



Glycated haemoglobin (HbA_{1c}) for postmortem diagnosis of diabetes

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ABSTRACT

The study was conducted at the Estonian Forensic Science Institute in 2008–2014 as continuous part of our previous study of alcohol and premature death in Estonian men. Autopsy data from 504 cases of male deaths (ages 19–79) were collected and blood and urine samples for glycated haemoglobin (HbA_{1c}), liver enzymes and alcohol concentration were analysed. The aim of our research was to find undiagnosed diabetes and diabetes risk cases postmortem on the basis of increased values of HbA_{1c}. HbA_{1c} was within the reference value 4.8%–5.9% (29–42 mmol/mol), in 88.1% (*n* = 444) of cases, below reference value in 2.4% (*n* = 12), in the risk group of diabetes, HbA_{1c} 6.0%–6.4% (42–46 mmol/mol) was within 5.8% (*n* = 29), and HbA_{1c} result of ≥6.5% (48 mmol/mol) manifested in 3.8% (*n* = 19) of cases. The higher the age, the more cases with HbA_{1c} value ≥6.0% (42 mmol/mol) occurred. In the group of external causes of death (*n* = 348), the HbA_{1c} value of ≥6.5% (48 mmol/mol) HbA_{1c} occurred in four cases. The HbA_{1c} value was ≥6.5% (48 mmol/mol) in 78.9% of 156 cases when the cause of death was disease, of which 58% were cardiovascular diseases. The prevalence of diabetes and diabetes risk was found lower compared to population-based study, as majority of the deceased were young and middle-aged males and no females were included. In the case of poisoning with narcotic substances, HbA_{1c} was within the reference range. A negative correlation occurred between alcohol intoxication and HbA_{1c} value. A positive correlation between ALT and HbA_{1c} was found – the higher stage of liver damage correlated with the higher HbA_{1c} level.

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Introduction

Postmortem diagnosis of glucose metabolism disorders include acute complications of diabetes mellitus as causes of death can be difficult and vague because of the lack of characteristic morphological findings. Diabetes mellitus is one of the most common chronic diseases in many countries, and its occurrence continues to increase. According to International Diabetes Federation data there were 425 million people aged of 20–79 years with diabetes in the world in 2017, and one of two adults with diabetes are undiagnosed (212 million). In Europe, the number of people with diabetes was estimated to be 58 million in 2017 [1].

Studies on alcohol consumption, pre-diabetes and type 2 diabetes risks have different and inconsistent results, showing either a protective or harmful effect or a more complex relationship. Data from epidemiological studies indicate a protective effect of moderate alcohol consumption. No beneficial effect can be attributed to high alcohol consumption [2,3]. Many studies showed that heavy alcohol consumption is a major cause of chronic pancreatitis and a risk factor for type 2 diabetes mellitus and pancreatic cancer [4]. Influence may also depend on specific alcoholic beverages. High

consumption of spirits and beer seemed to increase the risk of pre-diabetes and type 2 diabetes but high wine intake showed a reduced risk of pre-diabetes [5]. The persons having impaired fasting glucose (fasting serum glucose 6.1–6.9 mmol/L and 2 h glucose <7.8 mmol/L) or impaired glucose tolerance (fasting serum glucose <6.1 mmol/L and 2 h glucose 7.8–11.0 mmol/L) were referred to as pre-diabetes cases [5].

For diagnosing diabetes blood glucose should be determined, but as the blood glucose content decreases postmortem because of continuing metabolism, it is not practical to determine blood glucose. Glucose metabolism may be evaluated on the basis of the results of glycated haemoglobin which are not strongly affected by glucose metabolism postmortem [6–8]. The test plays a critical role in the management of a patient with diabetes, since it correlates well with both microvascular and, to a lesser extent, macrovascular complications and is widely used as the standard biomarker for the adequacy of glycaemic management [9]. The amount of HbA_{1c} in the blood is dependent on mean glucose levels present during the 8–12 weeks preceding measurement, as HbA_{1c} accumulates in red blood cells during their 120-day lifespan, and therefore it is useful for long-term blood

glucose control in diabetics [10–12]. For revealing undiagnosed or poorly managed diabetes in a post-mortem context, HbA_{1c} levels are useful for distinguishing diabetic ketoacidosis from starvation or alcoholic ketoacidosis, reveal death caused by hyperosmolar hyperglycaemic state [6,7,13–16] and diagnose fatal diabetic coma [17]. HbA_{1c} is relatively stable for analysing postmortem samples [18–20] and correlates well with clinical samples [19]. Ensuring the diagnosis of diabetes mellitus by morphological findings is very difficult and in many cases it remains undiagnosed. Therefore, it is important to determine if HbA_{1c} values are affected by other concomitant diseases in addition to diabetes and if it is associated with cause of death due to external causes (injury).

