Serum Cholesterol and Atherosclerosis in Man

By K. S. MATHUR, M.D., F.R.C.P., N. L. PATNEY, M.D., V. KUMAR, M.D., AND R. D. SHARMA, M.Sc.

HUMAN atherosclerosis has frequently been shown to be associated with derangement of some of the serum lipids. They may well have a bearing on the progression of the atherosclerotic process in man, but to establish this relationship is difficult.

Extensive work has been done on blood cholesterol level in clinically diagnosed cases of atherosclerosis. Cases of myocardial infarction and angina pectoris have been reported to be associated with hypercholesteremia.[1] High serum cholesterol values have been found in persons from a high socioeconomic group who are known to suffer a higher incidence of coronary heart disease.[2] But these studies are of doubtful significance as most of them have been concerned with serum cholesterol levels after, rather than before, myocardial infarction and angina pectoris. Clinical studies alone cannot with certainty establish a correlation between serum cholesterol levels and the extent of atherosclerosis, since the clinical manifestations of atherosclerosis are only the ultimate conflagrations in a long smoldering process. The so-called "normal" population groups also show atherosclerosis at autopsy. The beginning and mild forms of atherosclerosis, which would be of greatest value in studying the possible relationship to concentration of cholesterol in serum, present no symptoms and cannot be diagnosed.

A method more critical than clinical diagnosis is needed to establish a relationship between blood cholesterol concentration and the atherosclerotic process. This is possible by comparing the degree of atherosclerosis seen at autopsy with blood cholesterol levels. The present study was undertaken to correlate the concentration of cholesterol in blood serum with the degree of atherosclerosis seen at autopsy in the aorta and the coronary and cerebral arteries.

From the Cardiovascular Research Unit, Department of Medicine, Sarojini Naidu Medical College, Agra, India.

Material and Methods

Total serum cholesterol estimations were done on samples of blood collected before and after death in 20 unselected cases.

Two hundred cases were then selected for the present study out of 500 consecutive medicolegal autopsies during the period September 1958 to May 1960 at Sarojini Naidu Hospital, Agra. Included in this series are only those cases in which death was sudden, there was no history of recent illness, no evidence of organic disease at autopsy, and the sample of blood was available within 16 hours after death.

The following autopsy material was collected in each case for detailed analysis: aorta, from the valvular ring to its bifurcation; heart with the coronary arteries; brain with the cerebral arteries in situ; and 5 to 10 ml. of blood from the pulmonary trunk or the right side of the heart.

The specimens were carefully washed in tap water and fixed in 10 per cent formalin. The arteries were stained with Sudan IV by the technic of Holman et al.[3] The method recommended by the WHO study group[4] was utilized for the examination of the arteries. The amount and severity of atherosclerosis was assessed by grading and grouping the atherosclerotic lesions and expressing them in a simple formula or atherosclerotic profile, which was further reduced to "an atherosclerotic index" by assigning weights to the factors in the atherosclerotic profile as recommended by Gore and Tejada.[5]

The samples of blood were analyzed the same day for total cholesterol content by the technic of Zak et al.,[6] with use of ferric chloride reagent and slight modification in the preliminary extraction with a chloroform-methanol mixture.

Results

The values of serum cholesterol before and after death in the 20 cases are given in table 1. The period of collection of blood varied from 8 to 36 hours after death.

The total cholesterol in postmortem samples differed significantly from antemortem levels when the blood was collected 16 hours after

Table 1

Serum Total Cholesterol Levels in Twenty Cases

Case no.	Serum total cholesterol in mg. per cent Ante-mortem	Post-mortem	Time of collection of blood after death (hr.)
1	130	105	22
2	100	94	16
3	110	103	16
4	135	137	12
5	150	140	12
6	155	156	14
7	150	144	14
8	175	165	14
9	105	80	24
10	145	115	20
11	155	76	36
12	135	132	12
13	110	100	18
14	145	149	16
15	150	144	15
16	180	172	16
17	145	114	22
18	135	120	18
19	150	116	24
20	122	120	8

death, but the mean difference was not significant when the blood was collected sooner.

