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Alcohol, Cardiovascular Death and Postmortem Lipids (Total Cholesterol, HDL, Apo A1 And Apo B), A Case-Control Study

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Abstract

This study aimed to correlate postmortem TC, HDL, non-HDL, apo A1 and apo B levels with the presence of atherosclerotic cardiovascular disease and ethanol consumption before death. A total of 215 forensic autopsy cases (177 males and 38 females between 25–88 years of age) with a postmortem interval of 5.5–118.5 h were examined and divided into groups according to the presence and severity of atherosclerotic lesions and the cause of death. The presence of more advanced types of atherosclerotic lesions and a higher degree of coronary artery stenosis were associated with higher postmortem levels of Apo B and a higher apo B/apo A1 ratio. Deaths caused by acute and chronic ischemic heart disease differed from other causes of death by higher apo B levels. These findings occurred despite postmortem apo B levels being lower than the suggested clinical range in most cases. Alcohol consumption before death was not correlated with the postmortem levels of examined lipids and lipoproteins.

Key points

- Postmortem apo B levels were lower than the clinical range.
- Advanced atherosclerotic changes were associated with higher postmortem levels of Apo B and a higher apo B/apo A1 ratio.
- Ischemic heart disease as cause of death was associated with higher apo B levels compared to other causes of death.
- Alcohol consumption before death was not correlated with the postmortem levels of examined lipids and lipoproteins.

Introduction

Cardiovascular disease (CVD), especially coronary artery disease, remains the leading cause of death globally and is the most frequent cause of death in forensic autopsies in cases of sudden death [1–3]. The cause of the cardiovascular disease is multifactorial, but increased plasma lipids are among the main risk factors [4,5].

The cholesterol in plasma is bound to apolipoproteins to form lipoproteins. 60–70% of cholesterol is found in low-density lipoproteins (LDL) and 25–35% in high-density lipoproteins (HDL) [6]. Increased LDL values are causally related to CVD and low levels of HDL are associated with higher CVD risk [4,5]. Non-HDL (the difference between total cholesterol (TC) and HDL) comprises pro-atherogenic lipoproteins, predicts CVD better than LDL alone, and does not require fasting before sampling [4,7]. The major apolipoprotein of LDL is apolipoprotein B (apo B) and that of HDL is apolipoprotein A1 (apo A1). The apo B/apo A1 ratio is considered a strong risk marker for atherosclerosis [4,8–10].

The role of alcohol in CVD risk is controversial [4]. Moderate alcohol intake is associated with a lower risk of coronary heart disease, possibly through changes in lipids and hemostatic factors [4,11,12]. The cardioprotective effect of moderate alcohol consumption is not seen in binge drinking, the characteristic drinking pattern in Eastern Europe [13]. In women, hazardous alcohol consumption has been found to be an independent risk factor of CVD mortality [14].

This study aimed to correlate postmortem TC, HDL, non-HDL, apo A1, apo B levels with the presence of atherosclerotic cardiovascular disease, and ethanol consumption before death in men and women submitted to forensic autopsy.

Materials and Methods

Materials

A total of 215 forensic autopsy cases at the Estonian Forensic Science Institute were examined from the period of 2010-2014. The sample consisted of males (n = 177) and females (n = 38) between 25–88 years of age with a median of 44 years of age. The estimated postmortem interval (PMI) was 5.5–118.5 h with a median of 34.5 h.

The causes of death (COD) were determined based on macroscopical, microscopical, and toxicological findings, and divided into acute/chronic ischemic heart disease (n = 29, ICD-10 diagnoses I22 and I25), acute poisoning with ethanol (n = 23), other alcohol-related pathologies (n = 12, ICD-10 diagnoses K70 and K86.0) and other causes (n = 151, including other diseases and trauma).

In each case, the left anterior descending coronary artery (LAD), left circumflex coronary artery (LCX), and right coronary artery (RCA) were grossly graded for the degree of stenosis according to the most advanced atherosclerotic lesion as follows: 0 – no changes, 1 – stenosis affecting up to 50% of the lumen, 2 – stenosis of 51–75% of the lumen, and grade 3 – stenosis of more than 75% of the coronary artery lumen. The intimal surface of LAD, LCX, RCA, and the aorta was evaluated according to the type of the most advanced atherosclerotic lesion as follows: 0 – no changes, 1 – fatty streaks, 2 – atheromas and fibrous plaques, 3 – calcified lesions, and 4 – complicated lesions (lesions with ulceration and/or thrombus). All cases were divided into 2 groups: cases with atherosclerotic changes in the coronary arteries and/or aorta (SCL, n = 160), and cases without any signs of atherosclerosis (controls, n = 55). Case characteristics are summarized in Table 1.