The first aim of our research was to find undiagnosed diabetes cases and diabetes risk cases postmortem on the basis of increased values of glycated haemoglobin. The second aim was to find changes in the values of liver enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT), and the association between liver enzymes and glycated haemoglobin in chronic alcohol abusers in addition to liver damage diagnosed on the basis of macroscopic findings and to find out cases of possible secondary diabetes.

Materials and methods

The study was conducted at the Estonian Forensic Science Institute in 2008–2014 as continuous part of our previous study of alcohol and premature death in Estonian men. The study target population was all deaths in Estonia subject to forensic autopsy of men aged over 18 years. The criterion for inclusion into the present study was possibility to obtain vitreous humour and blood. At autopsy, blood was collected from peripheral vessels, usually from femoral vein, with 20 mL syringe and 14-gauge needle. Blood was collected into 10 mL vacutainer tubes – in an EDTA tube for glycated haemoglobin and one plain vacutainer for liver enzymes and with sodium fluoride for blood alcohol concentration. Urine was taken directly from the bladder with 20 mL syringe and 14-gauge needle. There was also one plain tube for urine. Urine samples were collected for alcohol and narcotics. Vitreous humour was aspirated from both eyes using a syringe.

The samples were refrigerated at 4 °C in the Forensic Science Departments of the Estonian Forensic Science Institute (EFSI) and dispatched at the same temperature conditions to the Southern Estonian Department within 24 h. For the determination of liver enzymes, blood was centrifuged at 2100 r/min for 15 min in Eppendorf cooled centrifuge 4 °C and thereafter the serum was removed. If it was not possible to get serum, this case was excluded from the study. Data from the remaining

504 cases was used in the analysis. EDTA-blood for the determination of HbA_{1c} and serum for the determination of liver enzymes were sent to the United Laboratories of the Tartu University Hospital. Vitreous humour was used for other studies. ALT and AST levels were measured using the kinetic photometric method as following: ALT and AST according to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) with pyridoxal phosphate activation (Cobas 6000, Roche) – *in vitro* tests for the quantitative determination of ALT and AST with pyridoxal phosphate activation in human serum and plasma on Roche/Hitachi cobas c systems. Both tests' measuring range is 5–700 U/L (0.08–11.7 μkat/L) and lower detection limit 5 U/L (0.08 μkat/L), and precision was determined using human samples and controls in an internal protocol with repeatability ($n = 21$) and intermediate precision (3 aliquots per run, 1 run per day, 20 days). The consensus measuring ranges (IFCC) for ALT and AST are <50 U/L in men and <35 U/L in women. GGT levels were measured with the kinetic colorimetric method as in the following: γ -Glutamyltransferase ver.2 Standardized against IFCC/Szasz (Cobas 6000, Roche) – *in vitro* test for the quantitative determination of γ -glutamyltransferase (GGT) in human serum and plasma on Roche/Hitachi cobas c systems. Measuring range is 3–1200 U/L (0.05–20.0 μkat/L) and lower detection limit of the test 3 U/L (0.05 μkat/L), and precision was determined using human samples and controls in an internal protocol with repeatability ($n = 21$) and intermediate precision (3 aliquots per run, 1 run per day, 21 days). The consensus measuring ranges (IFCC) for GGT are <60 U/L in men and <40 U/L in women. HbA_{1c} was measured using the Tina-quant Hemoglobin A1c Gen.3 – Hemolysate Application – Standardized according to IFCC transferable to Diabetes Control and Complications Trial/National Glycohemoglobin Standardization Program (DCCT/NGSP) (Cobas Integra 400 plus, Roche) – *in vitro* test for the quantitative determination of mmol/mol of hemoglobin A1c (IFCC) and percentage of hemoglobin A1c (DCCT/NGSP) in hemolysate prepared from whole blood on Roche clinical chemistry analysers. The HbA_{1c} determination is based on the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood. The final result is expressed as mmol/mol or percentage of HbA_{1c} and is calculated from the HbA_{1c}/Hb ratio. Measuring range of 23–196 mmol/mol HbA_{1c} (IFCC) and 4.2%–20.1% HbA_{1c} (DCCT/NGSP) at a typical haemoglobin concentration of 8.2 mmol/L (13.2 g/dL). Lower limits of measurement: accordingly limit of blank and limit of detection for Hb 0.31 mmol/L and 0.62 mmol/L and for HbA_{1c} 0.12 mmol/L and 0.18 mmol/L. Repeatability and

