ASSESSMENT OF ATHEROSCLEROTIC PLAQUE VULNERABILITY OF CORONARY ARTERIES IN CASES OF SUDDEN CARDIAC DEATH

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ABSTRACT

Objective: To study the plaque vulnerability in coronary arteries taken from autopsy specimens, of individuals dying of ischemic heart disease in our setup and to compare it with atheroma of those who died of non-cardiac causes.

Study Design: Case control study.

Place and Duration of Study: Study was carried out in the department of Pathology (Histopathology), Army Medical College, Rawalpindi and National University of Sciences and Technology (NUST), from June 2008 to June 2009.

Materials and Methods: Sixty coronary arteries having atherosclerosis, from autopsies of patients who died of sudden cardiac death were divided into case and control groups. Case group included thirty coronary arteries having atherosclerosis from autopsies of patients of whose death was attributable to Ischemic Heart Disease (IHD). Control group included thirty coronary arteries where atherosclerotic changes were found by chance (death not attributable to ischemic heart disease). Plaques were assessed for fibrous cap thickness, foam cells; mean percentage of inflammatory cells on Haemotoxylin & Eosin (H&E) stained slides whereas immunohistochemical (IHC) markers for T-Cells were done by IHC stain method.

Results: In present study, foam cells are significantly more in study group than in control group (P=0.007). Fibrous cap thickness fulfilling the criteria of vulnerable plaque was more in study group as compared to control group (P<0.001). The present study demonstrated that there was insignificant difference (P=0.152), in the mean percentage of inflammatory cells in case group and control group. An overall significant association was found between vulnerable plaque and death due to ischemic heart disease (P<0.001).

Conclusion: Patients dying of ischemic heart disease have more vulnerable plaque in their coronary arteries as compared to those dying from non ischemic cause. Although this is an autopsy study but the significance of in this study can be very important to guide cardiologists to identify patients at high risk of acute coronary syndrome and use new diagnostic modalities like intravascular ultrasonography and therapeutic strategies like genomic and proteomic techniques. This will help the early detection and treatment of such cases and may ultimately reduce the incidence of sudden cardiac death.

Key Words: Sudden cardiac death, atherosclerotic plaque, vulnerable plaque.

INTRODUCTION

Every year more than one million people in the United State and more than nineteen million worldwide, experience a sudden cardiac event (acute coronary syndromes and / or sudden cardiac death). [1] Sudden cardiac death (SCD) is an unexpected death due to cardiac cause occurring in a short time period (generally within one hour of symptom onset) in a person with known or unknown cardiac disease in whom no previously diagnosed fatal condition is apparent [2]. Ventricular fibrillation (VF) precipitated by ventricular tachycardia (VT) is a common mechanism of cardiac arrest leading to SCD. [3] Sudden cardiac death (SCD) according to Zipes, 2005, is related to underlying coronary artery disease in majority of cases [4].

Coronary atherosclerosis was also found to be the underlying cause of sudden cardiac death in the vast majority of cases as observed in one of the studies from Pakistan [5]. Coronary artery atherosclerosis is a disease which begins in early life, by third decade the advanced plaque is seen in most of western population [6]. The overall frequency of coronary artery disease in Pakistan is reported

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to be 26.9%, 23.7% in men, 30.0% in women [7]. A prospective observational study at Armed Forces Institute of Cardiology Rawalpindi Pakistan showed 72% had coronary artery disease.[8] in symptomatic females. In India, prevalence of coronary artery disease was reported to be 9.5% and in rural areas, 4% [9].

Atherosclerosis of the coronary arteries is a chronic inflammatory, fibroproliferative disease affecting primarily the intima of the major coronary vessels.[10] Atherosclerotic plaques particularly prone to rupture, so- called vulnerable plaque, are pivotal in the genesis of acute coronary syndromes.[11] Vulnerable plaque is most suitably to define as plaques susceptible to complications.[12] Vulnerable plaque is particularly prone to produce sudden major problems, such as a heart attack or stroke. These plaques are at increased risk of disruption leading to thrombus formation and have a higher likelihood of rupture leading to a major acute coronary event. Rupture of vulnerable plaques is the main cause of acute coronary syndrome and myocardial infarction. Identification of vulnerable plaque is, therefore, essential to enable the development of appropriate treatment modalities. The present study is designed to assess the morphological features of vulnerable plaque as its recognition and early detection has clinical implications in patients of sudden cardiac death. Aim of this study is to assess the plaque vulnerability in coronary arteries taken from autopsy specimens, in individuals dying of ischemic heart disease in our setup and to compare it with atheroma of those who died of non-cardiac causes.

