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Atheromatous Plaque Reflects Serum Total Cholesterol Levels: A Comparative Morphologic Study of Endarterectomy Coronary Atherosclerotic Plaques Removed from Patients from the Southern Part of India and Caucasians from Ottawa, Canada

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Summary

Background: Natives of South India have a very high incidence of coronary artery disease, despite low calorie and fat intake.

Hypothesis: This study was undertaken to determine whether morphologic features of atheromatous plaque reflect the serum total cholesterol.

Methods: Fifty-three endarterectomy specimens from patients (mean age 47 ± 9 years, mean cholesterol 203 ± 47 mg/dl) obtained from one cardiac surgeon working in a single institution in South India were evaluated. Morphologic findings were compared with 40 endoarterectomy specimens obtained from age-matched Caucasians from Ottawa, Canada, with a reported mean cholesterol of 262 ± 47 mg/dl. Morphometric measurements of the vessel size, percent stenosis, and the various components of the atherosclerotic plaque were determined by computerized planimetry.

Results: The vessel size was smaller in the Indian than in the Canadian population (4.6 ± 2.9 vs. 5.6 ± 3.0 mm^2, p = 0.07), the plaque area was less (4.3 ± 2.3 vs. 5.3 ± 2.8 mm^2, p = 0.055) and the calculated percent stenosis was significantly less (93 vs. 96%, p = 0.028). Of all the parameters evaluated, only necrotic core in the Indian population (7.1 ± 10.9% vs. Canadian 16.7 ± 19.7%, $p < 0.001$) and proteoglycan deposition (7.9 ± 11.2% vs. Canadian 3.7 ± 5.3%, $p < 0.023$) were significantly different. Despite the Indians having low total cholesterol, there was greater diffuse double and triple-vessel disease and at a younger age than in the Caucasians.

Conculsions: From our data, it appears that the mechanism of development of atherosclerotic disease in the Indians may be different because they have smaller vessels, smaller necrotic core, and greater proteoglycan deposition. Other etiologies, especially those related to a high carbohydrate diet (which is typical for South Indians), should be considered.

Key words: plaque morphology, total serum cholesterol, South Indian, Caucasian, coronary endarterectomy

Introduction

Immigrants from the Indian subcontinent have been shown to have a very high incidence of coronary artery disease throughout the world.[1] This high incidence has been reported from Fiji,[2] South Africa,[3] Trinidad,[4] the United Kingdom,[5] the USA,[6] and India.[7] Conventional risk factors do not explain this high incidence of coronary disease in these populations. In India itself, there is a significant difference in the incidence of coronary artery disease between the northern and southern part of the country.[7] The dietary habits of the North and South are markedly dissimilar. In the North, the fat consumption is 18 to 19 times higher than in the South, and the sugar consumption is 90 g per person per day in the North compared with only 10 g per person in the South.[7]

The major component of a South Indian diet is complex carbohydrates. Despite low caloric and fat intake, South Indians have a very high incidence of coronary artery disease. We set out to explore the paradox of a diet low in fat with the high incidence of atherosclerosis in South India. Our hypothesis

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was that a low fat diet should be reflected in the composition of the atherosclerotic plaque. In this study, we evaluated the morphologic characteristics of atheromatous plaque from endarterectomy specimens obtained from Madras, South India, where fat intake is low, and compared them with endarterectomy specimens from North America, where high fat intake is found to play a major role in the pathogenesis of atheromatous plaque.

Materials and Methods

We obtained endarterectomy specimens from 53 patients, aged 47 ± 9 years, from one cardiac surgeon working in a single institution in Madras, South India. Patient records were reviewed for the presence of coronary risk factor and extent of disease. These were compared with 40 endarterectomy specimens obtained from age-matched Caucasian patients from Ottawa, Canada. All specimens were cut transversely at 3–4 mm intervals and decalcified, when required, prior to cutting. All arterial segments were processed in graded series of alcohol and xylene, and embedded in paraffin. Sections were cut at 4–5 μm intervals and then stained by hematoxylin and eosin and Movat pentachrome stain.