intermediate precision were determined using human samples and controls in accordance with the Clinical and Laboratory Standards Institute (CLSI) EP5 requirements (2 aliquots per run, 2 runs per day, 21 days).

For the assessment of laboratory results, Tartu University Hospital's United Laboratories reference values for patients/living persons were used. The reference values for live persons are as follows: ALT <41 U/L, AST <38 U/L and GGT <61 U/L [20]. The reference values for HbA_{1c} are as follows: normal value 4.8%–5.9% (by the IFCC 29–42 mmol/mol), below reference value <4.8% (<29 mmol/mol), risk group for diabetes 6.0%–6.4% (42–46 mmol/mol), diabetes mellitus ≥6.5% (≥48 mmol/mol) [21].

Urine and blood alcohol concentrations (BAC) were measured at the Chemistry Laboratory of the EFSI using headspace gas chromatography. Urine samples were screened in EFSI for narcotics, using a rapid urine test (Multiscreen 10 MTD; Biomedical Diagnostics) for measure of amphetamine, methamphetamine, cocaine, ecstasy, morphine, methadone, cannabis metabolite Δ -tetrahydrocannabinole, benzodiazepines, barbiturates and tricyclic antidepressants. Where screening results were positive, further confirmatory analysis was undertaken in EFSI using combined gas chromatography and mass spectrometry.

For each case a study record was completed to which the record number, the EFSI Department, the autopsy number, and the deceased's date of birth, age, date of death and date of autopsy were entered using general data. Of more specific data, macroscopic, microscopic and laboratory findings (ethanol concentration, concentration of alcohol surrogates, illegal and legal drugs, liver enzymes and HbA_{1c}) were documented retrospectively. Cause of death according to ICD code, manner of death, main disease and concomitant diseases were added to the record summary. The causes of death were classified on morphological and toxicological bases. The data were analysed by the following age groups: ≤29 years, 30–39 years, 40–49 years, 50–59 years and ≥60 years.

Statistical analysis was performed using the Statistica 13.0 statistical program. Spearman rank order correlation (denoted as r_s), multiple response items and factorial dispersion analysis were used to assess bivariate relationships. The descriptive statistics were used to analyse cases' general data. A P -value <0.05 was considered statistically significant.

Results

Autopsy data from 504 cases of male deaths (ages 19–79) were collected and samples were analysed. HbA_{1c} was within the reference value 4.8%–5.9% (29–42 mmol/mol) in 88.1% ($n = 444$) of cases, HbA_{1c} was below reference value in 2.4% ($n = 12$), in the risk

group of diabetes, HbA_{1c} 6.0%–6.4% (42–46 mmol/mol) was within 5.8% ($n = 29$), and HbA_{1c} result of ≥6.5% (48 mmol/mol) manifested in 3.8% ($n = 19$) of cases. Previously was known to have diabetes in five males.

Age and HbA_{1c} values

Eighty-five per cent of the deceased were aged 30–60 years. The largest age group was 40–49 years old, 38.1% of all investigated deaths. The higher the age, the more cases with HbA_{1c} value of >6.0% (42 mmol/mol) occurred ($r_s = 0.2$, $P < 0.05$). In an age group ≤29 years there was only one case where the HbA_{1c} value was ≥6.5% (48 mmol/mol) (a 26-year-old youth who died of gastrointestinal haemorrhage and who had alcoholic liver steatosis as the main disease. There was a previous history of diabetes). In the 30–39 age group there were six cases with a HbA_{1c} value of > 6.0% (42 mmol/mol) and one case with a HbA_{1c} value of ≥6.5% (48 mmol/mol); in the 40–49 age group there were 12 cases with a HbA_{1c} value of > 6.0% (42 mmol/mol) and 4 cases with a HbA_{1c} value of ≥6.5% (48 mmol/mol); in the 50–59 age group there were 11 HbA_{1c} values of > 6.0% (42 mmol/mol) and 11 values of ≥6.5% (48 mmol/mol) (that is 45.8% of all values of > 6.0% (42 mmol/mol)); in the ≥60 age group there were two cases with a HbA_{1c} value of ≥6.5% (48 mmol/mol). The age distribution of subjects and HbA_{1c} values are presented in Table 1.