Biochemical Analyses

Blood was drawn from femoral or iliac veins at autopsy into whole blood tube with sodium fluoride for blood alcohol level (BAC) and serum vacuum tube for serum, additional blood samples were drawn for narcotics and medicines. The samples were stored at +4° C and transported to the laboratory within 24 h.

The whole blood alcohol level (BAC) was determined using headspace gas chromatography (Turbo Matrix 40 Headspace Sampler). The blood samples in the vacuum tubes were centrifuged to separate the serum and stored at -20° C until use. TC and HDL were measured using the enzymatic colorimetric method (Cobas 6000). Non-HDL cholesterol was calculated by the formula: Non-HDL= TC – HDL. Apo A1 and apo B were measured using the electrochemiluminescence method (Cobas 600 c 501).

Clinical reference values were as follows: 3.3 – 6.9 mmol/L for TC in subjects less than 50 years of age and 3.9 – 7.8 mmol/L for subjects aged 50 years and older; 0.8 – 2.1 mmol/L for HDL in men and 1.0 – 2.7 mmol/L for women; < 3.8 mmol/L for non-HDL; 37.0 – 72.0 µmol/L for Apo A1 in men and 38.6 –

Table 1. Case characteristics

Autopsycases (n =215)					
Atherosclerosis (n = 160)			Controls (n = 55)		P-value*
	Range	Median	Range	Median	
Male/Female (n)	135/25		42/13		NS
Age (years)	25-88	48	25-50	31	0.001
PMI (hours)	5.5-118.5	34	8-99	39	NS
BAC (mg/g)	0-6.3	0.7	0-4.9	0	NS

BAC, blood ethanol concentration. *Mann-Whitney U test. NS, not statistically significant

80.0 µmol/L for women; 2.5 – 5.0 µmol/L for Apo B in men and 2.3 – 4.4 µmol/L in women.

Statistical methods

Spearman's rank-order correlation (rs) was used to evaluate the relationship between pairs of parameters. A Kruskal-Wallis test was used to compare more than two groups; subsequent multiple pair-wise comparisons were adjusted using the Bonferroni adjustment. Comparisons between two groups were performed by Mann-Whitney U test. A P-value of less than 0.05 was considered statistically significant. Statistical analysis was performed using statistical software R (version 2.14.0).

Results

On gross inspection, coronary arteries and aorta showed no signs of atherosclerosis in 25% of cases. The most advanced type of atherosclerotic lesion was fatty streaks in 15% of all cases, plaques in 30%, calcified lesions in 21%, and ulcerated lesions in 9% of all cases. Coronary arteries were not significantly narrowed by 85% (no stenosis in 50% and less than 50% of stenosis in 35%) of cases. In 7.5% of cases, stenosis of 50-75% of the lumen was found and luminal narrowing of more than 75% was found in 7.5% of all cases.

After dividing all cases into the atherosclerosis group and control group, a statistically significant age difference emerged between the SCL group and controls (P< 0.001, Mann-Whitney U), with the persons in the SCL group being significantly older (Table 1). There was no difference in PMI or BAC between the SCL group and controls (P = 0.2 and P = 0.3, Mann-Whitney U).

Lipid levels are summarized in Table 2. In the SCL group, TC levels were above the suggested clinical limit in 47% of subjects less than 50 years of age and in 13% of subjects aged 50 years and older. In the control group, TC levels were above the suggested clinical limit in 15% of subjects less than 50 years of age. There were no subjects aged 50 years and older with TC levels above the suggested clinical limit in the control group.

In the SCL group, non-HDL levels were above the suggested clinical level in 70% of men and in 60% of women. In the control group, non-HDL levels were above the clinical level in 38% of men and 62% of women.

In the SCL group, HDL levels were below the suggested clinical minimum in 9% of men and 28% of women. In the control group, HDL levels were below the suggested clinical minimum in 7% of men. There were no women with HDL levels below the suggested clinical minimum in the control group.

In the SCL group, Apo A1 levels were below the suggested clinical minimum in 7% of men and 8% of women. In the control group, there were no men with Apo A1 levels below the suggested clinical minimum and Apo A1 levels were below the suggested clinical minimum in 15% of women.

There were no subjects with Apo B levels above the suggested clinical level. When comparing the SCL group to the controls, the levels of TC for subjects less than 50 years of age, non-HDL for men, apo B for men, and apo B/apo A1 ratio for men were significantly higher in the SCL group (P< 0.01, Mann-Whitney U).

