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RESEARCH ARTICLE

MORPHOLOGIC AND MORPHOMETRIC ANALYSIS OF ATHEROSCLEROTIC CORONARY ARTERIES IN MEDICOLEGAL AUTOPSIES

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ABSTRACT

Objective: Present study was aimed at evaluating the degree of severity of atherosclerosis by morphologic and morphometric indices. Study was also undertaken to correlate morphological grades with morphometric parameters.

Materials and Methods: Present study comprised of 80 heart specimens from consecutive medicolegal autopsy cases. Detailed gross examination of heart and four coronary arteries viz right coronary, left coronary, left anterior descending and left circumflex artery was done for evidence of infarction and atherosclerosis respectively. All the four arteries were assessed morphologically for 6 numerical grades as per the American Heart Association criteria. Thickness of intima, thickness of media, diameter of lumen and diameter internal to the media was measured under 400X using MICAPS software. Two morphometric parameters viz percentage of luminal narrowing and intima to media thickness ratio (IMTR) were evaluated for severity of atherosclerosis.

Result: Atherosclerosis was present in 81.87 % of cases. Females outnumbered males. Eccentric atheromas were seen in 67.81% of arteries. Left anterior descending artery had severe degree of atherosclerosis. Both parameters were directly proportional to the grades of atherosclerosis in all the four coronary arteries. Myocardial infarction was predominantly observed in grade VI atherosclerosis.

Conclusion: Comprehensive analysis of both morphologic and morphometric evaluation of arteries is a reliable tool to assess the severity of atherosclerosis.

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INTRODUCTION

Atherosclerosis is a disease of medium sized arteries characterized by hardening of vessel wall. The term is derived from greek word 'athero' means 'gravel' and sclerosis means hardening. Atheromatous or fibrofatty plaque consists of a central lipid core covered by a fibrous cap. Atheromatous plaque protrudes in the lumen and causes obstruction to blood flow. Besides this it is responsible for acute vascular catastroph due to complications of atheromatous plaque. Atherosclerotic coronary artery disease is the commonest cause of premature death in developed countries globally. By the year 2020 it will become the leading cause of death worldwide (Jain *et al.,* 2013). American Heart Association is a committee on vascular disease which has proposed a numerical six tier classification for the grading of atherosclerosis. Committee recommends the use of this numerical nomenclature as it avoids vagueness, ambuiguity and duplication of words (Jashnani *et al*., 2005 and Jeong *et al.,* 2010).

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Morphometric parameters like intima to media ratio and percentage of luminal narrowing is of great value in assessing the atherosclerosis in its subclinical state. (Kumar *et al.,* 2013)

Present study was undertaken for

- Evaluation of coronary artery atherosclerosis in autopsy cases by morphologic and morphometric analysis.
- To grade atherosclerosis as per AHA grades.
- Correlate AHA grades with morphometric parameters vizpercentage of luminal narrowing, intima to media thickness ratio (IMTR)

MATERIALS AND METHODS

Approval for conducting present study was obtained from the Institutional Ethics Committee. The present study comprises of 80 heart specimens of medico legal autopsies received in the Department of Pathology in tertiary care centre over a period of 6 months. Autolysed heart specimens were excluded from the study. Heart was fixed in 10% formalin. It was opened and dissected with Virchow's inflow outflow method. Right coronary and left coronary artery with its branches namely left

anterior descending and left circumflex were identified starting at their origin from aorta. Transverse cuts with sharp scalpel at intervals of 2 to 3 mm were given throughout the course of the artery till they enter the musculature. The exposed arteries were examined for thickening, narrowing, atheroma, thrombus and calcification. Representative bits from pathological areas from all the four arteries were taken and processed for paraffin embedding. Tissue blocks were cut at 5 micron thickness and stained with hematoxylin and eosin as per the standard protocol. Special stain like Verhoff's stain was employed where ever necessary.

Morphological Examination

All four vessels were examined and evaluated for presence of atherosclerosis and type of atherosclerotic plaque concentric or eccentric. Microscopic examination of cross section of all coronaries was done for morphological grading of atherosclerosis as per the American Heart Association criteria. American Heart Association criteria for grading atherosclerosis. (Jashnani *et a.,l* 2005, Jeong *et al.,* 2010 and Singh *et al.,* 2005)

- **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 Morphometric Evaluation and Analysis.

Computer assisted histomorphometric assessment was done using MICAPS (Microscope Imaging Captures Analysis and Processing System) software. All the four vessels were examined in the cross section for following morphometric parameters. Additional parameters were derived by mathematical calculations.

Following morphometric parameters were measured using MICAPS software

- Intimal and medial thickness – measured by method of Jashnani *et al.,* 2005and Jeong *et al.,* 2013. (Intima and media were measured at maximal intimal thickness)
- Diameter of lumen and diameter internal to media.
- No method is ideal. Present study adopted the method used by Jain *et al.,* 2013.

