

Progressive atherosclerosis in central India - A modern epidemic

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Abstract: *Background:* Atherosclerosis is the commonest and the most important of the arterial diseases commonly affecting aorta, coronaries and cerebral arterial systems. *Objective:* The present study was planned to know the prevalence of atherosclerosis in population of central India. *Methods:* The study duration was of 2 years from October 2006 to October 2008 & it includes 120 medicolegal postmortem cases. The atherosclerotic lesions if present in ascending aorta were noted. Related sections were taken from the areas for histopathological study. The major coronary arteries were sectioned transversely by sharp knife at intervals of not more than 0.3 cm as well as longitudinally and morphological lesions were noted. In each case sections were taken from coronary arteries and sent for histopathological study. *Results & Conclusion:* Overall prevalence of atherosclerosis was 74.2%. The percentage of affected arteries has shown a significantly rising trend with age, being highest in 60 and above age group (89.00%) and lowest in 10-19 years age group i.e.17.85%. The differences were also statistically significant. Males were affected more than females in all age groups. Advanced lesions were observed as early as in 3rd decade. As age advances more number of subjects was affected with atherosclerosis.

Keywords: Atherosclerosis, Prevalence, Coronary arteries, Ascending aorta.

Introduction

Atherosclerosis is the commonest and the most important of the arterial diseases. The most commonly affected arteries are the aorta, coronaries and cerebral arterial systems [1]. The basic lesion-the atheromas or fibrofatty plaque-consists of a raised focal plaque within the intima, having a core of lipid (mainly cholesterol, usually complexed to proteins, and cholesterol esters) and a covering fibrous cap. Apparently uncommon in antiquity, atherosclerosis became epidemic as populations increasingly survived early mortality caused by communicable diseases and malnutrition. Economic development, urbanization, habits of diet and diminished physical activity can favor atherogenesis. Such environmental factors have now become globalized, such that we face an epidemic of atherosclerosis that reaches far beyond Western societies [2]. Epidemic of atherosclerosis began at different times in different countries. In USA epidemic began in the early 1920s, in Britain in 1930s, in several European countries still later and now the developing countries are catching up [3]. Atherosclerosis is the leading cause of death and disability in the developed world. Many

generalized or systemic risk factors predispose to its development. Risk factors include- Non-modifiable- age, male gender, genetics and Modifiable- smoking, obesity, physical inactivity, lipid disorder, hypertension, diabetes, stress [3].

The classification of atherosclerosis was first described by Gore and Tejada (1957) and used on a large scale in the International Atherosclerosis Project [4]. The World Health Organization study group (1958) classified the atherosclerosis lesions as Fatty streak or spot, Fibrous plaque, Atheroma and Complicated lesions [5]. The American Heart Association (AHA) 1990, proposed a new morphological classification based on six lesion types designated by Roman numerals which indicate the usual sequence of lesion progression [1]. Stary (1992) described the compositions of eight morphologically characteristic types of atherosclerotic lesions in the sequence in which they may evolve in the course of a life [6]. Atherosclerosis affects various regions of circulation and yields distinct clinical manifestations depending on the particular

circulatory bed affected. Not all manifestations of Atherosclerosis results from stenotic occlusive disease. Ectasia and development of aneurysmal disease for example frequently occur in the aorta [7]. Before any atherosclerotic disorder manifests, there is a long prodrome (decades) of silent, slowly progressive coronary atherosclerosis [1]. The process of atherosclerosis begins in childhood as deposits of cholesterol and its esters; referred to as fatty streaks, in the intima of large muscular arteries [8]. More advanced lesions begin to develop after 21 years. Subsequently increased prevalence of advanced complicated lesions of atherosclerosis exists showing peak in 4th and 5th decade of life [9]. The clinical expressions of atherosclerosis may be chronic as in the development of stable, effort-induced angina pectoris or of intermittent claudication. Alternatively a acute clinical event, such as myocardial infarction, a cerebrovascular accident, or sudden cardiac death, may first herald the presence of atherosclerosis. Some may never experience clinical manifestations despite the presence of widespread atherosclerosis which is demonstrated in postmortem examination [7].

Material and Methods

This study was carried out in the department of Forensic Medicine and Toxicology in collaboration with the department of Pathology of our institute.