The atherosclerotic indices of the aorta and the coronary and cerebral arteries and the mean serum total cholesterol concentration were estimated in all the 200 cases under investigation. These have been analyzed with respect to age in decades (table 2).

The mean atherosclerotic index of the aorta and the coronary and cerebral arteries increased from low values in the early decades to maximal levels in the seventh decade. The mean atherosclerotic indices, i.e., the amount and severity of atherosclerosis in the aorta and the coronary and cerebral arteries, showed progressive increase with age.

The mean serum total cholesterol concentration rose progressively from the first decade to a maximum level in the fifth decade and subsequently declined. The coefficient of correlation was calculated between age and serum cholesterol concentration in each case up to the fifth decade and was found to be 0.19. The value of "t" calculated on the basis of this coefficient of correlation (r) at the 5

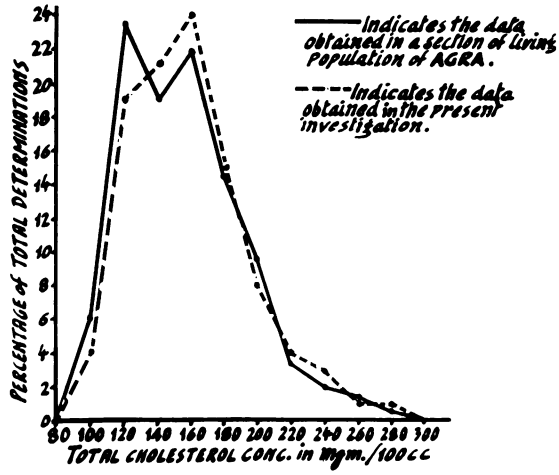

Figure 1

Frequency distribution of total cholesterol concentrations.

per cent level was statistically significant and therefore strongly favors the hypothesis that the concentrations of serum total cholesterol are directly related to age, up to the fifth decade.

To compare serum total cholesterol levels with the amount and severity of atherosclerosis in the arteries, all the cases were divided into nine arbitrary groups on the basis of the atherosclerotic index. To facilitate the correlation, the later groups were taken at broader intervals so as to minimize differences in sample size in the groups (table 3).

A continuous rise of mean serum total cholesterol levels for the successive groups of aortic atherosclerosis was observed from the first to the sixth group, but not in the later groups. In the case of coronary and cerebral arteries, there was no correlation.

The correlation of mean aortic atherosclerosis and mean serum total cholesterol may be due to the effect of age or to the pathologic process of atherosclerosis. What follows, is an attempt to dissociate these two processes in their effect on the serum total cholesterol levels.

Effect of age: Despite individual variations, there was definite increase in total cholesterol concentrations of serum for each successive decade up to the fifth. The significance of this

Table 2

Atherosclerotic Indices of Aorta, and Coronary and Cerebral Arteries and Serum Cholesterol Concentrations

Age period (yr.)	Mean age (yr.)	Sample size	Mean atherosclerotic index			Mean serum total cholesterol (mg. %)
			Aorta	Coronary artery	Cerebral artery	
1-10	5	28	0.021 (± 0.198)*	—	—	122 ± 36*
11-20	18	27	0.125 (± 0.05)	0.003 (± 0.04)*	—	141 ± 47
21-30	26	56	0.393 (± 0.28)	0.038 (± 0.05)*	—	158 ± 20
31-40	38	35	1.398 (± 1.17)	0.34 (± 1.48)	0.01 (± 0.25)*	162 ± 32
41-50	46	26	3.02 (± 2.96)	1.47 (± 1.30)	0.29 (± 0.31)	177 ± 38
51-60	56	17	8.95 (± 3.31)	7.44 (± 4.33)	0.43 (± 0.23)	159 ± 40
61-70	66	11	16.13 (± 6.92)	15.71 (± 7.15)	1.04 (± 0.41)	161 ± 20

*Standard deviations.

relationship has been shown previously by statistical analysis.

