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Plasma lipids, lipoproteins and apolipoproteins and sudden cardiac death

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Abstract To investigate the risk factors for sudden cardiac death, in particular that triggered by coronary heart disease, we analysed 17 different lipids, lipoproteins and apolipoproteins in the plasma of patients who had suffered sudden death, including sudden cardiac death. Studies were carried out on 107 cadavers comprising 78 subjects where the cause of death was diagnosed as sudden cardiac death and 29 subjects diagnosed with other causes of sudden death. All 107 cases were classified into four groups according to the degree of coronary stenosis and the degree of cardiac hypertrophy. Plasma levels of total cholesterol (T-CHOL), triglyceride (TG), β-lipoprotein (β-LIPO), free fatty acid, phospholipid, free cholesterol, high density lipoprotein cholesterol, lipoprotein(a) [Lp(a)], lipoproteins (VLDL, LDL, HDL) and apolipoproteins (apoAI, apoAII, apoB, apoCII, apoCIII, apoE) were determined. The level of apoB showed a significant difference and positive correlation with the degree of coronary stenosis by two different statistical methods, while the levels of T-CHOL, TG, β-LIPO, VLDL, apoCII, apoCIII and apoE showed significant differences with the degree of coronary stenosis by one statistical method. It was concluded that a high plasma level of apoB is a risk factor for coronary stenosis, with higher levels resulting in more severe degrees of coronary stenosis. Furthermore, a significant difference was found regarding only apoCIII both between the sudden cardiac death group and the sudden death from other causes group, and between each of the four groups classified according to the degree of coronary stenosis. It was thus concluded that a high plasma level of apoCIII indicates the possibility of a coronary stenotic origin for sudden cardiac death.

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Introduction

The close relationship between the severity and extent of coronary atherosclerosis and the development of coronary heart disease is now almost universally accepted [1–3]. One of the risk factors that contributes toward the development of this disease is plasma lipid levels [4]. It has been verified that high total cholesterol (T-CHOL) and high triglyceride (TG) levels, high Lp(a) levels, low HDL, high LDL and high VLDL levels in lipoprotein and high apoB levels in apolipoprotein each have a close relationship to coronary heart disease. Furthermore, the overlapping of these risk factors increases the chance of ischemic heart attack [5]. We wondered whether the overlapping of these risk factors also increases the fatality rate from coronary heart disease and this led us to analyse lipid, lipoprotein and apolipoprotein levels in the plasma of patients who had suffered sudden death, including sudden cardiac death and to investigate whether these levels are in fact risk factors for sudden cardiac death, in particular that triggered by coronary heart disease.

Materials and methods

Studies were performed on 107 cadavers with no emergency treatment and no medication before death, autopsied at the Department of Legal Medicine, Hirosaki University School of Medicine and Tokyo Medical Examiner's Office within 24 h of death. Analysis of blood, urine and gastric contents of all cadavers was undertaken, but no common drugs or alcohol were found. The population studied comprised 78 subjects (74 males and 4 females, the mean age was 52.27, SD 11.37 years, range 19–73 years) where the cause of death was diagnosed as sudden cardiac death: myocardial infarction or coronary atherosclerosis (66 cases) and congestive heart failure (12 cases), and 29 subjects (24 males and 5 females, the mean age was 43.54, SD 12.85 years, range 16–70 years) diagnosed with other natural causes of sudden death by means of macro- and microscopic examinations: ruptured cerebral aneurysm, epilepsy, bronchial asthma, gastrointestinal haemorrhage, etc. All of these