All sections were examined under light microscopy, and the cross section with the greatest luminal narrowing was selected, magnified (×15), digitized, and computerized morphometry was performed. The area of calcific deposit was defined by diffuse brown granular staining by Movat stained sections and by granular purple staining by hematoxylin and eosin stain. Only areas of confluent calcification could be measured. Sparse speckled calcification could not be outlined because of decalcification, and therefore the calcified areas measured may underestimate the true calcified area. Necrotic core (pultaceous debris) was identified by pale staining amorphous areas containing cholesterol clefts, with or without plaque hemorrhage. Areas rich in proteoglycans were identified by intense green staining on Movat stain. Smooth muscle cells were often interspersed in the proteoglycan matrix. Areas of foam cells were recognized when plump vacuolated cells were present in aggregates with or without associated few lymphocytes. Inflammatory infiltrate was defined as large numbers of lymphocytes (with or without plasma cells) with absence of or only rare foam cells. Morphometric measurements were performed with IP Lab™ image analysis software (Version 2.5). The following measurements were made or calculated: Area within the internal elastic lamina (IEL), lumen area and plaque area (IEL area - lumen area), and cross-sectional area percent stenosis = 100 × 1−(lumen area/IEL area). Plaque components consisting of calcified area, necrotic core, proteoglycan, foam cells, and inflammatory infiltrates were outlined and measured individually. The area occupied by collagenous fibrous tissue was derived [plaque area − (Ca^{++}area + necrotic core + proteoglycan area + foam cells +inflammation)]. In both groups, calcium, necrotic core area, proteoglycan, and area of fibrous tissue were expressed as a percentage of the total plaque area.

Results

Indian Population Characteristics

The Indian population (51 men, 2 women, n = 53) was young: the mean age was 47 ± 0.5 years. Weight of the individuals varied from 47 to 94 kg, with an average of 68 kg. Body surface area varied from 1.48 to 2.10 with an average of 1.75 kg/mm^2.

Coronary Risk Factors

Among Indians, 56% of the individuals were smokers, 54% had diabetes mellitus, and 41% had hypertension; 33% reported a family history of premature heart disease.

Lipoproteins among the Indian

The total serum cholesterol varied from 155 to 310 mg/dl with a mean of 203 ± 47 mg/dl; 49% of the population had a cholesterol < 200 mg/dl. High-density lipoprotein (HDL) in the population varied from 32 to 52 mg/dl with a mean of 40 ± 2 mg/dl, and low-density lipoprotein (LDL) varied from 82 to 201 mg/dl with a mean of 143 ± 35 mg/dl. Triglycerides varied from 123 to 270 mg/dl with a mean of 190.3 ± 44.5 mg/dl.

Angiographic Features

The majority of patients from India (63%) had multivessel disease; not a single patient had single-vessel disease.

Lipoproteins of Canadians (as Reported Previously)

The total cholesterol in the Canadian population was 262 ± 47 mg/dl.[8] Further demographics and the clinical data from the Canadian patients were not available.

Morphologic Features of the Plaque

Indians had smaller vessel size defined by the area enclosed within IEL (4.6 ± 2.9 vs. 5.6 ± 3.0, p = 0.07) and plaque area was also smaller (4.3 ± 2.3 vs. 5.3 ± 2.8, p = 0.05) than in Caucasian Canadians, but there was no significant difference in the lumen area. The various components of the atherosclerotic plaque showed significantly smaller necrotic core in the Indians, and a larger area was occupied by proteoglycans in the Indians compared with the Caucasian Canadians (Table I). When each component was expressed as a percent of the plaque, these differences in necrotic area and proteoglycan between the Indians and Canadians were highly significant (Table II).