From above findings following morphometric parameters were derived

- Intima to media thickness ratio (IMTR)
- Percentage of luminal narrowing (obtained by subtracting luminal area from internal elastic lamina area and the resultant intimal area was divided by from internal elastic lamina area and results were expressed in %)
- Percentage of luminal narrowing for all coronaries were calculated &categorized into <25%, 25-50%, 51-75%, >75%. Simple arithmetic mean of percentage of luminal narrowing and IMTR was calculated for each grade in all 4 coronaries to minimize the error.

Values thus obtained were compared and correlated

RESULTS

Heart specimens of 80 medicolegal autopsies were studied during six months of study period.

Table 1. Age and Sex Distribution

Sr No.	Age (years)	Males (No. of cases)	%	Females (No. of cases)	%	Total
1	0-20	2	2.5%	6	7.5%	8 (10%)
2	21-40	18	22.5%	27	33.75%	45 (56.25%)
3	41-60	12	15%	5	6.25%	17 (21.25%)
4	61 and above	5	6.25%	5	6.25%	10 (12.5%)
	Total	37	46.25%	43	53.75%	80

Table 2. Vessel Narrowing

Sr No.	Nature	No. of arteries	Percentage
1	Concentric	103	32.18%
2	Eccentric	217	67.81%

Table 3. AHA Grade Distribution

Artery		Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Grade 6
RCA	No. of cases	11	22	11	20	5	11	0
	%	13.75%	27.50%	13.75%	25%	6.25%	13.75%	-
LCA	No. of cases	10	17	13	16	8	14	2
	%	12.50%	21.25%	16.25%	20%	10%	17.50%	2.5%
LAD	No. of cases	20	19	6	23	2	9	1
	%	25%	23.75%	7.5%	28.75%	2.5%	11.25%	12.5%
LCX	No. of cases	17	14	13	17	1	15	3
	%	21.25%	17.5%	16.25%	21.25%	12.50%	18.75%	3.75%
Total		58 (18.12%)	72 (22.50%)	43 (13.43%)	76 (23.75%)	16 (5%)	49 (15.31%)	6 (1.87%)

Morphologic and morphometric analysis was done and following are the results obtained. In the present study females 43 (53.75%) outnumbered males 37 (46.25%). Maximum no. of cases were found in age group 21-40 years (56.25%). Eccentric type (67.81%) of luminal narrowing was more common than concentric (32.18%). Above table depicts that commonly affected artery was Left coronary sparing only 12.5% of cases. Commonest atherosclerotic change in RCA & LCA was grade 1 followed by grade 3 whereas in LAD & LCX it was grade 3 followed by grade 1. Overall grade 6 type of lesion (Figure 1) was found in 6 (1.87%) cases and all were associated with acute myocardial infarction.

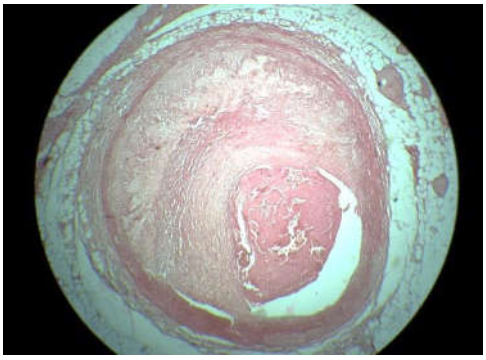

Figure 1. Photomicrograph(400X Verhoff's stain) shows >90% luminal occlusion

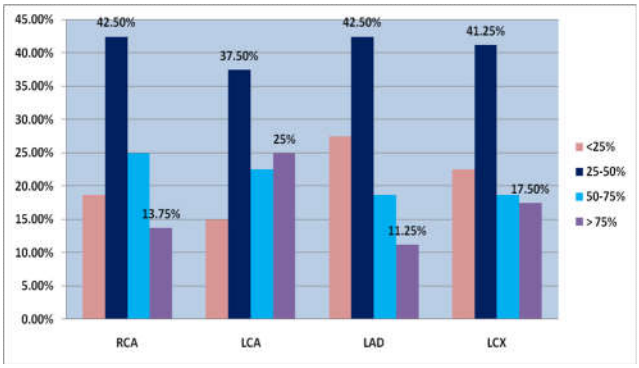

Chart 1. Percentage of luminal narrowing of all vessels

Above chart shows that luminal narrowing of 25-50% was the commonest finding in all four coronaries. Critical luminal occlusion (>75%) (Figure 2) was seen in 54 (16.87%) arteries of which LCA was the dominant victim followed by LCX.