individuals had no history of a significant medical condition before death and no other diseases at autopsy except for the cause of death. Blood was sampled from the right atrium and centrifuged at 3,000 r.p.m. for 10 min. Levels of T-CHOL, TG, β-lipoprotein (β-LIPO), free fatty acid (FFA), phospholipid (PL), free cholesterol (F-CHOL), high density lipoprotein cholesterol (HDL-C), Lp(a), lipoproteins (VLDL, LDL, HDL) and apolipoproteins (apoAI, apoAII, apoB, apoCII, apoCIII, apoE) were determined by the following methods: modification of the method of Allain et al. using paramax cholesterol reagent for CHOL; an enzymatic method for TG, FFA, PL and F-CHOL; immunoturbidimetry for β-LIPO and each apolipoprotein; direct measurement for HDL-C; latex immunoassay for Lp(a); and the lipophor system using polyacrylamide gel lipoprotein disc electrophoresis for each lipoprotein [6-9]. After weighing the heart in all 107 cases, the coronary arteries were dissected by serial transverse sectioning with a knife at frequent intervals. Areas of maximal narrowing in the main branches of coronary arteries (right, left circumflex and left anterior descending coronary arteries) were noted by specifying the degrees of reduction within the cross-sectional area of the lumen. Coronary stenosis was classified into four groups: 0–30% (24 cases), 31–50% (8 cases), 51–70% (13 cases) and >71% (62 cases), while cardiac hypertrophy was also classified into four groups according to Shigeta's coefficient, which was calculated from the cadaver's height, weight and heart weight [10]: –3–± (49 cases), +1 (18 cases), +2 (13 cases) and +3 (27 cases).

Statistical studies were performed by analysing the correlation coefficient of the levels of each factor, by Student's t-test on comparisons of each of 15 levels between the sudden cardiac death group (78 subjects) and the sudden death by other causes group (29 subjects) and by analysis of variance (ANOVA) and backward stepwise regression on comparison of each of 15 levels between each of the four groups classified according to the degree of coronary stenosis and between each of the four groups classified according to the degree of cardiac hypertrophy.

Table 1 Postmortem plasma levels (Mean ± SD) and plasma reference levels of lipids, lipoproteins and apolipoproteins

Tests	(Unit)	Postmortem plasma	In vivo plasma
T-CHOL	(mg/dl)	206 ± 72	130 –250
TG	(mg/dl)	235 ± 230	30 –150
β-LIPO	(mg/dl)	577 ± 293	140 –440
FFA	(mEq/l)	1274 ± 768	85 –650
PL	(mg/dl)	262 ± 83	140 –230
F-CHOL	(mg/dl)	58 ± 22	30 – 60
HDL-C	(mg/dl)	37.0 ± 19.1	♂42 – 63 · ♀48–60
Lp(a)	(mg/dl)	30.7 ± 25.4	0 – 30
VLDL	(%)	16.7 ± 7.8	9 – 22
LDL	(%)	57.9 ± 12.4	34 – 51
HDL	(%)	25.4 ± 11.3	31 – 51
apoAI	(mg/dl)	111 ± 33	112 –162
apoAII	(mg/dl)	34.4 ± 22.2	25.9– 37.7
apoB	(mg/dl)	109 ± 38	59 – 99
apoCII	(mg/dl)	4.5 ± 3.8	2.2– 4.6
apoCIII	(mg/dl)	12.4 ± 7.8	4.5– 10.5
apoE	(mg/dl)	7.8 ± 5.3	2.9– 5.3

Results and discussion

Postmortem plasma levels of HDL-C, HDL and apoAI were a little lower, while those of TG, β-LIPO, PL, Lp(a), LDL, apoB, apoCIII and apoE were a little higher than the reference levels in plasma before death. The postmortem plasma level of FFA was 3–5 times higher than the reference intervals in plasma before death (Table 1), therefore FFA was excluded from our subsequent statistical analysis. Moreover, a very high correlation (> 0.9) was seen between T-CHOL and F-CHOL, and T-CHOL and apoB in the correlation coefficient between each of 16 levels (Table 2). It is believed that there is a close relationship between some lipids, lipoproteins and apolipoproteins which form the constituent part of one another while it is also estimated that about 30% of CHOL is comprised of F-CHOL. For this reason, F-CHOL was also excluded from our subsequent analysis. However, since it is thought that no constitutional relationship exists between CHOL and apoB, apoB and the remaining 14 tested levels were used for the subsequent analysis.

