Histopathological Study of Coronary Atherosclerosis Using Special Stains and CD 34 Immunohistochemical Marker A Postmortem Study

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Summary:

Background: The coronary atherosclerosis received a great concern from the clinical aspect, but its pathological aspect is deficient in Iraq.

Objectives: To find a correlation between the type of the lesions that were grossly identified and their corresponding microscopical grades and Studying the effect of remodeling on preservation of the luminal area, 3) demonstrate the endothelial dysfunction in atherosclerosis.

Methods: fifty cases were gathered from the Medico-legal institute in Baghdad during the period from January to July 2004.The left anterior descending (LAD), left circumflex (LCX) and right coronary artery (RCA) from 50 postmortem cases were biopsied. Cryosectioning and staining with Oil-red O stain were done for twenty five specimens, then all the cases were embedded in paraffin blocks, and stained for hematoxylin and eosin (H&E) stain, Verhoff Van Gieson (VVG), twenty cases were stained with CD34 immunohistochemical marker. Cases were graded according to the American Heart Association (AHA) classification system.

Results: Seventy three per cent of grossly normal specimens were microscopically normal, while grossly flat fatty streaks correspond in 83% of cases to grade II. Raised fatty streaks were 100% grade III (intermediate lesions) and raised lesions were 100% advanced lesions (grade IV, V and VI). This study also shows that with progressive increment of plaque area, the total arterial cross-sectional area increased trying to preserve the lumen area. Endothelial dysfunction was also shown by decrease expression of CD34 immunohistochemical marker in diseased segments.

Conclusions: gross inspection of the vessel is a valuable method for detection of intermediate and advanced lesion, while the differentiation of early lesion from normal vessel needs microscopical examination. Remodeling has a great role in maintaining luminal patency in the major coronary arteries. This study also demonstrates the endothelial dysfunction overlying the atheroma in spite of endothelial integrity.

Key words: CD 34 immunohistochemical marker, coronary atherosclerosis, frozen section, postmortem study.

Introduction:

Arteriosclerosis: is an imprecise term, meaning thickening and loss of elasticity of arteries caused by conditions, such as hypertension, aging process, heavy cigarette smoking (1,2). Atherosclerosis: is the commonest form of arteriosclerosis characterized by intimal lesion called the atheroma (3). American Heart Association classifies atherosclerotic lesions into six grades according to certain criteria (4-8):

Grade 0: Normal artery with or without adaptive intimal thickening, no lipid.

Grade 1: Isolated macrophage foam cells (MFCs) containing lipid; no extracellular lipid; variable adaptive intimal thickening.

Grade 2: Numerous MFCs often in layers, with fine particles of extracellular lipid; no distinct pools; variable adaptive intimal thickening.

Grade 3: Numerous MFCs with >1 pool of extracellular lipid;

no well-defined core of extracellular lipid.

Grade 4: Numerous MFCs plus well-defined core of extracellular lipid with intimal surface covered relatively by normal intima.

Grade 5: Numerous MFCs, well-defined core of extracellular lipid, plus reactive fibrotic cap, vascularization, or calcium.

Grade 6: All f the above plus surface defect, hemorrhage, or thrombosis

AHA: American Heart Association   MFCs: macrophage foam cells.

An evolving plaque does not directly narrow the vessel lumen as it start to grow, progressive vessel enlargement compensate for a considerable period of time for intramural excess of tissue. With time outward bulging of arterial wall (remodeling) at the site atherosclerotic plaque maintaining the vessel cross-sectional area of the lumen (9,10). Luminal stenosis was only found to develop when 40% of its

potential area, defined by internal elastic lamina is occupied by the plaque (11-12) with a histological grade ≥ III AHA system (13). The biochemical mechanisms of arterial expansion are

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not known, release metalloproteinases which are responsible for extracellular matrix dissolution (14-15). Calcification correlates highly with plaque burden and may affect remodeling negatively (16).