Figure 2. Photomicrograph(400X H & E stain) shows critical luminal occlusion >75%

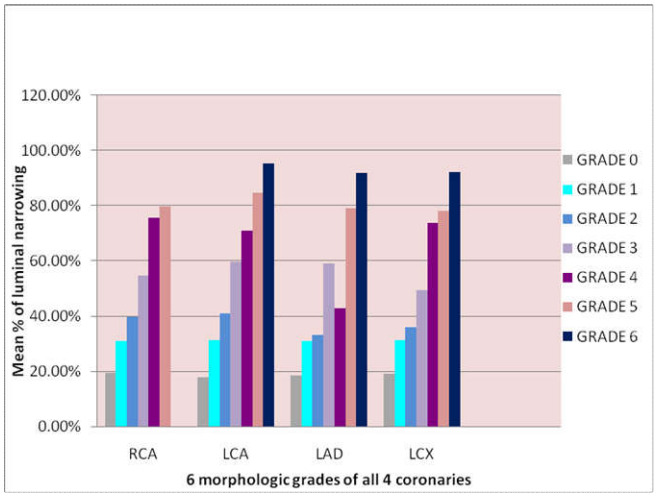

Chart 2. Values of mean percentage of luminal narrowing for each AHA grade

The above chart shows that the percentage of luminal narrowing went on increasing as the grade increased.

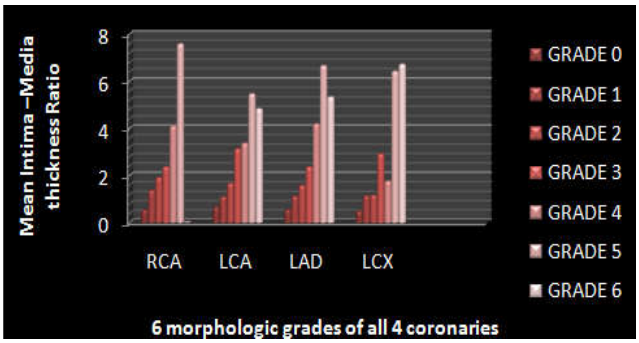

Chart 3. Values of mean IMTR for each AHA grade

This chart shows similar observations – severity of IMTR was directly proportional to the severity of atherosclerotic grade till grade 5. In grade 6 slight decline due to medial muscular hypertrophy which is a direct reaction to injury.

DISCUSSION

Autopsy studies showed atherosclerosis in coronary arteries as the commonest finding. Atherosclerosis is a chronic immune-inflammatory, fibro proliferative disease of large and medium sized arteries fuelled by lipids (Garg *et al*., 2011). Autopsy is a simple tool of assessment of different pathologies which are difficult to assess in living beings (Thej *et al*., 2012). The present study showed female preponderance whereas studies by Dhruva *et al*., 2012 (73.6%males), Puri *et al*., 2010 (80% males), Garg *et al*., 2011 (81% males), Thej *et al.,* 2012 (69%males), Singh *et al*., 2005(84% males) showed male preponderance. This disconcordence is due to difference in study design. Eccentric type of luminal narrowing was more common than concentric type. The similar observation was done by Waller B.F (1989). He found 73% eccentric type atherosclerotic plaque. Present study observed the highest frequency of atherosclerosis in left coronary artery whereas Jain *et al*., 2013, Vyas *et al*., 2015, Thej *et al*., 2012 observed it in LAD. This is due to difference in the study design- they did not include LCA in their study. In present study Grade 3 lesion was common. This finding is in accordance with Garg *et al*., 2011. Jain *et al*., 2013 found that atherosclerotic LADs and LCXs showed 25-50 % lumen narrowing while atherosclerotic

Table 1.Comparison of frequency of atherosclerosis in left coronary artery (LCA)

Name of Author	Singh *et al.*(2005)	Puri *et al.*(2010)	Dhruv *et al.*(2012)	Kumar *et al.* (2013)	Vyas *et al.*(2015)	Present study(2016)
Frequency of Atherosclerosis (%)	78%	86%	23.30%	80%	73.45%	81.88%

Table 2. Comparison of incidence of acute myocardial infarction

Name of Author	Garg *et al.* (2011)	Dhruva *et al.* (2012)	Vyas *et al.*(2015)	Present study (2016)
Acute Myocardial Infarction	3%	9.72%	10.80%	10%

RCAs showed <25 % lumen narrowing. Critical narrowing (>75%) was seen in 7 LADs, 4 LCXs and 2 RCAs respectively. However in the present study all the atherosclerotic vessels showed luminal narrowing between 25-50%. Critical narrowing (>75%) was seen in 25% of LCAs. This discordance was due to difference in the study design. Above table shows that the incidence of acute myocardial infarction in present study was 10% which is in accordance with Vyas *et al*. (2015), Dhruva *et al*. (2012). The present study showed that percentage of luminal narrowing andinitma to media thickness ratio went on increasing as the AHA grade increased. Similar observations were done by Jain *et al*., 2013. Morphometry showed direct relationship of increasing grades of atherosclerosis with increasing percentage of luminal narrowing and Intima to media thickness ratio which is well in accordance with already published data. Hence the study showed correlation between morphologic grades and morphometric parameters.

Conclusion

Subclinical atherosclerosis is a latent precursor of cardiovascular disease. Autopsy highlights the severity of lesion and gives insight for adopting the preventive measures and healthcare strategies. In the living B-Mode ultra sound can be used as a tool to measure IMTR which helps in identifying atherosclerosis and its burden in high risk cardiovascular subjects.

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