Necrotic Core

Necrotic core size was evaluated as a percent of the plaque. In the Indians, the mean necrotic core of the atherosclerotic plaque was 7.1 ± 10.9%, whereas in the Canadians it was 16.7

TABLE I Comparison of morphometric analysis of coronary endartectomy specimens from Indian and Canadian patients with ischemic heart disease

Group	IEL area (mm^2)	Lumen area (mm^2)	Plaque area (mm^2)	% Stenosis	Necrotic core area (mm^2)	Proteoglycan area (mm^2)	Calcified area (mm^2)	Fibro-collagenous area (mm^2)
Indian	4.58 ± 2.86	0.29 ± 0.31	4.29 ± 2.30	92.9 ± 0.01	0.71 ± 0.83	0.61 ± 0.59	0.78 ± 0.98	3.16 ± 2.21
Canadian	5.59 ± 3.01	0.26 ± 0.41	5.33 ± 2.83	95.5 ± 0.05	1.68 ± 1.7	0.27 ± 0.24	0.84 ± 0.83	3.62 ± 1.94
p Value	0.074	0.604	0.055	0.028	0.004	0.010	0.802	0.256

Abbreviation: IEL = internal elastic lamina.

TABLE II Comparison of the composition of the atherosclerotic plaque expressed as percent of plaque area in Indian and Canadian patients with ischemic heart disease

Group	Plaque area (mm^2)	% Necrotic core	% Proteoglycan	% Fibrocollagen tissue	% Calcified tissue	% Foam cells	% Inflammatory cells
Indian	4.29 ± 2.30	7.1 ± 10.9	7.9 ± 11.2	74.7 ± 18.4	8.7 ± 16.2	0.96 ± 2.67	0.52 ± 2.75
Canadian	5.33± 2.83	16.7 ± 19.7	3.7 ± 5.3	71.0 ± 18.2	8.0 ± 11.8	0.45 ± 2.55	0.13 ± 0.62
p Value	0.055	0.001	0.023	0.293	0.810	0.291	0.36

± 19.7% ($p < 0.001$). The necrotic core was then analyzed in patients whose cholesterol was >200 mg/dl and compared with those with levels <200 mg/dl (Table III). Even though it is not statistically significant, there is a trend showing that if the cholesterol is <200 mg/dl, there is a tendency for the necrotic core to be smaller.

Proteoglycan

This component was also evaluated as a percent of the plaque. This was significantly different in the two populations. In the Indian population, proteoglycan was 7.9 ± 11.2%; in the Canadian population, it was 3.7 ± 5.3% ($p < 0.023$). Figure 1 illustrates the differences in necrotic core and proteoglycan in the two populations.

TABLE III The relationship of cholesterol level and the amount of necrotic core present in the athersclerotic plaque of the Indian population

Cholesterol	Number of patients	Necrotic core area mm^2	% Necrotic core
≤200 mg/dl	26	0.174 ± 0.3	6.05 ± 11.8
>200 mg/dl	27	0.45 ± 1.0	9.8 ± 12.5
p Value		0.3	0.3

Discussion

Many studies show severe atherosclerosis in young Asian Indians,[9,10] and the present report is no exception. In our very selective population from India, 12% were <40 years of age, whereas the incidence of coronary artery disease <40 years of age in the west is usually <2%.[11] In our Indian population, 62% were ≤50 years of age. This high incidence of young Indians who are affected by coronary artery disease has been reported from other parts of the world. A study from the United Kingdom noted that a myocardial infarction occurring below the age of 40 is ten-fold higher in Asian Indians than in Caucasians.[12] In Qatar, 71% of the men admitted with myocardial infarction below the age of 40 were Asian Indians.[13] Reports from India also confirm this high incidence in the young. In a series reported from Christina Medical College, Vellore, South India, 12% of patients undergoing coronary arteriography for coronary artery disease were below the age of 40.[14] This figure is similar to the presently reported series.