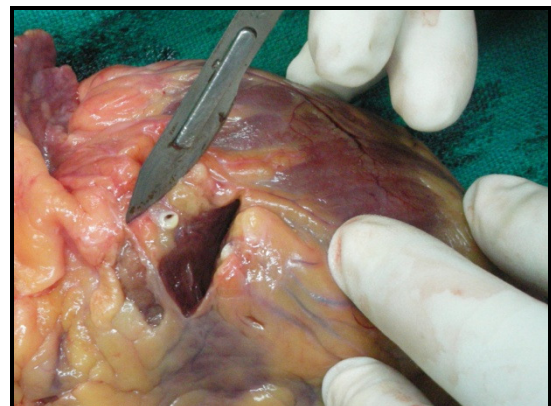
Methodology: The study duration was of 2 years from October 2006 to October 2008. The study includes 120 medicolegal postmortem cases conducted at our institute. Data (age, sex, occupation, education, alcoholism, smoking, dietary habits etc) was recorded from the short history of each case. The specimens (heart and ascending aorta) were collected randomly from these medicolegal autopsies. They were fixed in 10% formalin. In each specimen, the lesions in aorta if present were noted. Sections were taken from these areas for histopathological study. Origins of coronary arteries were identified and their course within the epicardium was followed. Major coronary arteries were sectioned transversely by sharp knife at intervals of not more than 0.3 cm as well as longitudinally upto its ostia, and morphological lesions were noted. [10]. Representative sections were taken from each coronary artery and preserved in 10% formalin in separate labelled bottles (Bottle 1-

Right coronary artery, Bottle 2- Left coronary artery, Bottle 3- Circumflex artery, and Bottle 4- Ascending aorta). Then these were sent for histopathological study. This was done in the department of pathology. Same procedure was repeated on all the specimens.

Fig-1: Macroscopic changes of atherosclerosis in ascending aorta.



Fig. 2: Macroscopic changes of atherosclerosis in coronary artery.



Histopathological examination [11]:

Following procedure was used:

- a) **Fixation:** Fixation of the tissue was done in 10% formal saline (10% Formalin in 0.9% NaCl) for not less than 24 hours.
- b) **Processing:** Thin sections of tissues were cut for microscopic examination for which the tissues were embedded in medium that allows the tissue to be cut. This processing involve following steps:
 - i) **Dehydration-(using alcohol):** Paraffin wax will not penetrate tissues in the presence of water so dehydration is an

essential preliminary in the process. The tissues are transferred from a solution of lower to higher concentration of alcohol to prevent shrinkage of tissue that occurs by rapid dehydration.

- ii) *Clearing*: As the alcohol and wax are not miscible, the alcohol which occupies the tissue spaces is to be replaced with the solvent of the wax in which the tissue is to be later embedded before it can be cut. Majority of wax solvents make a tissue more or less transparent and this effect has led to the use of term clearing. Clearing was done using Xylene-1 and Xylene-2.
 - iii) *Impregnation*: After clearing, the tissues were transferred to molten wax in a paraffin embedding oven (58-60° C) to impregnate the tissue completely with the molten paraffin. Tissue was kept in molten paraffin wax for 2-10 hours. Good quality paraffin wax was used with melting point 54-56° C, free from dust and other foreign matter. Volume of wax used was 20-50 times the volume of tissue.
 - iv) *Embedding or blocking or casting*: Leukhart's L-shaped pieces, made up of brass, a rust proof metal were used for moulding purpose. Paraffin used for making blocks.
- c) *Section cutting using rotary microtome*:
- i) Trimming of the block- it was done by cutting excess of paraffin wax by taking 15-20 micron thin sections.
 - ii) Final cutting- 5-6 micron thin sections were cut.
 - iii) *Floating sections*: Sections were then lifted gently using a fine brush in a hot water bath kept to temperature below the melting point of wax. A clean slide was lowered gently into the water to which egg albumin was applied so that the section glided off.
- d) *Mounting of section on the slides*: The slides were dried off by placing them on a tray in paraffin wax oven for 15-30 minutes or at 37°C in an incubator for 24 hours. Thus, the sections were fixed to the slide firmly and is ready for staining.

e) *Staining of slides*: Mayer's or Ehrlich's haematoxylin and Eosin stains were used.

f) *Screening of slides*: This was done in the department of pathology under the guidance of pathologist.

The relevant data was collected and analyzed according to Kagan and Uemura (1976) [12]. The age, sex and disease distribution of patients were evaluated by means of Chi-square test (χ^2) and Fisher exact probability test both of which compare the proportions of cases falling into various categories in one group with the proportions of cases falling into the same categories in another group. The Chi-square test (χ^2) was applied to those groups, which contain more than 40 cases and Fisher test was applied when smaller groups were involved. The probability level of significance for the entire statistical test was arbitrarily set as P=0.05.