A significant difference ($p < 0.05$) was found only for apoCIII between the sudden cardiac death group and the sudden death from other causes group by Student's t-test on each of the 15 levels (Table 3). Significant differences

Table 2 Correlation coefficients between each of 16 plasma levels

	T-CHOL	TG	β-LIPO	PL	F-CHOL	HDL-C	Lp(a)	VLDL	LDL	HDL	apoAI	apoAII	apoB	apoCII	apoCIII
TG	0.541														
β-LIPO	0.85	0.818													
PL	0.82	0.573	0.807												
F-CHOL	0.923	0.59	0.842	0.847											
HDL-C	0.162	–0.142	–0.131	0.132	0.112										
Lp(a)	0.313	0.02	0.22	0.182	0.238	–0.052									
VLDL	0.131	0.592	0.391	0.248	0.168	–0.209	–0.117								
LDL	0.368	–0.098	0.26	0.148	0.283	–0.396	0.33	–0.516							
HDL	–0.524	–0.347	–0.601	–0.359	–0.453	0.617	–0.292	–0.184	–0.745						
apoAI	0.35	0.151	0.169	0.401	0.24	0.698	–0.041	0.074	–0.246	0.223					
apoAII	0.304	0.32	0.314	0.365	0.327	0.134	–0.003	0.219	–0.051	–0.11	0.267				
apoB	0.917	0.421	0.817	0.779	0.85	–0.025	0.382	0.04	0.527	–0.634	0.17	0.248			
apoCII	0.602	0.646	0.642	0.582	0.627	0.154	–0.008	0.428	–0.11	–0.202	0.282	0.456	0.481		
apoCIII	0.582	0.734	0.679	0.617	0.595	0.253	–0.05	0.41	–0.2	–0.086	0.393	0.495	0.45	0.874	
apoE	0.595	0.729	0.722	0.715	0.7	–0.041	–0.002	0.365	–0.038	–0.237	0.115	0.322	0.518	0.763	0.686

Table 3 *P* values by Student's t-test on each of 15 plasma levels between the acute cardiac death group and the sudden death from other causes group

Tests	*P* value
T-CHOL	0.2422
TG	0.4407
β-LIPO	0.1617
PL	0.9519
HDL-C	0.3867
Lp(a)	0.1671
VLDL	0.0615
LDL	0.9259
HDL	0.1556
apoAI	0.5454
apoAII	0.4865
apoB	0.2597
apoCII	0.3609
apoCIII	0.0473
apoE	0.3138

Table 4 *P* values by the ANOVA test on comparisons of each of 15 plasma levels between each of the four groups classified according to the degree of coronary stenosis and between each of the four groups classified according to the degree of cardiac hypertrophy

Tests	Coronary stenosis	Cardiac hypertrophy
T-CHOL	0.0077	0.1959
TG	0.0215	0.2051
β-LIPO	0.0098	0.1601
PL	0.0679	0.2796
HDL-C	0.5525	0.8288
Lp(a)	0.1166	0.3844
VLDL	0.0396	0.1592
LDL	0.8064	0.6381
HDL	0.1959	0.6536
apoAI	0.0999	0.9634
apoAII	0.5305	0.1567
apoB	0.0196	0.1001
apoCII	0.0033	0.5904
apoCIII	0.0114	0.6399
apoE	0.0342	0.2851

($p < 0.05$) were found regarding T-CHOL, TG, β-LIPO, VLDL, apoB, apoCII, apoCIII and apoE between each of the four groups classified according to the degree of coronary stenosis. However, no significant positive correlation was found between each of the four groups classified according to the degree of cardiac hypertrophy by ANOVA (Table 4).

Further statistical analysis comprising backward stepwise regression was carried out on each of the 15 levels between each of the four groups. Unlike ANOVA, a positive regression coefficient was only found for apoB (coefficient: 0.029, Std. Error: 0.007, Std. Coeff.: 0.877, F-value: 18.093) between each of the four groups classified according to the degree of coronary stenosis. No positive regression coefficient was found between each of the four groups classified according to the degree of cardiac hypertrophy. It would therefore seem that there is a positive correlation between the four steps of progression of coronary stenosis and the level of apoB.