In these young individuals from Southern India, the coronary disease tends to be diffuse: 63% of our population had multivessel disease, a similar high incidence has been reported previously. In a study from the United Kingdom, Asian Indians had a higher incidence of triple-vessel disease (54%) than Caucasians (21%).[15] The incidence of triple-vessel disease is also high in reports from India. A recent study from Vellore reported a 55% incidence of triple-vessel disease compared with a 21% incidence of single-vessel disease.[14] Therefore, the disease in Asian Indians has two main characteristics: it occurs in the young and, when present, the coronary disease tends to be diffuse.

Can conventional risk factors explain this high incidence in India? In our population, we evaluated the conventional risk factors such as diabetes, hypertension, smoking, and family history of premature coronary heart disease: 54% had diabetes, 56% smoked, and 44% had hypertension. Diabetes mellitus is higher in the Asian Indians than that reported in the Framingham study,[16] but the incidence of hypertension and smoking is the same or less in Asian Indians. Total cholesterol

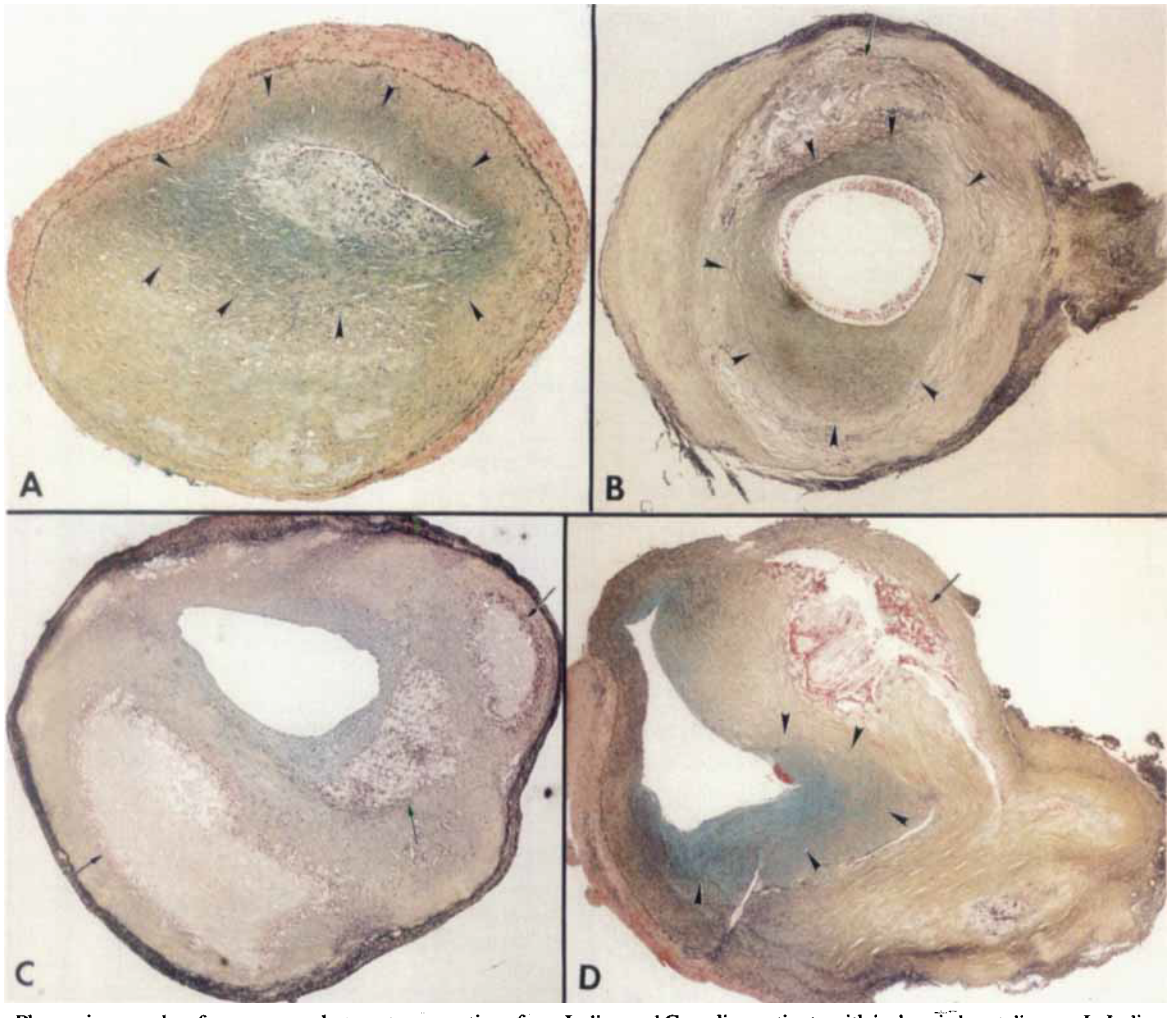

FIG. 1 Photomicrographs of coronary endarterectomy sections from Indian and Canadian patients with ischemic heart disease. In Indian specimens, large areas of atherosclerotic plaque are occupied by smooth muscle cells in a proteoglycan matrix (areas enclosed by arrow heads) as shown in A and B. Canadian specimens, C and D, show large areas of necrotic core (arrows) which are most prominent in C. Only focal presence of proteoglycans is seen in D. Movat pentachrome stain A × 58, B × 24, C × 36, and D × 29.

levels are significantly lower in the Asian Indian group than in the West. The average cholesterol in our study group of Asian Indians was 203 mg/dl with a range of 155–310 mg/dl; 49% of our study group had cholesterol ≤ 200 mg/dl. These levels are very similar to coronary artery disease patients studied in Vellore, India.[14] The mean cholesterol of the patients with coronary artery disease was 205 mg/dl, and in the control population without coronary artery disease it was 186 mg/dl. In the population with coronary artery disease, 15% had total cholesterol of < 150 mg/dl, and 45% had cholesterol levels <200 mg/dl. Furthermore, Asian Indians have low HDL levels and high levels of triglycerides.[15] In our population, the average HDL was 41 mg/dl (range 32–50 mg/dl), LDL was 120 mg/dl (range 82–201 mg/dl), with an average triglyceride of 192 mg/dl. These levels are not significantly different from those reported in the West.[16]

