

A COMPARATIVE ANALYSIS OF CORONARY ADVENTITIAL T-LYMPHOCYTES —AN AUTOPSY STUDY

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Background: Recent clinical and histopathologic data suggests that inflammation plays a key role in coronary artery plaque instability and subsequent occlusive thrombosis. The intima has received much attention as a site of inflammation, while the adventitia has remained relatively unexplored. The aim of the present study was to investigate the frequency of inflammatory activity in the cap and shoulder region of unruptured, atherosclerotic lesions in coronary arteries and to correlate these findings with distribution of inflammatory cells in adventitia. **Methods:** The study was carried out in Histopathology Department, Army Medical College, Rawalpindi and National University of Sciences & Technology (NUST), from August 2008 to July 2009. Sixty-seven autopsy cases performed at Military Hospital Rawalpindi, Pakistan were selected. The cases were divided into study group and control group. Case group (n=35) included those where cause of death was ischemic heart disease. Those coronary arteries were taken as control (n=32) where atherosclerotic changes were found by chance (death without history of ischemic heart disease). Plaques in each group were assessed by light microscopy and by immunohistochemistry. **Results:** The ages of the deceased ranged from 38 to 49 years. Within study group, adventitial lymphocytes exhibited strong correlation with erosion, thrombus formation in culprit plaque ($p=0.001$). No correlation was found between adventitial T-lymphocytes and erosion of plaque ($p=0.700$) in control group. In 72% of culprit plaques moderate staining for T-lymphocytes was observed in adventitia as well as intima. In control group, most of the cases contained scattered cells. Few cases of stable plaques revealed lymphocytes as clusters, both in adventitia and in intima. **Conclusion:** Adventitial inflammation may play a pivotal role for atherosclerotic lesion histology and atheroma instability. With the help of these autopsy findings, we hope to be able to reduce the incidence of culprit plaques related to inflammatory reaction in patients of ischemic heart disease.

Keywords: Atherosclerosis, Culprit plaque, Adventitial inflammation, T-lymphocytes

INTRODUCTION

Recent clinical and histopathologic data suggests that inflammation plays a key role in coronary artery plaque instability and subsequent occlusive thrombosis.¹ Much experimental and clinical evidence links inflammation and atherosclerosis; for example, systemic indices of inflammation may play a role in predicting risk of coronary events.² Reports have attributed the inflammation, in the cap and shoulder of the atherosclerotic lesion, a fundamental pathogenetic role in plaque disruption and consequent acute thrombotic complications.^{3,4}

A few pathological studies have addressed the involvement of inflammation in the media or adventitia.^{5,6} Furthermore, adventitial inflammation may also contribute to intimal disease.⁷ Role of inflammation in atherosclerosis could involve not only the pathogenesis of atherosclerotic plaques, but also their rupture. Concerning this last point, the intima has received much attention as a site of inflammation⁸, while the adventitia has remained relatively unexplored.

Cell types that take part in plaque inflammation are macrophages, T-lymphocytes, mast cells and dendritic cells. The predominant leukocytes in atherosclerotic plaques are heterogeneous populations of macrophages/macrophage-derived foam cells and T-cells.⁹ Data of one study indicates that the major T-cell

compartment of the arterial wall is the lamina adventitia rather than the lamina intima.¹⁰ Among these, the participation of T-cells in atherogenesis is relevant as T-cells are immune regulatory cells, which, once activated are capable of modulating the intensity of the inflammatory response. These cells also influence synthesis and degradation of extracellular matrix proteins, implying that they may affect the stability of the plaque.

So far no histopathologic study has yet revealed whether plaque inflammation should be considered a local event or it is also related to presence of inflammatory infiltrate in adventitia.

The aim of the present study was to investigate the frequency of inflammatory activity in the cap and shoulder region of unruptured, atherosclerotic lesions in coronary arteries and to correlate these findings with distribution of inflammatory cells in adventitia.

MATERIALS AND METHODS

Study was conducted in the department of histopathology Army Medical College, Rawalpindi and National University of Sciences & Technology (NUST), in a period of one year from August 2008 to July 2009. The study was designed to assess 67 autopsy cases performed at Military Hospital Rawalpindi, Pakistan. The cases were divided into study group and control group. Case group (n=35) included those specimens

where cause of death was ischemic heart disease (IHD). Those coronary arteries were taken as control (n=32) where atherosclerotic changes were found but death was due to non-cardiac causes.