CD34 endothelial immunohistochemical marker description: a single chain transmembrane glycoprotein is selectively expressed on human lymphoid and myeloid hematopoietic progenitor cells. CD 34 function is membrane staining. (17). Antibody to CD34 also reacts with vascular endothelial cells in normal tissues and in benign and malignant proliferations (18).

Materials and Methods

Segments from the three coronary arteries were collected from 50 non-selected cases that were autopsied within 48 hours of death (12) including all ages and both sexes. Cases gathered from the Medico-legal Institute in Baghdad, during the period from January 2004 to July 2004. For each coronary artery, the lesion was classified grossly into flat fatty streak, raised fatty streak, raised lesion and normal (if no lesion can be identified) (19). Specimens were divided into 2 halves, one half was frozen at −35°C. the other half was embedded in paraffin and sections were stained with: I) Hematoxylin and eosin stain to study the morphology. II) Verhoff Van Gieson stain to demonstrate the integrity of internal elastic lamina and then measuring intimal area. III) Oil Red O Stain was done for frozen specimens to demonstrate neutral lipids in atheroma (20).

IV) CD34 immunohistochemical marker was used to demonstrate endothelial dysfunction in which twenty selected cases from the 3 coronary arteries with different grades showing neither endothelial rupture nor erosion were chosen.

A total 50 cases of LAD coronary artery were histologically graded according to the AHA classification system (table-1). For each section the cross-sectional area percent stenosis was determined by the following formula: 100X (1-lumen area/ arterial size) (21). Then the severity of ATH was classified into: severe: if cross-sectional area stenosis was more than 75% moderate: if cross-sectional area stenosis was 50-75% mild: if cross-sectional area stenosis was less than 50% (22).

Statistical Analysis: Data were collected and analyzed using SPSS version 10.0 for Windows (SPSS, Chicago, Illinois, and USA). Differences between groups were examined by ANOVA (Analysis of variance of mean); correlation and Chi-squire test were done to detect significance of relation between various variables. P. value < 0.05 was considered as statistically significant, while P. value < 0.01 was considered as highly significant. Data were also expressed as mean +/- standard deviation.

Results:

Out of the total 50 cases, 38 cases (76%) were males and 12 (24%) cases were females. Their ages ranged from 5-58 years (mean; 36+/- 12.6 years). Cases were divided into 6 age groups at 10 years intervals

Table-1: Age and sex distribution in 50 cases.

Age/years	Male No. (%)	Female No. (%)	Total No. (%)
5-14	1(2%)	1(2%)	2(4%)
15-24	3(6%)	1(2%)	4(8%)
25-34	11(22%)	3(6%)	14(28%)
35-44	11(22%)	4(8%)	15(30%)
45-54	11(22%)	3(6%)	14(28%)
>54	1(2%)	0	1(2%)
Total	38(76%)	12(24%)	50(100%)

Correlation was done between gross and microscopical findings in 50 cases of LAD coronary artery trying to evaluate the significance of the gross observation (Table 2). From 26 cases grossly classified as normal 19 cases (73%) were found to be normal microscopically and 5 cases (19%) were grade I and 2 cases (8%) were grade II. The 6 cases which had grossly flat fatty streak, their microscopical sections showed that 5 cases (83%) were grade II and 1 case (17%) was grade I. In 5 cases the lesions were grossly found to be raised streaks, their microscopical sections showed 100% grade III lesion, while the 13 cases with grossly raised lesion, their microscopical sections revealed 84% grade V, 8% grade IV and another 8% grade VI. Figure 3 and 4 show grade Vb lesions by H&E and Oil-red-O stains respectively.