Cause of death and HbA_{1c} values

On the basis of the cause of death we established the following study groups: (1) external causes of death ($n = 348$): injury, other external causes (hypothermia, electrocution), drowning, hanging, other asphyxias (e.g. asphyxia with foreign bodies, inhalation of gastric contents), alcohol poisoning, illegal drug poisoning, fire fatality (e.g. carbon monoxide poisoning, burns of multiples body regions), (2) diseases ($n = 156$): cardiovascular diseases (chronic ischaemic heart disease, myocardial infarction, alcoholic and hypertrophic cardiomyopathy, hypertensive diseases), alcoholic liver disease and other diseases (pneumonia, haemorrhagic gastritis). Cause of death, age and levels of HbA_{1c}, ALT, AST, GGT and BAC are presented in Table 2.

Table 1. The age distribution of subjects and HbA_{1c} values.

Age group (years)	<i>N</i>	<4.8%	4.8%–5.9%	6.0%–6.4%	≥6.5%
≤29	66	1 (1.5%)	64 (96.7%)	0	1 (1.5%)
30–39	113	2 (1.8%)	104 (92.0%)	6 (5.3%)	1 (0.9%)
40–49	192	6 (3.1%)	170 (88.5%)	12 (6.3%)	4 (2.1%)
50–59	124	3 (2.4%)	99 (79.8%)	11 (8.9%)	11 (8.9%)
≥60	9	0	7 (77.8%)	0	2 (22.2%)
Total	504	12 (2.4%)	444 (88.1%)	29 (5.8%)	19 (3.8%)

Table 2. Cause of death, age and levels of HbA_{1c}, ALT, AST, GGT and BAC.

Cause of death	n	Age (years)		HbA _{1c} (% (mmol/mol))		ALT (U/L)		AST (U/L)		GGT (U/L)		BAC (mg/g)	
		Range	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range	Median
Injury	82	21–54	39.5	4.8–6.4 (29–46)	5.4 (36)	35–28 900	689	99–19 850	983.5	15–1 455	58	0–4.3	2.0
Other external causes	19	32–58	45.0	4.7–6.2 (28–44)	5.5 (37)	51–9 374	273	82–5 717	814	26–947	59.5	0–3.2	0.0
Drowning	26	26–54	41.0	4.8–5.9 (29–42)	5.4 (36)	32–4 792	268	172–2 039	747	21–348	90	0–3.9	2.3
Hanging	91	19–68	44.0	4.9–8.4 (30–68)	5.5 (37)	57–439 500	1 342	134–195 200	1 112	21–327	76.5	0–4.0	1.4
Others asphyxias	34	20–66	41.0	5.0–6.6 (31–47)	5.5 (37)	112–5 919	639	156–6 208	897	30–1 608	124	0–6.3	1.5
Fatal alcohol abuse	43	29–54	46.0	4.8–6.9 (29–52)	5.4 (36)	40–17 000	483	63–9 984	945	29–725	124	0–6.6	3.9
Drug intoxication	36	25–43	28.0	4.5–5.9 (26–42)	5.3 (34)	165–42 580	1 833	287–24 540	1 664	34–1 102	123	0–2.7	0.0
Fire fatality	17	26–58	46.0	4.3–5.7 (24–39)	5.3 (34)	45–29 898	745	116–17 653	613	34–1 102	123	0–4.4	2.7
Cardiovascular diseases	87	31–79	47.0	4.3–10.7 (24–93)	5.6 (38)	29–8 363	906	122–9 817	1 026	20–1 884	89	0–3.8	0.0
Alcoholic liver disease	35	26–60	48.0	4.5–6.9 (26–52)	5.5 (37)	101–13 793	1 078	237–13 050	1 847	47–988	340	0–2.6	0.0
Others diseases	34	29–54	47.0	4.7–9.9 (28–85)	5.6 (38)	49–11 172	771.5	106–9 596	1 266.5	23–900	82	0–2.8	0.0

BAC: blood alcohol concentration.