Fig-3: Aorta showing grade VII changes on histopathology (H&E x 40).

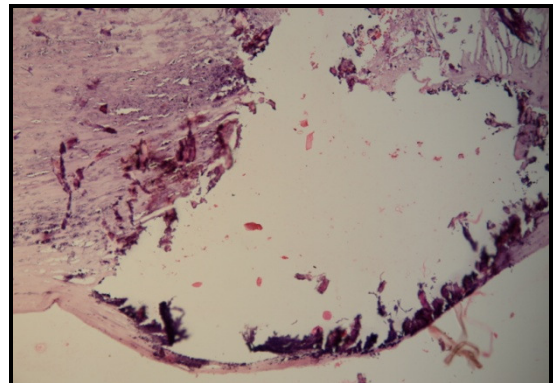
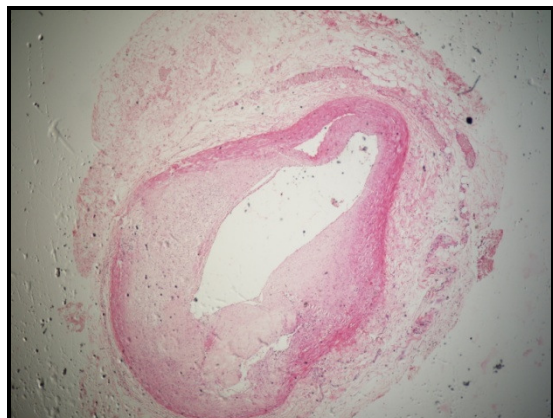


Fig-4: Coronary artery showing grade V changes on histopathology (H&E x 10).



Results

The results of the study are described in various tables given below-

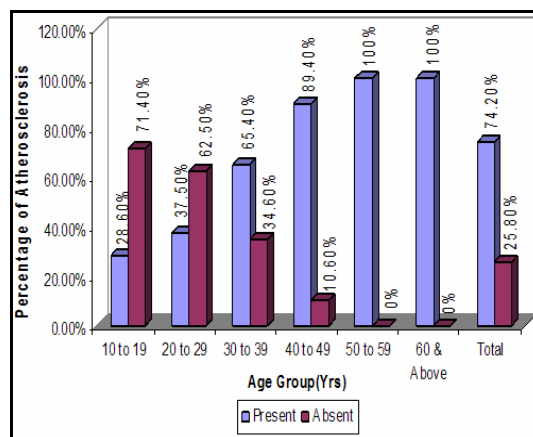
Age group (years)	Male	Female	Total
10-19	4	3	7
20-29	16	8	24
30-39	18	8	26
40-49	15	4	19
50-59	17	2	19
60 & above	20	5	25
Total	90	30	120

Total numbers of subjects were 120 (90 males and 30 females).

It can be observed that the atherosclerotic changes increase with age, being lowest in 10-19 years age group (28.6%) and highest in 60 and

above age group (100.0%). The differences were also statistically significant. The overall prevalence was 74.2% among this population. Higher proportion of atherosclerosis was found in males (76.6%) than in females (66.7%).

Fig-5: Percentage of atherosclerotic changes in different age groups.



Sl. No.	Variable	Atherosclerosis changes			Statistical significance
		Present (%)	Absent (%)	Total (%)	
1.	Age group				$\chi^2=43.13$ $p<0.0001$ Significant
	10-19	02 (28.6)	05 (71.4)	07 (100)	
	20-29	09 (37.5)	15 (62.5)	24 (100)	
	30-39	17 (65.4)	09 (34.6)	26 (100)	
	40-49	17 (89.4)	02 (10.6)	19 (100)	
	50-59	19 (100)	00 (0.0)	19 (100)	
	60 & above	25 (100)	00 (0.0)	25 (100)	
Total	89 (74.2)	31 (25.8)	120 (100)		
2.	Sex				1.17 $p\text{-value}=0.27$ NS, $p>0.05$
	Male	69 (76.6)	21 (23.4)	90 (100)	
	Female	20 (66.7)	10 (33.3)	30 (100)	
	Total	89 (74.2)	31 (25.8)	120 (100)	

(Figures in parenthesis represent percentage)

The level of atherosclerosis in all the four arteries in each subject was added to find out the total level of atherosclerosis in each subject. The total levels of atherosclerosis of all the subjects in the same age group were added to find out the total level of atherosclerosis in that particular age group. Then the mean levels of atherosclerosis in all the age groups were calculated. The degree of overall atherosclerosis in all arteries examined has shown a statistically significant rise with age.