Morphometric measurements of the endarterectomy specimens revealed that the Indians had smaller vessels than the Caucasians from Canada, although only of borderline significance. However, this difference in vessel size may explain why the Indians manifest the disease at a younger age than their counterparts from the West. In most individuals, the atherosclerotic plaque accumulation occurs at a very slow rate, taking decades before producing symptoms. Because the vessel size is smaller in the Indians, the amount of plaque required to produce severe narrowing is also less than in larger arteries; therefore, coronary artery disease will require less time to become manifest as significant luminal narrowing may occur sooner in the Indians than in the Caucasians. The plaque area was significantly smaller in the Indians than in the Canadians when the two populations were age matched. To our knowledge, it has only been previously hypothesized, but never proven, that the Indians may have smaller size coronary arteries; the present study is the first to demonstrate that the Indians have smaller vessels than their counterparts in the West.

The various components of the atherosclerotic plaque in the two populations have not been previously quantitated and compared. It is of great interest to determine whether the

atheromatous lesions reflect the significantly different lipid profile pattern seen in the two populations: high cholesterol in the Caucasians from Canada and low cholesterol in the South Indians. There were significant differences in two components of the plaque in the two populations: the necrotic core which is rich in cholesterol and cholesterol esters, and proteoglycans which are a large component of the eroded plaque and are often seen in patients with unstable angina. If the lipid plays a role in the genesis of atheromatous plaque, it may be expected that increased cholesterol levels are reflected in the increased necrotic core size. As expected in the South Indian group with a low cholesterol level, the necrotic core area as a percent of the plaque (7.1 ± 10.9%) was much less compared with the high cholesterol group from Ottawa, Canada (16.7 ± 19.7%).