A statistically significant difference emerged in the levels of apo B and apo B/apo A1 ratio according to the degree of maximal coronary artery stenosis (p < 0.001 for both, Kruskal-Wallis). Cases without stenosis had significantly lower apo B levels and apo B/apo A1 ratio compared to cases with stenosis (both P< 0.001, Mann-Whitney U). Cases with stenosis affecting up to 50% of the lumen had significantly lower apo B levels and apo B/apo A1 ratio compared to cases with more than 50% of

Table 2. Lipid levels

		Atherosclerosis (n = 160)		Controls (n = 55)		Pvalue*
Lipid	Clinicalreference	Range	Median	Range	Median	
Totalcholesterol (mmol/L), age< 50 years	3.3-6.9	2.4-11.7	6.7	2.3-12.1	5.1	0.001
Totalcholesterol (mmol/L), age ≥ 50 years	3.9-7.8	2.0-11.5	6.3	5.5	5.5	NS
HDL (mmol/L), men	0.8-2.1	0.4-5.2	1.3	0.4-4.1	1.3	NS
HDL (mmol/L), women	1.0-2.7	0.5-3.1	1.4	0.9-2.4	1.4	NS
Non-HDL (mmol/L), men	< 3.8	0.61-15	3.5	0.5-11.0	2.5	0.01
Non-HDL (mmol/L), women	< 3.8	1.42-11.37	3.1	1.1-6.5	3.1	NS
Apo A1 (µmol/L), men	37.0-72.0	28.4-153.8	63.4	36.6-107.2	58.4	NS
Apo A1 (µmol/L), women	38.3-80.0	21.6-102.6	65.4	35.8-91.4	70.3	NS
Apo B (µmol/L), men	2.5-5.0	0.6-4.3	2.0	0.7-3.0	1.3	0.001
Apo B (µmol/L), women	2.3-4.4	0.8-2.8	2.0	1.3-2.3	1.8	NS
Apo B/ apo A1, men		0.009-0.075	0.030	0.010-0.055	0.025	0.001
Apo B/ apo A1, women		0.014-0.062	0.033	0.017-0.062	0.027	NS

*Mann-Whitney*U* test. NS, notstatisticallysignificant

lumen stenosis (P = 0.02, P< 0.001, Mann-Whitney U). Cases with stenosis of more than 75% of coronary artery lumen had significantly higher apo B/apo A1 ratio compared to cases with less stenosis or no stenosis at all (P< 0.01, Mann-Whitney U).

We found a statistically significant relationship between the type of most advanced atherosclerotic lesion in the coronary arteries and/or the aorta and the level of TC, non-HDL, apo B and apo B/apo A1 ratio (P = 0.01, P< 0.01, P< 0.001 and P< 0.001, respectively, Kruskal-Wallis). Cases without any signs of atherosclerosis had significantly lower levels of TC, non-HDL, apo B and a lower apo B/apo A1 ratio compared to cases with atheromas/fibrous plaques and calcified lesions (P< 0.01 for all, Mann-Whitney U) and significantly lower levels of apo B and apo B/apo A1 ratio compared to cases with complicated lesions (P< 0.01 and P< 0.001 respectively, Mann-Whitney U). Significantly lower levels of apo B were found in cases with fatty streaks compared to cases with calcified lesions and complicated lesions (P = 0.01 and P< 0.01, Mann-Whitney U).

When comparing COD to lipid levels, a significant relationship was found for TC, non-HDL, apo B, and Apo B/apo A1 ratio (P = 0.03, P = 0.03, P = 0.03 and P< 0.001, respectively, Kruskal-Wallis), but not for apo A1. The levels of TC, non-HDL, apo B, and the apo B/apo A1 ratio were significantly higher in the CVD group compared to other diseases and trauma cases (P = 0.04 and P = 0.02, Mann-Whitney U). The levels of TC and non-HDL were significantly higher in cases with acute ethanol poisoning as COD compared to other diseases and trauma cases (P = 0.02 and P = 0.04, Mann-Whitney U). Apo B/apo A1 ratio was significantly higher in the CVD group compared to acute ethanol poisoning as COD. However, the subjects in the CVD group were significantly older compared to subjects in the acute ethanol poisoning group and other disease/trauma group (P = 0.04 and P< 0.001, Mann-Whitney U).

The correlation was not found between PMI or the age of the subjects and the levels of TC, HDL, non-HDL, apo A1, apo B, apo B/apo A1 ratio, and BAC. Statistically significant differences in lipid levels were not found between genders.

We did not find any correlation between blood ethanol concentration and the levels of TC, HDL, non-HDL, apo A1, apo B or apo B/apo A1 ratio. There was no statistically significant relationship between BAC and the type of most advanced atherosclerotic lesion or the degree of coronary artery stenosis (P = 0.15, P = 0.29, P = 0.33, respectively, Kruskal-Wallis).

Discussion

We evaluated the levels of total cholesterol, HDL, non-HDL, apo A1, apo B, and Apo B/apo A1 ratio from postmortem serum in the context of atherosclerosis and blood alcohol concentration.