According to the above results, the plasma level of apoB showed a significant difference and positive correlation with the degree of coronary stenosis by the two statistical methods of ANOVA and backward stepwise regression. However, the plasma levels of CHOL, TG, β-LIPO, VLDL, apoCII, apoCIII and apoE showed significant differences with the degree of coronary stenosis only by the ANOVA method. Postmortem plasma levels of apoB had a tendency to increase in line with the degree of coronary stenosis, however, the other 7 levels of the 51–70% coronary stenosis group were higher than those of the >71% coronary stenosis group (Table 5). These results suggest that high plasma levels of T-CHOL, TG, β-LIPO, VLDL, apoCII, apoCIII and apoE are effective markers for indicating when the maximal narrowing of coronary arteries exceed 50%. Our results regarding apoB are in agreement with a paper by Valenzuela et al. [11] who determined lipoproteins and apolipoproteins in postmortem pericardial fluid. They concluded that a significant increase in apoB was found in cases with a positive diagnosis of myocardial infarction and that the determination of apoB in pericardial fluid could be a useful parameter to be included in biochemical analysis for the postmortem diagnosis of myocardial infarction related to coronary atherosclerosis. It was concluded from our results that a high plasma level of apoB is a risk factor for coronary stenosis, with higher levels resulting in more severe degree of coronary stenosis.

For the levels of plasma lipids, lipoproteins and apolipoproteins in the living body, varying results have

Table 5 Postmortem plasma levels (Mean ± SD) of eight tests of each of four groups classified according to the degree of coronary stenosis. Only the level of apoB increased in line with the degree of coronary stenosis

Tests (Unit)	Degree of coronary stenosis			
	0–30%	31–50%	51–70%	>71%
T-CHOL (mg/dl)	170 ± 74	178 ± 84	237 ± 72	220 ± 64
TG (mg/dl)	164 ± 171	119 ± 66	417 ± 489	244 ± 168
β-LIPO (mg/dl)	454 ± 276	446 ± 203	751 ± 456	618 ± 244
VLDL (%)	14.5 ± 9.2	11.4 ± 3.8	19.8 ± 6.8	17.8 ± 7.3
apoB (mg/dl)	90 ± 33	98 ± 45	112 ± 36	118 ± 37
apoCII (mg/dl)	2.4 ± 2.4	4.2 ± 3.6	7.0 ± 6.9	5.0 ± 3.2
apoCIII (mg/dl)	8.6 ± 7.7	9.3 ± 4.4	15.7 ± 7.1	13.8 ± 7.8
apoE (mg/dl)	5.6 ± 3.7	9.3 ± 9.2	10.7 ± 6.4	8.0 ± 4.8

been obtained regarding risk factors for coronary heart disease depending upon the race, age and sex of the subjects and the statistical method used [12, 13]. It is clear that significant coronary atherosclerosis is present in most cases of naturally occurring sudden death and consequently coronary heart disease is the leading cause of sudden death [14]. Some studies on the postmortem applications of biochemical markers involved in sudden death have been published [15]. Recently, genetic studies have been carried out for the LDL receptor [16], apoE [17] and Lp(a) [18] in the living body. To date however, there have been only two reports on the determination of plasma lipid levels in corpses as fatal risk factors for sudden cardiac death [19, 20]. In the present study, a significant difference was found only for apoCIII both between the sudden cardiac death group and the sudden death from other causes group and between each of the four groups classified according to the degree of coronary stenosis. It was thus concluded that a high plasma level of apoCIII indicates the possibility of a coronary stenotic origin for sudden cardiac death.

There was no test which produced a significant positive correlation or positive regression coefficient with the four groups classified according to the degree of cardiac hypertrophy. This result suggests that there is no relationship between plasma lipid, lipoprotein and apolipoprotein levels and the degree of cardiac hypertrophy and that cardiac hypertrophy progresses independently of plasma lipids, lipoproteins and apolipoproteins.

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