MATERIALS AND METHODS

This case control study was carried out from June 2008 to June 2009 in department of Histopathology, Army Medical College, Rawalpindi. Sixty coronary arteries having atherosclerosis from autopsies of patients who died of sudden cardiac death were divided into case and control groups. Case group included thirty coronary arteries having atherosclerosis from autopsies of patient's death attributable to Ischemic Heart Disease (IHD). Control group included thirty coronary arteries where atherosclerotic changes were found by chance (death without history of ischemic heart disease). Tissue showing autolytic changes were not included in this study. Histological evaluation was done on formalin fixed, paraffin embedded sections of atherosclerotic plaque of coronary arteries, stained with H &E stain. Coronary arteries were examined for various components of vulnerable plaque (Table-1) as proposed by Forrester [13]. Immunohistochemical technique was used to stain inflammatory cells (T-Lymphocytes) in atherosclerotic plaque by using CD45RO immunomarker.

Number of foam cells and inflammatory cells were counted under 40 x objectives by using an eyepiece reticule on which a square was engraved. One side of square was calibrated against a stage micrometer and then area of the square was calculated. The cells were counted on an H&E stained sections within the square of an eyepiece reticule measuring 0.01mm² areas. Thickness of fibrous cap was calculated by ocular micrometer. Five fields were chosen for morphometric analysis of fibrous cap. The ocular micrometer scale was superimposed on the fibrous cap and the mean thickness of the fibrous cap was calculated. Calcification and intraplaque hemorrhage were assessed by simple microscopy. IHC indirect technique was used for detection of T lymphocytes in atherosclerotic plaque of coronary arteries. This technique was applied on sixty slides of atherosclerotic plaque. Results were obtained by means of counting stained T lymphocytes by CD45RO immunomarker. Scoring was as, zero for no score, +1 for a few scattered cells and +2 scattered cells and clusters of >10 cells [14].

The data had been analyzed using SPSS version 15. Descriptive statistics were used to describe the data. Association between vulnerable and stable plaque between case and control groups was calculated by using chi-square test. Results were considered significant if P-value is less than 0.05.

RESULTS

Sixty autopsy specimens of coronary atherosclerosis were included in the study.

Thirty autopsy specimens from cases of death attributable to ischemic heart disease and their coronary artery had atherosclerosis. Another 30 coronary arteries were included in this study where atherosclerotic changes were found by chance, and their cause of death was not ischemic heart disease. All were males with a age range of 20-60 years and median age of 36.00 (Table-2).

Presence of foam cells (Figure-1) is one of the important criterion (singular) for vulnerable plaque. In present study foam cells were found in 53.32% cases of case group as compared with control (20%) (P=0.007). In 24 (80%) cases of case group fibrous cap thickness was in range of 65-150µm fulfilling the criteria for plaque vulnerability compared with the control group, where only 4 (13.3%) had fibrous cap thickness in range of 65-150µm (P<0.001) (Figure-2).

The present study demonstrated that when mean percentage of inflammatory cells was calculated in case and control group, heigher percentage was found in case group i.e 36.7% compared with control group i.e 20% (P=0.152) (Figure-3),

CD45RO Immunomarker (Figure-4) was found positive in 23 (76.7%) autopsy specimens and negative in 7 (23.3%) autopsy specimens in case group. Seventeen (56.7%) autopsy specimens showed CD45RO positivity and 13 (43.3%) autopsy specimens showed no staining (Negative staining) in control group (P=0.100). In present study, mean percentage of calcification was calculated in case and control group. Heigher percentage was found in case group i.e 36.7% compared with control group i.e 3.3% (P=0.001). No intraplaque hemorrhage was seen in atherosclerotic plaque of case and control group.