The levels of examined lipids were mostly within the suggested clinical range for living persons except for apo B. We did not notice any apo B levels above the suggested clinical range of 2.5–5.0 μmol/L, in most cases (in 80% of all men and 63% of all women) apo B was below the suggested clinical limit. The postmortem levels of lipids are stable in some previous studies. Freedman et al [15] found that the postmortem levels of total cholesterol, LDL, and HDL were representative of antemortem levels. Takeichi et al. [16] studied postmortem plasma sampled up to 12 h after death and found it to be appropriate for measuring lipids and lipoproteins. On the contrary, Särkioja et al. [17] studied the stability of plasma lipids and apolipoproteins during the early postmortem period by taking four duplicate blood samples from eight cadavers 2, 6, 12, and 24 h after death, and the results showed unpredictable fluctuations in plasma lipid and apolipoprotein values. Valenzuela et al. [18] investigated biomarkers from postmortem pericardial fluid and found that levels of LDL, HDL, apo A, and apo B were lower than the antemortem reference interval in plasma before death. Hart et al. [19] have reported average postmortem cholesterol levels 13% lower than antemortem levels. Postmortem degradation and idiosyncrasy of the measurement systems used are suggested as the reasons for lower postmortem lipid levels [18,19]. PMI in our cases was considerably longer compared to the aforementioned previous studies (up to 118.5 h in our study). However, we did not find a correlation between PMI and the levels of examined lipids. Lower than usual lipid and apolipoprotein levels have been found in several acute clinical conditions, such as myocardial infarction, stroke, extensive traumas, diabetes, and liver disease [6]. The presence of co-morbid conditions might explain the low post-mortem lipid levels to some degree. Keeping this in mind, the results from our study suggest that the clinical range of apo B cannot be directly used in interpreting postmortem samples.

In spite of unusually low apo B levels, we found apo B and apo B/apo A1 ratio to be most promising for assessing cardiovascular risk. We found a significant difference in the levels of apo B and apo B/apo A1 ratio between the atherosclerosis group and the controls. Both of those markers were significantly higher in ischemic heart disease as a cause of death compared to other diseases. Also, the apo B/apo A1 ratio was significantly higher in ischemic heart disease as a cause of death compared to trauma and acute ethanol poisoning. Subjects in the atherosclerosis group and those who died of ischemic heart disease were significantly older compared to the control group and subjects whose cause of death was another disease, acute ethanol poisoning or trauma. This seemed to have little effect on the results since we found no substantial association between age and the levels of examined markers.

The levels of apo B and apo B/apo A1 ratio were statistically different between cases with different types of atherosclerotic lesions and the degree of coronary artery stenosis, with higher apo B and apo B/apo A1 ratio in more advanced lesions and stenosis. Apo B is a valid indicator of cardiovascular risk in previous postmortem studies [18,20,21]. The higher apo B/apo A1 ratio in cases with both acute and chronic ischemic heart disease as the cause of death is consistent with studies by Valenzuela et al. [18], Sidorenkov et al. [14] and Rashid et al. [21], and has been explained by elevated apo B levels in these cases.

As for the other markers, non-HDL was significantly different between cases and controls for men and TC for cases and controls in subjects aged under 50 years. TC and non-HDL were also significantly higher in CVD as the cause of death compared to other diseases and in acute ethanol poisoning compared to other diseases and trauma.

The effect of alcohol consumption on the postmortem levels of biochemical markers in Estonian men has been investigated previously [22]. In the present study, we were not able to show a correlation between postmortem blood ethanol concentration and the levels of total cholesterol, HDL, non-HDL, apo A1, apo B, and apo B/apo A1. These results are somewhat unexpected, as the common drinking pattern in Eastern Europe is thought to be drinking in binges, which is associated with significant increases in atherogenic LDL and apo B [13]. However, the exact drinking pattern of the subjects in this study is unknown.

Limitations

This study has several limitations. The number of cases is relatively low, which may interfere with the results. Only cases submitted to forensic autopsy were included, which explains the high number of deaths due to external causes and also limits the available information about subjects' other cardiovascular risk factors, the use of lipid-lowering medication, fasting status, and

ethanol consumption pattern.

Conclusion

The results of this study support the use of apo B and apo B/apo A1 ratio as markers for atherosclerotic cardiovascular disease. We found that the presence of atherosclerotic change and more advanced atherosclerotic lesions in coronary arteries and the aorta is associated with higher postmortem levels of Apo B and apo B/apo A1 ratio. Deaths caused by acute or chronic ischemic heart disease differed from other causes of death by higher apo B levels. Alcohol consumption before death did not affect the postmortem levels of examined markers. Although the levels of examined lipids were not influenced by PMI, postmortem B levels were in most cases lower than the suggested clinical range, making the use of clinical reference values questionable on postmortem samples.

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