After receiving the heart specimens in histopathology department, the four major epicardial coronary arteries (left main, left anterior descending, left circumflex, and right coronary arteries) and their major branches were carefully dissected from the heart. Sections of those coronary arteries were selected where atherosclerosis was apparent on gross inspection. Sections were processed routinely for histopathologic study.

All coronary plaques from both groups were evaluated by light microscopic examination. Each case was analysed for the presence of thrombus, cap erosion and plaque area.

Plaques in each group were classified according to the modified American Heart Association atherosclerosis classification¹¹ and as per consensus document of the American Heart Association.¹² Two categories were recognised culprit plaques having an acute thrombus associated with plaque rupture or plaque erosion. Plaques with thin fibrous cap (65–150 µm) were included in this category; the remaining plaques were classified as stable plaques. Fibrous cap thickness was quantified in micrometers by manual ocular micrometer.

The immunohistochemistry was performed on all arterial sections. Arteries were stained with anti-CD45RO to characterise and quantify T-lymphocytes within the plaque and adventitia. The lymphocytes present in all 40× microscopic fields of the adventitia were counted in each of the selected sections. The total amounts were divided by the number of fields and divided by 0.22 mm² (which is the area of each microscopic field)¹³ to obtain the mean adventitial density of lymphocytes/mm². Lymphocytes located within the plaques were also counted; their numbers were divided by the area of the plaque (discounting the fat area, which was devoid of lymphocytes) to obtain density of lymphocytes/mm² in plaque.¹⁴

To find out degree of inflammation, the number of immunohistochemically positive cells for anti-CD45RO antibody was evaluated at a magnification of 40× in the plaque and adventitia. Cells were assessed in 5 fields showing maximum density and graded semi-quantitatively:

- (no cells present), + (few scattered cells present), ++ (scattered cells and clusters with >10 cells).¹⁵

RESULTS

Samples from a total of 67 autopsy cases (35 with ischemic heart disease; study group and 32 without ischemic heart disease; control group) were studied.

Their ages ranged from 38 to 49 years.

In study group, adventitial lymphocytes exhibited strong correlation with erosion, thrombus formation in culprit plaque ($p=0.001$, Table-1). The data of control group, where atherosclerosis was found by chance showed no correlation between adventitial T-lymphocytes and erosion of plaque ($p=0.700$, Table-2). In study group, 72% of culprit plaques demonstrated moderate staining for T-lymphocytes in adventitia as well as intima (Table-3). Whereas, in control group, most of the cases contained scattered cells. Few cases of stable plaques revealed lymphocytes as clusters, both in adventitia and in intima (Table-4).

Table-1: Correlation of T-Lymphocytes density in adventitia and intimal plaque in Case Group

	Mean density of T-lymphocytes in intimal plaque (mm ²)	Mean density of T-lymphocytes in adventitia (mm ²)	r	p-value
Culprit plaque (n= 18)	34.97±48.89	11.07±10.80	.736	0.001*
Stable plaque (n=17)	69.93±90.90	14.43±13.39	.436	0.119

* p-value is significant

Table-2: Correlation of T-Lymphocytes density in adventitia and intimal plaque in Control Group

	Mean density of T-lymphocytes in intimal plaque (mm ²)	Mean density of T-lymphocytes in adventitia (mm ²)	r	p-value
Culprit plaque (n= 7)	54.24±40.25	27.19±21.17	0.238	0.700
Stable plaque (n=25)	31.15±35.05	10.73±10.40	0.558	0.004*

*p-value is significant

Table-3: Percentages of immunostaining for CDRO45 (Case Group)

	Degree of CDRO 45 staining		
	-	+	++
Culprit plaque n=18			
Intimal plaque area n (%)	3 (16.6 %)	13 (72.2 %)	-
Adventitia n (%)	3 (16.6 %)	13 (72.2 %)	-
Stable plaque n=17			
Intimal plaque n (%)	2 (11.7 %)	12 (70.5 %)	-
Adventitia n (%)	2 (11.7 %)	12 (70.5 %)	-