As per laid down criteria 24 (80%) autopsy specimens showed features of vulnerable plaque and 6 (20%) autopsy specimens showed stable plaque in case group. In control group 4 (13.3%) autopsy specimens showed vulnerable plaque and 26 (86.7%) autopsy specimens showed stable plaque. An overall significant association was found between vulnerable plaque and death due to ischemic heart disease (P<0.001).

DISCUSSION

Coronary atherosclerosis is the most frequent cause of ischemic heart disease. The composition and vulnerability of the atherosclerotic plaque determines the development of acute coronary syndromes [15]. The risk for plaque disruption depends more on plaque vulnerability. Lipid-rich and soft plaques are more vulnerable and prone to

Table-1: Components to be assessed for a vulnerable plaque

S No	Major Criteria	S No	Minor Criteria
1.	Increase foam cells (>40% of total lesion area)	1.	Calcification
2.	Thin fibrous capsule (65 – 150 µm)		
3.	Presence or absence of mononuclear cells (≥25 cells /40xobjective)	2.	Intraplaque hemorrhage
4.	Immunohistochemistry for the status of T cells		

Table-2: characteristics of the sixty autopsy specimens of coronary atherosclerosis included in the study

Variable (unit of measurement)	Case group	Control group
Age in years; (median/range)	37 (21-58)	35 (25-50)
Sex(M/F)	Male	Male
Patients with and without Ischemic Heart Disease(N)	30	30
Foam Cell (mean percentage)	53.3%	20%
Fibrous Cap (mean percentage)	80%	13.3%
Inflammatory Cells (mean percentage)	36.7%	20%
Immunohistochemistry for T cells (positive cases)	76.7%	56.7%
Calcification	36.7%	3.3%
Intraplaque Hemorrhage	Absent	Absent
Vulnerable Plaque	80%	13.3%
Stable Plaque	20%	86.7%

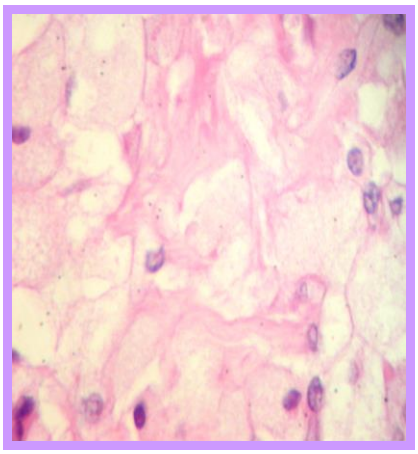

Figure 1: Photomicrograph having many foam cells H*E x 100.

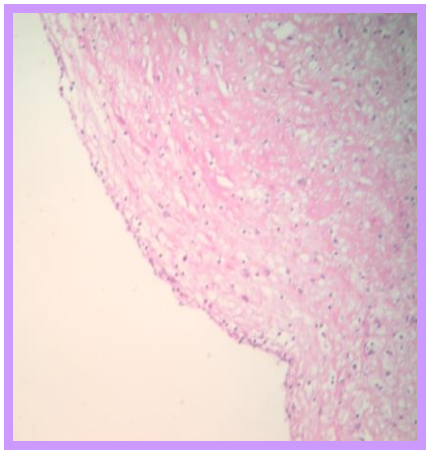

Figure-2: Photomicrograph Showing atheroma plaque with fibrous cap.