It is lowest in the age group 10-19 years (0.35) and highest in 60 and above year age group (4.19). The mean level of atherosclerosis in the age group 20-29 years was 0.58, in the age group 30-39 was 1.34, in the age group 40-49 was 2.43 and in the age group 50-59 was 3.52. Higher level of atherosclerosis is found in males (2.36) than in females (1.90), the difference being statistically insignificant.

Table-3: Overall atherosclerotic changes by selected variables				
Sl. No.	Variable	Total subjects	Mean level of atherosclerosis in all arteries examined	Statistical Significance
1.	Age group			
	10-19	07(5.9)	0.35±0.62	F=21.33 p=0.000 S,p<0.05
	20-29	24(20.0)	0.58±0.91	
	30-39	26(21.7)	1.34±1.50	
	40-49	19(15.8)	2.43±1.82	
	50-59	19(15.8)	3.52±1.73	
	60 & above	25(20.8)	4.19±1.62	
Total	120(100.0)	2.19±2.03		
2.	Sex			
	Male	90(75.0)	2.36±2.00	t=1.07 p-value=0.28 NS,p>0.05
	Female	30(25.0)	1.90±2.12	
	Total	120(100.0)		
(Figures in parenthesis represent percentage)				

Table-4: Percentage of atherosclerotic changes by selected variables				
Sl. No.	Variable	Total arteries Examined	Number of arteries affected (%)	Statistical Significance
1.	Age group			
	10-19	28	05 (17.85)	41.46 p<0.0001 Significant
	20-29	96	21 (21.87)	
	30-39	104	48 (46.15)	
	40-49	76	49 (64.47)	
	50-59	76	67 (88.15)	
	60 & above	100	89 (89.00)	
Total	480	279 (58.13)		
2.	Sex			
	Male	360	218 (60.5)	0.95 p-value=0.32 NS,p>0.05
	Female	120	61 (50.8)	
	Total	480		
(Figures in parenthesis represent percentage)				

From each subject 4 samples from coronary arteries like RCA, LCA, circumflex and ascending aorta were sent for histopathological examination. It was observed that in the age group 10-19 years, out of 28 samples of the arteries sent for histopathological examination, only 5 were affected with atherosclerosis. In the age group 20-29 years, out of 96 samples 21 were affected with atherosclerosis. In the age group 30-39 years, out of 104 samples 48 were affected. In the age group 40-49 years, out of 76 samples 49 were affected. In the age group 50-59 years, out

of 76 samples 67 were seen with atherosclerotic changes and in the age group 60 and above years, out of 100 samples 89 were affected with atherosclerosis. Thus the percentage of affected arteries has shown a significantly rising trend with age, being highest in 60 and above age group (89.00%) and lowest in 10-19 years age group i.e.17.85%. Higher percentage of the arteries were affected in males (60.5%) than in females (50.8%).

Sl. No.	Author	Year of study	Sample size	Sex		Results %		Total %
				Male	Female	Male	Female	
1.	A study by R. Subramaniyam et al, Madras	1964	492	317	175	52% (In coronary arteries)	32% (In coronary arteries)	70%
2.	A study by S. Padmavati et al	1968	555	413	142	71% (In coronary arteries)	63% (In coronary arteries)	67.3%
3.	A Study by H. Singh, SS Oberoi, RK Gorea, MS Bal	2005	200	170	30	80% (In coronary arteries)	66.6% (In coronary arteries)	78%
4.	Present Study	2008	120	90	30	76.6%(In coronary arteries & aorta)	66.7%(In coronary arteries & aorta)	74.2%

A study done by Subramaniyam et al in 1964, with 492 samples consisting of 317 males and 175 females revealed that atherosclerosis was present in 52% males and 32% females (total 70%). S. Padmavati et al in 1968 with a sample size of 555, consisting of 413 males and 142 females, observed atherosclerosis in 71% males and 63% females (total 67.3%) [13]. H. Singh et al in 2005, in their study of 200 cases (170 males and 30 females) observed prevalence of atherosclerosis in 78% cases (80% in males and 66.6% in females) [14]. In our study out of 120 cases (90 males and 30 females) 74.2% were found affected with atherosclerosis (In males 76.6% and in females 66.7%).

Discussion

The study is based on grading system that takes into account both the area of most severe involvement and total extent of intimal involvement of the coronary arteries and ascending aorta. The lesions noticed on gross examination were subjected for the histopathological examination. The results were statistically analyzed.