Table-4: Percentages of immunostaining for CDRO45 (Control Group)

	Degree of CDRO 45 staining		
	-	+	++
Culprit plaque n=7			
Intimal Plaque n (%)	-	7 (100 %)	-
Adventitia n (%)	-	7 (100 %)	-
Stable plaque n=25			
Intimal Plaque n (%)	-	22 (88 %)	3 (12 %)
Adventitia n (%)	-	23 (92 %)	2 (8 %)

DISCUSSION

The role of adventitial inflammation in coronary atherosclerosis is not clear. The relationship between adventitial inflammation and culprit plaque morphology has not been studied in depth. Features associated with plaque instability are associated with significantly greater degrees of adventitial inflammation.¹⁶

The predominant leukocytes in atherosclerotic plaques are heterogeneous populations of macrophages/macrophage-derived foam cells and T cells.⁹ The presence of T cells within the adventitia of atherosclerotic lesions has been confirmed by multiple investigators.¹⁷

Plaque rupture is the principal cause of luminal thrombosis occurring in 75% of patients dying of an acute myocardial infarction. In up to 25% of cases, thrombosis may result from superficial erosion over a plaque.¹⁸ Inflammatory activity has been associated with plaque erosion and may have a role in the pathogenesis of endothelial damage. In a study, erosion of thrombosed coronary atherosclerotic plaques was characterized by an inflammatory infiltrate regardless of plaque structure.¹⁹

In the outer layer of the adventitia of infarct-related coronary arteries in patients with myocardial infarction, lymphocytes and macrophages were found. Adventitial inflammation may play a pivotal role for atherosclerotic lesion development and atheroma instability. Although macrophages are well-recognized to play a proatherogenic role, the impact of lymphocyte subpopulations remains to be fully understood.

In a study it was found that the number of T-cells present in human plaques is highly variable and also relates to the overall plaque morphology.⁹ This information, obtained mainly in the aorta, suggests an active role of an adventitial lesion in generating an immune response. In the abdominal aorta, the rate of inflammation was found to be higher in the adventitia underneath ruptured plaques than in the adventitia under fatty streaks or fibrous plaques.²⁰ Few studies have been conducted for the coronary arteries. Kohchi *et al*²¹ and Stratford *et al*²² observed a significant increase in the rate of adventitial inflammation in patients with fatal acute myocardial infarction (AMI). Neither group correlated the adventitial infiltrate with the plaque type. We demonstrated the role of adventitial T-lymphocytes in plaque erosion. In cases where cause of death was ischemic heart disease, mean density of T-lymphocytes in adventitia of culprit plaque, was more closely related to mean density of same in intima ($p=0.001$). In contrast, control group, where atherosclerosis was found by chance; did not show any correlation between adventitial T-lymphocytes and erosion of plaque ($p=0.700$).

A focal infiltration of inflammatory cells into the coronary adventitia is a well-recognized pathologic alteration in the atherosclerotic coronary artery. According to previous data,²¹ such focal infiltrations were encountered more frequently in cases of coronary death than in the patients with angina who died of non-cardiac causes. Houtkamp *et al*²³ demonstrated the presence of follicular aggregates composed of B and T cells in the aortic adventitia. The pattern of these cells was different in the present data. Instead of focal clusters, we found scattered lymphocytes in maximum number of patients who died due to ischemic heart disease. Our study effectively demonstrated T lymphocyte population in atherosclerotic arteries by immunostaining. This data, in the context of the earlier studies, indicates that degree of staining does not have any significant difference in eroded or stable plaques. Although these results do not match with a recent data by Higuchi *et al*²⁴, who demonstrated significantly more lymphocytes in coronary culprit lesions than in stable lesions in patients with fatal AMI.

CONCLUSIONS

Compared with stable plaques, culprit plaques have an increased incidence of adventitial inflammation. Therefore, adventitial inflammation may play a pivotal role for atherosclerotic lesion histology and atheroma instability. With the help of these autopsy findings, we hope to be able to reduce the incidence of culprit plaques related to inflammatory reaction in patients of ischemic heart disease.

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