 (74%)
LCX	26	35	19	10	12	102 (68%)
RCA	40	25	12	6	7	90 (60%)

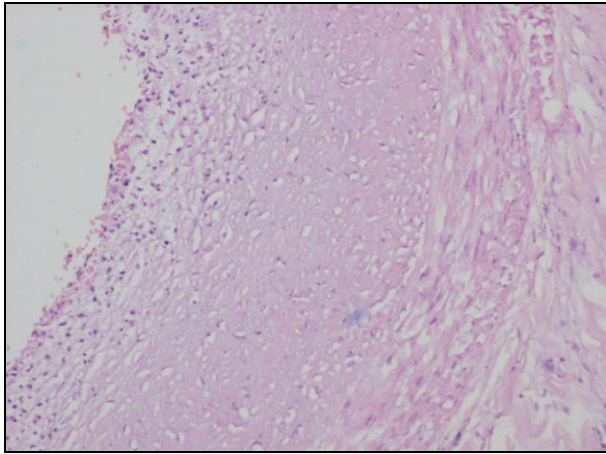


Figure 1: Photomicrograph of Coronary artery showing intimal thickening with foam cell aggregates and underlying medial thinning: AHA Grade 2 atherosclerosis (H & E, 200x)

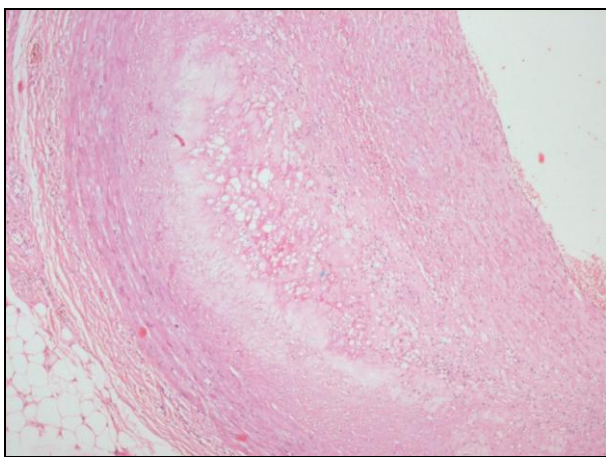


Figure 2: Photomicrograph of Coronary artery showing Atheroma composed of lipid core, foam cells, fibrous cap composed of SMCs and collagen with thinned out underlying media: AHA Grade 4 atherosclerosis (H & E, 100x)

3.4 Morphometric Analysis

Majority of atherosclerotic LADs and LCXs showed 25-50 % lumen narrowing while most atherosclerotic RCAs showed <25 % lumen narrowing. Critical narrowing (>75%) was seen in 7 LADs, 4 LCXs and 2 RCAs respectively (Table 3).

Table 3: Showing Percentage of luminal narrowing for all three coronaries

Coronary Artery	Percentage of Luminal Narrowing				Total
	<25%	25-50%	50-75%	>75%	
LAD	33	59	12	7	111
LCX	37	49	12	4	102
RCA	54	30	4	2	90

3.5 Correlation between morphometric parameters and morphological grades

Comparison result of both mean Percentage of luminal narrowing and mean IMR value for each AHA grade of atherosclerosis showed a linear increase in the values of both these parameters with each higher grade of atherosclerosis in all three coronaries. Thus, the morphometric parameters correlated well with the grade of atherosclerosis (Table 4, 5).

Table 4: Showing values of Mean Percentage of luminal narrowing for each AHA Grade in all three coronaries

Coronary Artery	Atherosclerosis Grade				
	1	2	3	4	5
LAD	25%	27%	31%	46%	55%
LCX	24%	26%	27%	55%	59%
RCA	22%	22%	26%	39%	61%

Table 5: Showing values of Mean IMR for each AHA Grade in all three coronaries

Coronary Artery	Atherosclerosis Grade				
	1	2	3	4	5
LAD	2.2	2.9	3.8	6.9	13.2
LCX	2.5	2.2	3.2	9.5	12.6
RCA	2.2	2.4	3.2	5.6	11.6

4. Discussion

Atherosclerosis of coronaries is the most common cardiac pathology found in autopsies [9]. Atherosclerosis is a chronic immunoinflammatory, fibroproliferative disease of large and medium-sized arteries fuelled by lipid [10]. Autopsy is a tool of real value for assessment of pathologies that are difficult to assess in the living [11].