In the group of external causes of death, an HbA_{1c} value of $\geq 6.5\%$ (48 mmol/mol) HbA_{1c} occurred in four cases (1.1%): in two cases the cause of death was mechanical asphyxia (hanging), in one case other asphyxiation (aspiration of vomit) and in one case ethanol poisoning. In the case of acute alcohol poisoning the HbA_{1c} value was 4.8%–6.9% (29–52 mmol/mol) (median 5.4% (36 mmol/mol)) and in the case of poisoning with narcotic substances, and in the injury and hanging groups, HbA_{1c} was within the reference range (median 5.4% (36 mmol/mol)). A negative correlation occurred between alcohol intoxication and HbA_{1c} value ($r_s = -0.1$, $P < 0.05$). In the whole study group 54.6% of the deceased ethanol was found in blood.

In the diseases group, in 78.9% ($n = 123$) of cases the HbA_{1c} value was $\geq 6.5\%$ (48 mmol/mol), of which 57.9% were cardiovascular diseases. In the alcoholic liver damage group, the HbA_{1c} of value $\geq 6.5\%$ (48 mmol/mol) occurred in two cases. In previously known diabetes patients ($n = 5$) HbA_{1c} values were as in the following: 9.9% (85 mmol/mol) in male whose cause of death was ischaemic heart disease, 10.7% (93 mmol/mol) in male with myocardial infarction, 5.6% (38 mmol/mol) in male with other unspecified heart disease, and 6.2% (44 mmol/mol) and 9.44% (78 mmol/mol) in two cases with pneumonia. On the basis of forensic autopsy findings diabetes was diagnosed only in one case where it was previously undiagnosed and where the direct cause of death was myocardial infarction; the biochemical characteristics were: HbA_{1c} 8.93% (74 mmol/mol), ALT 2 415 U/L, AST 1 439 U/L and GGT 28 U/L.

Comorbidities and HbA_{1c} values

Cardiovascular diseases occurred in 75% of all deceased, of which 47.2% ($n = 238$) were atherosclerosis of the aorta and coronary arteries, 4.8% ($n = 24$) hypertension, 3.2% ($n = 16$) alcoholic cardiomyopathy, 3.2% ($n = 16$)

dilating cardiomyopathy, 4.5% ($n = 23$) hypertrophic cardiomyopathy, 11.7% ($n = 59$) chronic ischaemic heart disease and 0.4% ($n = 2$) other cardiovascular diseases (valve defect, myocarditis). Of the cases, 19.8% ($n = 100$) were without concurrent cardiovascular pathology and in 26 cases no data were reported about cardiovascular pathology. Of the deceased with HbA_{1c} value $\geq 6.0\%$ (42 mmol/mol), 33.4% ($n = 15$) had ischaemic heart disease as well as atherosclerosis of the aorta and coronary arteries, 11.1% ($n = 5$) had hypertrophic cardiomyopathy and other pathologies occurred as single cases. In two cases with an HbA_{1c} value $\geq 6.0\%$ (42 mmol/mol) no cardiovascular diseases occurred.

Liver diseases occurred in 58% of subjects, of which focal steatosis made up 22.6% ($n = 114$), diffuse steatosis 25.8% ($n = 130$), incomplete fibrosis 2.4% ($n = 12$), incomplete cirrhosis 3.8% ($n = 19$) and alcoholic liver damage 3.6% ($n = 18$). Without liver pathology were 34.9% ($n = 176$) and in 35 cases had no microscopically data. With an HbA_{1c} value ≥ 6.0 (42 mmol/mol) there was focal liver steatosis of 35.6%, diffuse steatosis of 28.9%, cirrhosis of 4.5% and alcoholic liver damage of 2.2%. Of these, 24.2% had no liver pathology (in other cases the microscopically pathology were not determined).