Effect of atherosclerosis: The mean serum total cholesterol showed a progressive rise from the first to sixth groups of aortic atherosclerosis, but, at the same time, the mean age for each group also increased. Since the amount of atherosclerosis in the aorta increased with age and the serum cholesterol concentration also rose up to the fifth decade, it is important to determine if the significant correlation between the concentration of serum total cholesterol and aortic atherosclerosis is a correlation with severity of atherosclerosis per se or is merely due to the effect of age, or both. In order to determine the significance of the effect of age and atherosclerosis on the serum cholesterol concentration, the coefficient of partial correlation between aortic atherosclerosis and serum cholesterol with age excluded was calculated. No correlation could be found between the two, indicating that, when the age factor was removed, the positive correlation between aortic atherosclerosis and serum total cholesterol is statistically insignificant.

The effect of the severity of aortic atherosclerosis on the serum total cholesterol was tested further by drawing scattergrams in which the aortic atherosclerotic index was plotted against the serum cholesterol level in each case and analyzed by decades. The points were scattered at random, showing that there is no correlation between the serum total cholesterol concentration and the amount and severity of aortic atherosclerosis.

Discussion

Contradictory reports exist on the relationship of blood cholesterol concentration and atherosclerosis,[7-13] but Katz et al.[14] have reported normal values.

No unanimity exists even on the relationship of serum cholesterol to age. The combined evidence, however, points to a rising value of serum cholesterol with age from youth through middle age and to lower levels in older age groups.

Practically no information is available in regard to the validity of total cholesterol determinations in blood obtained post mortem Shope[15] noted the absence of cholesterol esters in blood collected post mortem and concluded that a very active cholesterol-splitting enzyme is present in tissue. Recent evidence indicates, however, that the proportion of combined cholesterol in total cholesterol of serum may be markedly reduced in the presence of infection, and it seems likely that the abnormal

Table 3

Serum Cholesterol Levels and Aortic, Coronary, and Cerebral Atherosclerotic Index

Case no.	Atherosclerotic index	Aorta		Coronary arteries		Cerebral arteries	
		Mean age (yr.)	Mean serum total cholesterol (mg. %)	Mean age (yr.)	Mean serum total cholesterol (mg. %)	Mean age (yr.)	Mean serum total cholesterol (mg. %)
1	No atherosclerosis	6 (11)	125 ± 32*	17 (54)	134 ± 29*	27 (128)	152 ± 41*
2	0 — 0.1	11 (32)	130 ± 45	35 (40)	168 ± 36	43 (18)	175 ± 40
3	0.1— 0.3	17 (35)	149 ± 45	37 (36)	164 ± 37	45 (28)	171 ± 33
4	0.3— 0.5	27 (24)	167 ± 25	48 (18)	167 ± 21	55 (18)	161 ± 21
5	0.5— 1.0	36 (24)	171 ± 16	48 (28)	166 ± 18	55 (4)	159 ± 32
6	1.0— 5.0	45 (23)	175 ± 31	57 (6)	175 ± 24	60 (4)	173 ± 30
7	5.0—10.0	58 (25)	160 ± 39	62 (6)	175 ± 21		
8	10.0—20.0	63 (12)	163 ± 16	60 (4)	190 ± 32		
9	20.0—30.0	61 (14)	162 ± 7	61 (8)	183 ± 26		

The figures in parentheses below mean age indicate the number of cases in the group.
*Standard deviations.

cholesterol picture observed by Shope in the serum was present before death and did not occur post mortem. Certainly it cannot be concluded from Shope's single observation that there is a change in serum cholesterol levels following death. [16, 17]

The frequency distribution (calculated as a percentage) of the concentration of total cholesterol in our selected cases has been compared (fig. 1) with those we obtained in a study of serum cholesterol levels in a comparable group of healthy men of similar economic status and age.[2] Comparison of the two curves offers strong support for our assumption that no significant change in the antemortem level of total cholesterol occurred after death, provided the blood samples were collected within 16 hours after death.