I. Distribution of cases:

Age and sex: In the present study out of 120 cases, there were 75% males and 25% females, which are more or less similar to most of the studies conducted in the past. Murthy et al had taken 150 cases in their study, out of which 123

(82%) were males and 27(18%) were females [15]. Padmavati and Sandhu observed 74.5% males and 25.5% females in their study [13]. Bhargava and Bhargava observed 75.8% males and 24.2% females in their study [9]. The reason for male subjects being higher in all these studies is that as the males are the bread earners and females usually doing the household work, thus making the males more vulnerable to accidents, violence, stress etc.

The mean age in our study is 43.98 years in males and 38.56 years in females as we have considered age groups ranging from 10-19 years to 60 and above years. Kagan and Uemura had mean age in their study 40-69 years as they have considered age group from 10-99 years while Murthy MSN et al considered age group ranging from 1 year to above 70 years in their study [12, 15].

II. Atherosclerotic changes in different variables:

1. *Atherosclerosis and age:* The advance of atherosclerosis with age is universally observed. In the present study we found that more number of subjects were affected with atherosclerosis as age advances. In 10-19 years age group 28.6% of the subjects were observed affected with atherosclerosis. In the age group 20-29 years 37.5% subjects, in the age group

30-39 years 65.4% subjects, in 40-49 years age group 89.4%, in the age group 50-59 years 100% and in the age group 60 and above years age group 100% subjects were seen affected with atherosclerosis. The total prevalence was found to be 74.2%. In our study the total mean level of atherosclerosis in all the arteries examined was found to be 0.35 in the age group 10-19 years, 0.58 in the age group 20-29 years, 1.34 in the age group 30-39 years, 2.43 in the age group 40-49 years, 3.52 in the age group 50-59 years and highest i.e. 4.19 in the 60 and above age group. It shows that the severity of atherosclerotic lesion also increases with advancing age.

We observed the lesion starting from age 18 years. Harkirat Singh et al in their study also observed atherosclerotic changes starting from age of 17 years [14]. Mathur et al also reported initial lesion in the second decade of life. Grade II (fatty streak) lesions were found in all the affected arteries in the age group 10-19 years. Grade V (advanced lesions) lesions were found in 4.2% of the subjects affected with atherosclerosis in the age group 20-29 years (Grade IV to grade VIII lesions are combined as advanced lesions). Thus advanced lesions are also found in the age group 20-29 years in our study. Grade VI, grade VII and grade VIII lesions are known as complicated lesions and include complicated fibroatheroma, calcific lesions and fibrotic lesions respectively. Complicated lesions were found from 30-39 years age group onwards. Complicated lesions were seen in 16.3% males and 35% females in the age group of 60 and above years.

Harkirat Singh et al observed in their study the incidence of atherosclerosis to be 78%. The commonest type of lesion in their study was fatty streak (45%). They also observed that the severity of atherosclerotic lesions increases with increased age [14]. Strong and McGill found that grossly visible lesions were rare in the first 10 years of life, but increased rapidly in the succeeding decades until their presence was almost universal in adults. They also observed fatty streak in most of the cases at younger ages and after age 40 fibrous plaques were present in most

cases in all sex race group [16-17]. Wig et al reported initial lesion in the second decade and a peak in the sixth decade of life. They found significant atheroma in 2/3rd of the cases above the age of 20 years. Severe sclerosis was found in 38% of men after the age of 40 years. Tandon et al reported atherosclerosis in 2nd and 3rd decade (14.3%) and after that there was a steep rise [14]. In our study it was observed that, in the 2nd decade i.e. in the age group 10-19, atherosclerosis was present in 5.8% cases and in the 3rd decade i.e. in the age group 20-29, it was observed in 37.5% cases.

2. *Atherosclerosis and sex:* In the present study the overall atherosclerotic changes were observed in 76.6% of male subjects and 33.3% of female subjects. The mean level of atherosclerosis in males was 2.36 and in females it was 1.90. Thus the prevalence and severity is found to be more in males than in females.

Strong and McGill in their study found that males had approximately 3 times greater proportion of the intimal surface involvement than the females in atherosclerosis. He concluded that all the age specific male-female differences in coronary lesions were insignificant [16-17]. Roberts WC et al observed that significantly higher proportion of men had vascular catastrophes than did women. However, there was no sex difference demonstrated in the distribution and severity of atherosclerosis [18]. Thus we conclude that-

- Overall prevalence of atherosclerosis was 74.2%. Males were affected more than females in all age groups.
- Lesions were seen as early as in second decade of life (18 years).
- Advanced lesions were observed as early as in 3rd decade.
- As age advances more number of subjects were affected with atherosclerosis.

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