Pancreatic diseases were found in 14.5% ($n = 73$), of which there were 5 cases of acute pancreatitis and 68 cases of chronic pancreatitis; in 65.9% ($n = 332$) cases no pathology occurred (in 99 cases it was not possible to determine pancreatic pathology). In subjects with HbA_{1c} value $\geq 6.0\%$ (42 mmol/mol) acute pancreatitis occurred in 2.2% ($n = 3$) and chronic pancreatitis in 15.6% ($n = 7$), no pancreatic pathology occurred in 64.4% of the cases (in other cases the microscopically pathology were not determined).

In the case of mechanical asphyxiation, atherosclerosis of the aorta and coronary arteries was diagnosed as a concomitant disease, in one case hypertension and

liver lipodosis; in the case of other asphyxiation the concomitant diseases were dilating cardiomyopathy and liver lipodosis; and in the case of alcohol poisoning ischaemic heart disease was diagnosed as the concomitant disease.

The positive correlation between ALT and HbA_{1c} ($r_s = 0.1393$, $P < 0.05$) was found: the higher the stage of damage the higher the HbA_{1c} level. High GGT values occurred in the liver alcoholic damage group. Liver alcoholic damage occurred in 35 cases with a GGT activity median of 340 U/L. The value of the GGT value was also significantly higher than in other cause of death groups ($P < 0.05$). The increase of GGT activity correlates with the risk of death due to atherosclerosis of the coronary arteries and other heart diseases. Table 3 shows the association of liver pathology with HbA_{1c} value and ALT, AST and GGT.

Discussion

The postmortem determination of complications of diabetes mellitus is difficult depending on the duration of disease. The changes as pancreas islet loss, atherosclerosis, microangiopathy, retinopathy, nephropathy and neuropathy are non-specific signs. Qualitative tests with stripes and tablets (glucose, bilirubin or acetone) or also by using electronic test devices may help to confirm or exclude certain suspected diagnoses [17]. In one study it was found that the calvariae of the diabetics showed reduced brightness and degree of the darker colour was correlated with the duration of illness [22]. The other researchers did not detect colour differences between diabetics and non-diabetics, but found correlation between yellowish bone colour and the subjects' age [23]. The recent study has shown significant correlation of bone yellowing with HbA_{1c} and

age [24] and it is suggested that it is due to accumulation of advanced glycation end products produced in the Maillard or browning reaction by non-enzymatic means [25]. Histological examinations can reveal diabetic glomerulosclerosis (Kimmelstiel Wilson syndrome or nodular glomerulosclerosis) due to angiopathy of capillaries of renal cortex and glycogen nephrosis in cases long-lasting high hyperglycaemia (Armani-Ebstein cells in straight sections of the proximal tubules) [26,27]. Biochemical analyses, including blood glycated haemoglobin, fructosamin [8,20,28] and anhydroglucitol [29,30], determination, may complement postmortem investigations and provide useful information for determining the cause of death even in corpses with advanced decomposition. In post-mortem specimens, HbA_{1c} was found stable from 4 to 36 h [18] or for at least 72 h (72 or more hours following death) [19,20], and EDTA was found to be preferable preservative, as samples can be measured after four weeks storage at 4 °C [13]. But interpretation of biochemical analyses is difficult because of postmortem blood alterations involving glucose metabolic pathways. For comparison of the clinical and postmortem results, the measurement of blood glycated haemoglobin was chosen because fructosamin or anhydroglucitol is not used for glycaemic control in clinical laboratories in Estonia.

In Estonia, the adults (20–79 years old) with diabetes were estimated to be 55 000 people, diabetes age-adjusted comparative prevalence 4.0%, adults with undiagnosed diabetes 19 800 and diabetes-related deaths 544 [1].

By International Expert Committee report on the use of the HbA_{1c} assay in the diagnosis of diabetes, diabetes should be diagnosed when HbA_{1c} is $\geq 6.5\%$ (48 mmol/mol). Individuals with an HbA_{1c} level $\geq 6\%$ (42 mmol/mol) but $< 6.5\%$ (48 mmol/mol) are at the highest risk for progression to diabetes [31].

In our study, most HbA_{1c} values were within the limits of the reference value 4.8%–5.9% (29–42 mmol/mol)); 5.8% of cases had values indicating diabetes risk and 3.8% with diabetes mellitus. At the same time, we saw that the higher the age the more frequently the HbA_{1c} value is $\geq 6.0\%$ (42 mmol/mol). As majority of the deceased were young and middle-aged males and no females were included in our study group, therefore, prevalence of diabetes and diabetes risk was found lower compared to population-based study.