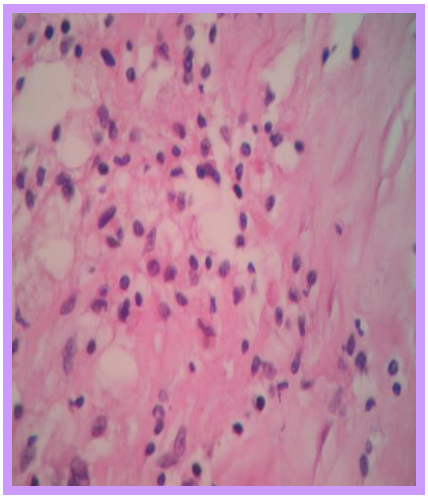

Figure-3 atheroma plaque with numbers inflantomy cells. H*E x 100

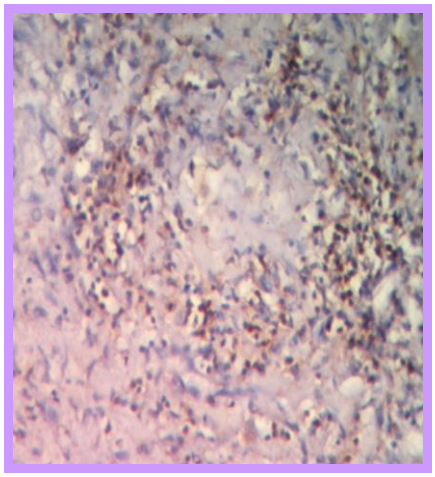

Figure-4 CD45RO Positive steiving in atheroma plaque.

rupture than collagen-rich and hard plaques. There seems to be three major determinants of a plaque's vulnerability to rupture, the size and consistency of the lipid-rich atheromatous core, the thickness of the fibrous cap covering the core, and ongoing inflammation and repair processes within the fibrous cap [16].

Our results show a strong relationship between coronary atherosclerotic plaque vulnerability and ischemic heart disease which is almost similar to previous studies conducted by Cheruvu et al in 2007 on 50 whole hearts taken from patients who died of cardiovascular (n=33), non cardiovascular (n=13) and unknown causes (n=4) [17]. They found that frequency of vulnerable and ruptured plaques were greater in individuals dying of cardiovascular cause than in those dying of non cardiovascular or unknown cause. Similarly in another study, 41 patients had vulnerable plaque out of 54 [18].

Thin cap vulnerable plaques are more frequent in patients dying of acute myocardial infarction and are least common in incidental non coronary deaths [19]. Virmani et al in 2002 also demonstrated that the vulnerable plaques are most frequent in coronary arteries of patients dying of acute myocardial infarction, and same was observed in present study [20].

Presence of foam cells is one of the important criteria for vulnerable plaque. In present study significant difference was found between case group and control group and same was observed by others [21]

The present study showed higher percentage of fibrous cap thickness in the range of 65-150µm in case group as compared to

control group. Same was observed in another autopsy study of Virmani et al in 2002 which demonstrates the presence of vulnerable plaque having a necrotic core with an overlying thin fibrous cap of 65µm in patients dying of acute myocardial infarction [20].

Increased inflammatory cells are also seen in vulnerable atherosclerotic plaque as compared to stable plaque. In an autopsy study of 74 coronary arteries of elderly people, macrophages were observed in the cap and shoulder in 118 of 282 (42%) of non ruptured plaques in coronary arteries [14]. Similarly another study stated that when compared with lesions underlying chronic stable angina, the lesions of patient with unstable coronary syndromes contain significantly larger amounts of inflammatory cells [22]. An interesting relationship was also seen between the amounts of inflammatory cells in the lesions and the severity of various unstable ischemic syndromes [22].

The present study demonstrated that mean percentage of inflammatory cells and CD45RO positive T cells were significantly more in case group as compared to control and similarly in previous study conducted by Pasterkamp et al in 1999 [23], immunohistochemical markers CD68 and CD45RO showed more T lymphocytes in the atherosclerotic cap and shoulder of the unruptured plaques of coronary arteries.

CONCLUSIONS

Patient dying of ischemic heart disease have more vulnerable plaque in their coronary arteries as compared to those dying from non ischemic cause. From post mortem observations we have learned that large lipid core and thin cap with increased inflammatory cell are frequently observed phenomena in non ruptured atherosclerotic lesions. Although this is an autopsy study but the significance finding found in this study can be very important to guide cardiologists to identify patients at high risk of acute coronary syndrome and use new diagnostic modalities like intravascular ultrasonography and therapeutic strategies like genomic and proteomic techniques. This will help the early detection and treatment of such cases and may ultimately reduce the incidence of sudden cardiac death.

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