In the present study, the interrelationship between blood cholesterol levels and the amount and severity of atherosclerosis has been evaluated by comparison of blood cholesterol levels with the amount of atherosclerosis seen on direct visual examination of the blood vessel at autopsy.

Our findings of increased serum cholesterol levels from youth through middle age and to lower levels in older persons is in accordance with the results of most workers. The fall of cholesterol levels in old age does not necessarily mean that cholesterol level actually declines. Keys et al.[18] explain this point by stating that the elderly persons with low cholesterol levels were perhaps the survivors of a larger population.

In the present study, we did not find any significant correlation between the blood serum total cholesterol and atherosclerotic index as a representation of the extent and severity of atherosclerosis for any of the vessels studied. The mean serum total cholesterol concentration in the six groups of aortic atherosclerosis showed a successive rise but, when the age factor was taken into consideration, the correlation between atherosclerosis and serum cholesterol in these same groups was found statistically insignificant. In this respect our findings failed to substantiate the findings of Gertler et al.,[9] Morris et al.,[11] and others, who have reported higher values of blood cho-

lesterol with increasing severity of atherosclerosis.

On the other hand, our findings are in accordance with those of Lande and Sperry[16] and Paterson et al.,[19] who determined blood cholesterol levels at intervals during life and compared them with the amount of atherosclerosis found at autopsy. Gofman et al.[20] arrived at similar conclusions.

Iljinski[21] observed high cholesterol levels in cases that showed progressive atherosclerosis with fresh deposits at autopsy but found normal levels in persons with regressive or fibrous modifications of atheromatous changes. Steiner and Domanski[22] found the blood cholesterol of cases with atherosclerosis inconstant and widely fluctuated, whereas values in normal subjects remained relatively constant. Since the normal content of serum cholesterol is close to its saturation point,[22] deposition of cholesterol may follow slight elevations above normal levels, while between times the blood cholesterol levels may be apparently normal.

Summary

Two hundred cases were selected from medicolegal autopsies for a study of the relationship of serum cholesterol to the amount and severity of atherosclerosis in the aorta and the coronary and cerebral arteries. A preliminary study of cholesterol before and after death in 20 cases showed a close parallel between the two when the sample of blood was taken within 16 hours of death.

The mean serum total cholesterol showed a tendency to rise from 122 mg. per cent ±16 in the first decade to 176 mg. per cent ±28 in the fifth decade. A statistically significant correlation was found between serum total cholesterol levels and age up to the fifth decade.

No correlation could be observed between the serum cholesterol level and the amount and severity of atherosclerosis in the arteries. When all the cases were divided into arbitrary groups according to the amount of atherosclerosis, a rise in the levels of mean serum total cholesterol was seen in the first six successive groups of aortic atherosclerosis. But when age was excluded from the correlation between atherosclerosis and serum cholesterol, the interrelationship between the two was found to be statistically insignificant.