Chen et al. [32] have found that hyperglycaemia was frequently detected in mechanical asphyxiation, salt-water drowning, cerebrovascular disease, ischemic heart disease and electrocution. In deaths caused by external causes we found high HbA_{1c} values only in individual cases, all with concomitant cardiovascular diseases; therefore, it is reasonable to think that the increase of HbA_{1c} is caused by the diseases, not external causes. Although the relationship between cardiovascular disease and glycaemia is believed to represent

Table 3. The association of liver pathology with HbA_{1c} value and ALT, AST and GGT mean value.

Liver pathology	Hb1Ac (%) (mmol/mol)	ALT (U/L) Mean	AST (U/L) Mean	GGT (U/L) Mean
Without pathology	4.8–5.9 (29–42)	2 802.1	2 075.9	79.6
	6.0–6.4 (42–46)	6 333.4	4 510.4	86.0
	>6.4 (46)	1 264.0	1 375.0	47.5
Focal steatosis	4.8–5.9 (29–42)	8 321.0	4 663.4	121.3
	6.0–6.4 (42–46)	4 009.0	2 788.3	100.3
	>6.4 (46)	3 449.6	1 590.6	126.0
Diffuse steatosis	<4.8 (29)	1 173.5	2 895.5	475.0
	4.8–5.9 (29–42)	2 382.8	2 837.8	277.5
	6.0–6.4 (42–46)	1 797.8	1 554.9	146.5
Fibrosis	>6.4 (46)	1 997.3	3 643.0	178.0
	4.8–5.9 (29–42)	1 350.1	1 513.3	279.0
	<4.8 (29)	203.0	931.0	787.5
Cirrhosis	4.8–5.9 (29–42)	3 186.7	4 614.8	280.7
	6.0–6.4 (42–46)	4 687.0	2 384.0	199.0
	>6.4 (46)	72.0	151.0	410.0
Alcoholic liver damage	<4.8 (29)	420.0	1 562.0	947.0
	4.8–5.9 (29–42)	779.1	1 338.5	284.3
	6.0–6.4 (42–46)	247.0	909.0	352.0

a continuum without a threshold effect, HbA_{1c} might offer more advantages in terms of prognostic information, as it is a more stable, accurate parameter of glucose homeostasis.

Persistent elevations in blood sugar (therefore HbA_{1c}) increase the risk of long-term vascular complications of diabetes such as coronary disease, heart attack, stroke, heart failure, kidney failure, etc. [33,34]. HbA_{1c} is a good marker of glycated proteins, which play a contributory role in atherosclerosis in both diabetic and non-diabetic individuals. We also achieved the similar result, as the HbA_{1c} values were in range 4.3%–10.7% (24–93 mmol/mol), median 5.6% (38 mmol/mol) occurred in 87 cases with cardiovascular diseases, an HbA_{1c} value $\geq 6.0\%$ (42 mmol/mol) occurred with ischaemic disease, atherosclerosis of aorta and coronary arteries (33.4%) and when the HbA_{1c} value was $\geq 6.5\%$ (48 mmol/mol), 58% of cases included cardiovascular diseases. Our study also showed that the increase in HbA_{1c} value 6.0%–6.4% (42–46 mmol/mol) occurs together with concomitant affecting cardiovascular diseases: atherosclerosis of the aorta and coronary arteries, hypertension and dilating cardiomyopathy.

Among adults per capita alcohol consumption (in litres of pure alcohol) by type of alcoholic beverage was 41% beer, 37% spirits, 11% wine and 11% other in 2010 [35]. The “other alcohol” group contains surrogate non-beverage alcohols (ethanol-containing liquids not intended for consumption), which are drunk particularly among men in Estonia [36,37]. In a previous study of association of alcohol with the mortality of Estonian men aged 25–54 years, alcohol was found in blood in concentrations ≥ 0.2 mg/g in 55% cases of death [38]. Results of that study of men also showed evidence of alcohol-induced pathologies in 75% of cases, and 32% had pathologies in two or more organs [39]. Some types of alcohol-related purchases were associated with a lower prevalence of type 2 diabetes (T2D), and respondents who purchased the greatest volumes of wine or beer – but not liquor – were less likely to report being diagnosed with T2D in 2011–2012 than non-drinkers [40].