The necrotic core has recently been shown to play a significant role in plaque rupture and thrombosis in patients with acute myocardial infarction and sudden death.[17,18] A large necrotic core with a thin fibrous cap has been defined as a vulnerable plaque and is likely a forerunner of plaque rupture and thrombosis. Gertz and Roberts have shown in patients with acute myocardial infarction that the necrotic core is significantly larger (32 ± 14%) in the regions of plaque rupture than in arteries without plaque rupture (12 ± 10%, $p = 0.02$).[19] It is possible that the Indian population may not manifest coronary artery disease from plaque rupture as the necrotic core is much smaller than that seen in the population from the West. It would seem reasonable that other etiologies for the atherosclerotic process should be sought in this South Indian population.

Proteoglycan, on the other hand, forms a significant portion of the atheromatous plaque from the South Indian group. The mean value was 7.9 ± 14.2% compared with 3.7 ± 5.2% for the Ottawa population, which is also highly significant ($p < 0.023$). Foam cells and inflammatory cells were slightly higher in the Indian population, but this was not statistically significant. This difference in plaque morphology may be a reflection of the high incidence of diabetes (54%) in the Indian population. Cellular content of the diabetic plaque may be different.

Does the analysis of plaque truly reflect the blood and the tissue levels of lipoprotein and dietary intake of fats? In 1991, Rapp *et al.* evaluated the polyunsaturated fatty acids derived from fish oil in the human coronary arteries[20] and found a good correlation between the fatty acids in the plaque and those in dietary intake of fish oil. Recently, Felton *et al.*[21] studied the relationship between dietary polyunsaturated fatty acids and its effect on the formation of atheromatous plaque. They studied fatty acids from serum, adipose tissue, and the plaque in the aorta and found a direct correlation between them. This clearly demonstrates that dietary intake of fat plays a major role in atheromatous plaque formation.

Our study shows that a low serum cholesterol level and a low fat intake are associated with very low content of fat in the atheromatous plaque. Despite low fat intake, these South Indians have severe coronary artery disease. From the above data, it appears that lipids may not play an equally sigificant role in the etiology of atherosclerosis in all populations. Therefore, it is important to look for other etiologies for malignant atherosclerosis seen in this South Indian population. Recently, Sandhyamani reported mucoid vasculopathy involving human coronary arteries of young males from South India.[22] In this entity, there is significant mucoid thickening of intima and media that results in narrowing and occlusion of the vessels. These vessels are devoid of any atheromatous plaque. Analysis of dietary intake of these individuals showed a high carbohydrate and low protein diet.[22] Such lesions have also been produced experimentally in bonnet monkeys by using a protein-deficient diet.[23] The above findings suggest that diet may have modified the coronary atherosclerotic plaque in the South Indians in the presence of other risk factors such as smoking, diabetes melllitus, and hypertension.

Study Limitations

This is a single time point evaluation of coronary artery disease; therefore, it is difficult to determine the mechanisms of the atherosclerotic process in the South Indian population. The atherectomy specimens from Canada did not have detailed risk factor analysis. Therefore, we have used historical control data from North America (Canada). Despite this, one can draw some basic conclusions that plaque morphology of the South Indian population is significantly different from the Caucasian Canadians.

Conclusions

Coronary artery disease affecting South Indians is quite different from that affecting Canadians. The disease affects the young, is extensive, and often involves all three vessels. The coronary vessel size tends to be smaller than that of Caucasians. Traditional risk factors such as high cholesterol level do not seem to be as important in this population. The low serum cholesterol is reflected in the morphologic features of the atheromatous plaque of this population. Since the traditional risk factors do not explain the malignant nature of this disease in the young South Indian population, other etiologic factors should be considered.

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