Table-2: Correlation between the type of the lesion grossly and its microscopical grade in the LAD. (P. value < 0.001)

Micro- scopica Grade Gross	0	I	II	III	IV	V	VI	Total N (%)
N	19(73%)	5(19%)	2(8%)	0	0	0	0	26(100%)
FFS	0	1(17%)	5(83%)	0	0	0	0	6(100%)
RFS	0	0	0	5(100%)	0	0	0	5(100%)
RL	0	0	0	0	1(8%)	11(84%)	1(8%)	13(100%)
Total	19	6	7	5	1	11	1	50

Normal FFS   flat fatty streak RFS raised fatty streak RL raised lesion

Discussion:

Very limited number of studies dealt with the pathology of coronary atherosclerosis in Iraq, in spite of the enormous number of such studies abroad. This may be attributed to the difficulties in obtaining a coronary artery biopsy. Postmortem dissection may be the only source to get these biopsies in Iraq. In western countries both postmortem and ante-mortem biopsies are available from patients who underwent allograft cardiac transplant procedures.

Gross observation of the coronary artery is a valuable method to detect intimal lesion. This is especially true for intermediate and advanced lesions. The main problem lies in the identification of early lesions from normal vessels. Some of the normal vessels were graded microscopically as I or II, this is explained by the fact that grade I lesion contains few scattered foamy macrophages identified under the microscope. Grade II lesions sometimes are seen by the naked eye only after immersing the biopsy in a lipid stain, Suddan IV stain is widely used for this purpose, in which grossly the fatty streaks appear black. Grossly identified flat fatty streaks were found microscopically to be grade I and II. This is explained by the fact that grade II lesions are usually seen by the naked eye as flat fatty streaks, while grade I lesions rarely persist to adulthood. Al-Saudi, Al-Hadithi and Baker studied the cardiac changes in sudden natural death (23) and Al-Habba S.Y and Al-Kaptan I. A studied the relation of coronary pathology to the risk factors of atherosclerosis in young Iraqi population(24) . Both studies mentioned that the gross examination of the coronaries is not sufficient for correct diagnosis of mild coronary atherosclerosis which is consistent with this study.

This study showed that an increase in the intimal area has little or no effect on the luminal area but it has a great influence on the total arterial cross-sectional area in the same vessel segment. This is explained by the effect of remodeling on the vessel lumen, so that with progressive intimal thickening and increment in the plaque area, the IEL area increased, trying to preserve the lumen area Burke AP, Kolodgie FD, Farb A et al. in 2002 (25) studied the morphological predictors of arterial remodeling, and used the computerized morphometry to measure the lumen area, plaque area and IEL area and found a linear relationship between plaque area and IEL area for the three arteries while they found no relationship between plaque area when plotted against lumen area in the LAD and RCA. This is quite consistent with this study.

In the current study the absence of staining or just low staining intensity was clearly demonstrated in all the lesions selected regardless the grade or the percent of stenosis in spite of their endothelial integrity, in comparison with the normal staining intensity observed in the endothelium of vasavasorum of the same section, or in segments distal to the lesion in the same vessel. Laszik ZG, Zhou XJ, Ferrell GL, Silva FG, Esmon CT. in 2001(22) worked on six patients who underwent heart transplantation, from each case 3 segments were taken from LAD and RCA showing different grades of the lesion with moderate and severe percentages of stenosis. Cases were immunostained with CD34 and CD31. They ended with quite similar results and stated that the observation of a decrease in expression of these endothelial markers suggests that thrombomodulin and endothelial cell protein C receptor may be down regulated in these regions, which favors local thrombus formation (22).

Conclusions:

Gross inspection of atherosclerotic lesion gives 100% correct result in interpretation of intermediate and advanced lesions. The main difficulty lies in the distinction of early lesion from normal,this study demonstrates the effect of remodeling on luminal patency and it is reflected by an increase in the total cross-sectional area, trying to preserve the luminal patency with progression of atherosclerotic grade from 0-VI and the endothelial dysfunction has been well-demonstrated immunohistochemically in the vessels with advances ATH lesions, reflected by decreasec expression of endothelial cell marker CD 34.

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