We found a negative correlation between alcohol intoxication and HbA_{1c} value ($r_s = -0.1$, $P < 0.05$), as deceased in the acute alcohol intoxication group were relatively younger. The effects may be also caused by alcohol-induced hypoglycaemia [41]. In the whole study group 54.6% of the deceased ethanol was determined in blood.

Lower-than-expected levels of HbA_{1c} can be seen in people with shortened red blood cell lifespan, such as with glucose-6-phosphate dehydrogenase deficiency, sickle-cell disease or any other condition causing premature red blood cell death. A low HbA_{1c} value occurred with carbon monoxide poisoning (2.4%) which may be associated with the ability of haemoglobin to bind carbon monoxide.

In the case of poisoning by narcotic substances there are different opinions in the literature about HbA_{1c} values, e.g. according to Chen et al. [32] the HbA_{1c} value is lower in fatal methamphetamine abuse, probably from the starvation during the long-term abusing of methamphetamine. Palmiere et al. [15] showed that cocaine consumption provokes hyperglycaemia due to increases of adrenalin/noradrenalin level. Probably the HbA_{1c} normal values found on the basis of this study may be explained by the fact that the fentanyl typically used in Estonia [42]. Fentanyl does not affect glucose metabolism or that mostly young people die as a result of poisoning with narcotic substances.

The liver is an accumulator of glycogen and a regulator of glucose metabolism. Therefore when liver damage worsens, glucose metabolism is also impaired and the HbA_{1c} level increases. With an HbA_{1c} value $\geq 6.0\%$ (42 mmol/mol), 35.6% had focal liver steatosis, 28.9% had diffuse steatosis, 4.5% cirrhosis and 2.2% alcoholic liver damage. Liver damage was absent in 24.2% of cases. With an HbA_{1c} value $\geq 6.0\%$ (42 mmol/mol) of pancreas damage acute pancreatitis occurred in 2.2% and chronic pancreatitis in 15.6% of cases. At the same time we know that autolysis makes the assessment of morphological findings of the pancreas difficult. Therefore, we see every day that it is not possible to assess pancreas findings.

The increasing trends in alcoholic liver cirrhosis mortality in Estonia over the past two decades represent trends for chronic liver disease and cirrhosis in northern European countries [43]. Drastic reduction of the level of liver enzymes ALT and AST in the case of cirrhotic liver damage indicates liver failure in live subjects, but on the basis of these reference values it is not possible to find associations of ALT and AST with cause of death groups, as the same reference ranges cannot be used in deceased subjects. At the same time there was a positive correlation between ALT (and the enzyme associated more with liver damage than AST) and HbA_{1c} in cause of death groups. An AST to ALT ratio over 2:1, characteristic to alcohol abuse, also occurred in our study in alcoholic liver disease groups. In the study by Ringmets et al. [38] it was stated that liver enzymes, used widely to detect liver damage in living subjects, have a limited postmortem value. AST and ALT are increased by haemolysis and hypoxia and so are subject to differences in the process of dying. GGT, as a marker of liver fibrosis and cirrhosis, is less affected by these processes.

When determining the cause of death, only the GGT result of liver enzymes can be used to determine the cause of death (e.g. cirrhotic liver, chronic alcohol abuse). A strong dose-response relationship of serum GGT to cardiovascular mortality has been found [44]. The association with high GGT was consistent over all subgroups of disease with the exception of acute/

subacute forms of coronary heart disease in men. However, this latter association was significant in men aged <60 years. Additionally, GGT was significantly associated with established risk factors such as triglycerides, body mass index and cholesterol. Adjustment for these variables in multiple risk factor regression analyses confirmed GGT as an independent predictive factor for cardiovascular mortality.

However, the results in postmortem biochemistry in general do not have an absolute value, and they must be interpreted in the context of pathological findings and the circumstances of the death, and with information from multiple marker systems, to maximize their diagnostic value.

Conclusion

Since these diabetic complications are often involved in the causes of sudden death or contributors to other casualties, postmortem diagnosis of diabetes mellitus is important in routine forensic autopsy. HbA_{1c} in blood is useful to investigate not only fatal metabolic disorders involved in diabetes mellitus but also death processes due to other causes.

Compliance with Ethical Standards

This article does not contain any studies with human participants or animals performed by any of the authors.

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