The present study included 76% males and 24% females. This is in concordance with the data of other similar autopsy studies by Dhruva et al, [12] (73.6% males and 26.4% females), Puri et al, [13] (80% males and 20% females), Garg et al, [14] (81% males and 19% females), Thej et al, [15] (69% males and 31% females) and Singh H et al, [16] (84% males and 16% females). Higher number of autopsied males was the feature in our study and also other similar studies as well. This is because most autopsied cases include the victims of events such as road traffic accidents which commonly involve the males rather than females.

Prevalence of coronary atherosclerosis in the present study was found to be 81.3%, which matches with the data given by Kumar et al, [17] Puri et al, [13] and Singh H et al, [16] as 80%, 86% and 78 % respectively. But Thej et al, [15] have reported a prevalence rate of 66.3% which is due to the fact that they have used the Modified AHA classification where early lesions like intimal thickening and intimal xanthoma are not considered as atherosclerotic lesions.

Among all three major coronaries, LAD had the highest incidence of atherosclerotic involvement (74%), which is in concordance with data given in studies by Kumar et al, [17] and Yazdi et al, [18] as 68% and 60% respectively. LAD is the most commonly involved coronary vessel in atherosclerosis, possibly due to the hemodynamic stress that it undergoes through.

In the present study, AHA Grade 2 atherosclerotic change was the most common type of atherosclerotic lesion seen in both LAD and LCX while in RCA, AHA Grade 1 atherosclerotic change was most common. But in studies by Dhruva et al, [12] and Garg et al, [14] the commonest type of atherosclerotic change was AHA Grade 4 and Grade 3 respectively. This variation possibly, could be due to the fact that most autopsy cases in our study were in the 30-40 years

age group.

Degree of lumen narrowing in majority of the atherosclerotic LADs and LCXs was between 25-50 %, while most atherosclerotic RCAs showed <25 % lumen narrowing. But in study by Kumar et al, [17] majority showed lumen narrowing in the range of 50-75%. In the present study, the intimal area, rather than the thickness of the intima was measured in order to allow an accurate evaluation of eccentric or irregular atherosclerotic lesion.

In the present study, the values of both morphometric parameters derived from morphometry of atherosclerotic lesions showed a linear increase with each higher AHA grade of atherosclerosis reflecting the severity of atherosclerotic involvement. The mean percentage of luminal narrowing was highest for grade 5 lesions. The fibromuscular caps in AHA Grade 5 lesion contain a greater proportion of collagen fibers which oppose outward expansion of the vessel wall and thus narrowing of the lumen is a prominent feature of Grade 5 lesions [19]. The increase in mean IMR value with each higher grade of atherosclerosis reinforces the fact that medial thickness underlying the diseased intima (atherosclerotic plaque) is considerably thinner [20]. However, in the case of living subjects, measurement of Intima-Media thickness (IMT) by B-mode ultrasonography is used for determining the extent of atherosclerosis and for assessing the cardiovascular risk [21]. The major limitation of our study is absence of correlation of the study findings with the cardiovascular risk factors such as smoking and lifestyle habits, as there was no information available on these at autopsy.

5. Conclusion

The values of both mean percentage of luminal narrowing and mean IMR show linear increase with each higher AHA grade of atherosclerosis consistently in all the three coronaries. Hence, both these morphometric parameters correlate well with the morphological grades of atherosclerosis. These parameters, thus can serve as reliable indices for assessing the severity of atherosclerotic lesions supplementing the histomorphological analysis. Our study has attempted a comprehensive analysis of both morphological and morphometrical data on coronary atherosclerotic lesions. Future research on larger samples is suggested to confirm the findings of our study. It is also proposed to study the clinical correlates and associated risk factors of coronary atherosclerosis.

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