References

1. Mathur, K. S., Malhotra, K. K., and Sharma, R. D.: Serum lipids in coronary heart disease. Indian Heart J. 12: 28, 1960.
2. Mathur, K. S., Wahi, P. N., Malhotra, K. K., Sharma, R. D., and Srivastava, S. K.: Dietary fats, serum cholesterol and serum lipid phosphorus in different socio-economic groups in Uttar Pradesh. J. Indian M. A. 33: 303, 1959.
3. Holman, R. L., McGill, H. C., Jr., Strong, J. P., and Geer, J. C.: Technics for studying atherosclerotic lesions. Lab. Invest. 7: 42, 1958.
4. World Health Organization Technical Report Series No. 143, October, 1957.
5. Gore, I., and Tejada, C.: The quantitative appraisal of atherosclerosis. Am. J. Path. 33: 875, 1957.
6. Zak, B., Dickenman, R. L., White, E. G., Burnett, H., and Cherney, P. J.: Rapid estimation of free and total cholesterol. Am. J. Clin. Path. 24: 1307, 1954.
7. Barker, N. W.: The plasma lipoids in arteriosclerosis obliterans. Ann. Int. Med. 13: 685, 1909.
8. Steiner, A.: Cholesterol in arteriosclerosis with special reference to coronary arteriosclerosis. M. Clin. North America 34: 673, 1950.
9. Gertler, M. M., Garn, S. M., and Bland, E. F.: Age, serum cholesterol and coronary artery disease. Circulation 2: 517, 1950.
10. Steiner. A., Kendall, F. E., and Mathers, J. A. L.: The abnormal serum lipid pattern in patients with coronary atherosclerosis. Circulation 5: 605, 1952.
11. Morrison, L. M., and Johnson, K. D.: Cholesterol content of the coronary arteries and blood in acute coronary artery thrombosis. Am. Heart J. 39: 31, 1950.
12. Little, J. A., Shanoff, H. M., Van der Flier, R. W., and Rykert, H. E.: Serum lipids in carefully selected "atherosclerotic" and "normal" males. Abstract, Circulation 10: 585, 1954.
13. Page, I. H.: Atherosclerosis—An introduction. Circulation 10: 1, 1954.
14. Katz, L. B., Rhodes, G. J., George, R. S., and Moses, C.: Total serum cholesterol, cholesterol-lipid phosphorus ratio and Sf 12-20 concentrations in hypertension, diabetes and coronary artery disease. Am. J. M. Sc. 120: 111, 1953.

15. SHOPE, R. E.: Cited by Katz et al.[14]
16. LANDE, K. E., AND SPERRY, W. H.: Human atherosclerosis in relation to cholesterol content of blood serum. Arch. Path. **22**: 301, 1936.
17. GREENBLATT, I. J., BRADESS, V. A., AND SPAIN, D. M.: Postmortem studies on serum lipoproteins in one-hundred consecutive autopsies. Abstract, Circulation **10**: 605, 1954.
18. KEYS, A., MICKELSON, O., MILLER, E. O., HEYS, E. R., AND TODD, L. L.: The concentration of cholesterol in the blood serum of normal man and its relation to age. J. Clin. Invest. **29**: 1347, 1950.
19. PATERSON, J. C., CORNISH, B. R., AND ARMSTRONG, E. G.: The serum lipid in human atherosclerosis—an interim report. Circulation **13**: 224, 1956.
20. GOFMAN, J. W., LINDGREN, F. T., LYON, T. P., ELLIOTT, H. A., AND STRISOWER, B.: Blood lipids and human atherosclerosis. Circulation **2**: 161, 1950.
21. ILJINSKI, B. V.: Pathogenesis of atherosclerosis. Abstract, Circulation **15**: 609, 1957.
22. STEINER, A., AND DOMANSKI, B.: Serum cholesterol level in coronary atherosclerosis. Arch. Int. Med. **71**: 397, 1943.

The Spirit of Adventure

Investigators do not march straight to their goal with ease and directness. In their imagination they see a possible fact and they set forth to learn whether their foresight can be realized. Or they come upon something which is puzzling and challenging and which they wish to explain; then they try in various ways to relate it to other phenomena that would solve the riddle. The search for understanding is an adventure or, more commonly, a series of adventures. If an attempt in one direction fails, the failure is not discouraging to an eager explorer. When the goal is reached, there is occasion for joy and exultation. A conquest has been achieved. New knowledge has been gained which deeply satisfies both the explorer's adventurous spirit and his persistent curiosity.—WALTER B. CANNON, M.D., *The Way of An Investigator*. New York, W. W. Norton & Company, Inc., 1945, p. 22.

Serum Cholesterol and Atherosclerosis in Man

K. S. MATHUR, N. L. PATNEY, V. KUMAR and R. D. SHARMA

Circulation. 1961;23:847-852
doi: 10.1161/01.CIR.23.6.847
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7322. Online ISSN: 1524-4539