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Ethylene glycol poisoning may be associated with elevated post-mortem vitreous glucose level

Pirkko Kriikku [a,b,*], Ilkka Ojanperä [a,b], Philippe Lunetta [c,d]

[a] Forensic Toxicology Unit, Finnish Institute for Health and Welfare, P.O. Box 30, 00271 Helsinki, Finland
[b] Department of Forensic Medicine, University of Helsinki, P.O. Box 40, 00014 Helsinki, Finland
[c] Department of Biomedicine, Forensic Medicine, University of Turku, Finland
[d] Department of Forensic Medicine, Research Unit of Internal Medicine, University of Oulu, Finland

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ABSTRACT

Ethylene glycol (EG) is a toxic chemical that is sometimes used as ethanol substitute. Besides the desired intoxicating effects, the intake of EG may often lead to death unless timely treatment measures are provided by medical professionals.

We examined 17 fatal EG poisonings between 2016 and March 2022 in Finland in terms of forensic toxicology and biochemistry results and demographic information. Most of the deceased were male and the median (range) age was 47 (20–77) years. Of the cases, 6 were suicides, 5 accidents and in 7 cases the intent remained undetermined. In all cases, vitreous humour (VH) glucose was above the limit of quantitation 0.35 mmol/L (mean: 5.2 mmol/L; range 0.52–19.5 mmol/L). Other markers of the glycaemic balance were within the normal range in all except one case.

As EG is not routinely screened for in most laboratories but only analysed in cases where the intake of EG is suspected, some fatal EG poisonings may remain unrecognised in post-mortem (PM) investigations. Although various conditions may induce hyperglycaemia, it is worthwhile keeping in mind that elevated PM VH glucose levels that cannot be otherwise explained may suggest intake of ethanol substitutes.

1. Introduction

Ethylene glycol (EG) is an odourless and colourless dihydric alcohol that is non-flammable and sweet-tasting. It is widely used as an industrial chemical but also in antifreeze products and hydraulic brake fluids. There is a long history of especially the automotive liquids being used as a cheap substitute for ethanol. In the US, the American Association of Poison Control Centers' National Poison Data system reported 7 242 cases with EG exposure in 2019 [1]. Furthermore, 12 deaths by automotive EG and an additional 12 deaths by other forms of EG were reported [1]. In Finland, the yearly number of fatal EG poisonings ranged between 1 and 8 in 2014–2017 [2].

EG toxicity is for the most part related to its metabolites that cause metabolic acidosis, cardiovascular failure, acute renal failure and central nervous system dysfunction [3] although death due to acute toxic effects of EG has also been reported [4]. After the intake of EG, the hepatic enzyme alcohol dehydrogenase turns EG into glycoaldehyde, which is further oxidised to glycolic acid, glyoxylic acid and finally oxalic acid and formic acid [5]. In many laboratories, glycolic acid (GA) is determined in addition to EG since their combination better illustrates the severity of the EG poisoning [6]. Oxalic acid precipitates as calcium oxalate crystals in the kidneys and other tissues and is thus responsible for the kidney toxicity commonly occurring after the intake of EG [7,8].

In most laboratories EG is not routinely analysed in post-mortem (PM) cases but the analysis is performed when there is a reason to believe that the deceased may have ingested ethanol substitutes. Thus, if such background information is not available and the microscopic examination overlooks or displays no calcium oxalate crystals in the kidney tissue, fatal EG poisoning may remain unnoticed. The appearance of typical oxalate crystals in kidney is time-dependent and rely on the interval between EG intake and death [9], and the presence of such crystals is not specific for EG poisoning [10].

Elevated vitreous humour (VH) glucose levels in fatalities are usually connected to complications of diabetes such as diabetic ketoacidosis or hyperosmolar hyperglycaemic state [11,12,13,14]. In addition, high blood glucose levels have been connected to methanol poisoning

* Corresponding author at: Forensic Toxicology Unit, Finnish Institute for Health and Welfare, P.O. Box 30, 00271 Helsinki, Finland.
E-mail address: pirkko.kriikku@thl.fi (P. Kriikku).

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[15,16,17,18]. Hyperglycaemia has even been suggested to be a significant predictor of a fatal outcome in methanol poisoning cases entering the hospital for treatment [17]. However, even though there are sporadic reports of hyperglycaemia in EG poisonings [19,20], there is very little scientific literature on the effects of EG on blood sugar, and to the best of our knowledge the phenomenon has so far only been described in clinical settings [21,22,23].

We aimed to fill this gap by presenting a series of fatal EG poisonings in which PM VH glucose was also determined. In addition, we sought to investigate whether elevated VH glucose levels, in cases where other markers of sugar balance are within the normal range, could be used as an additional indication for the analysis of EG.

2. Material and methods

Data was extracted from the forensic toxicology database maintained by the Finnish Institute for Health and Welfare (THL) in which all results of the PM toxicological analyses in medico-legal investigations nationwide as well as information from the death certificates are collected.

All cases in which the cause of death was fatal EG poisoning (ICD-10 code T53.8) between 2016 and March 2022 were included. Blood and urine concentrations of EG and GA and VH glucose levels were collected together with demographic information of the deceased. For comparison, we reviewed during the same study period all cases in which VH glucose and *beta*-hydroxybutyrate (BHB) either in PM blood or VH was analysed.

Femoral blood, urine, and VH samples had been collected during the autopsy on average 7.8 days after death (range 4–12 days) and stored refrigerated at 4 °C until the analysis. Sodium fluoride was used as a preservative in the blood samples.

All EG poisoning cases had been analysed by a headspace in-tube extraction gas chromatography-mass spectrometry method for EG, GA and formic acid in blood and urine. The analytical method has been described in detail elsewhere [24]. The cases had been selected for analysis based on suspected intake of EG before death. The suspicion had been raised either due to background information from the police or post-mortem histological detection of calcium oxalate crystals in kidneys.

Glucose and lactate levels and BHB concentrations were determined in VH by standard enzymatic assays as described elsewhere [25,26].

3. Results

There were 28 fatal EG poisonings between 2016 and March 2022. The 17 EG poisonings in which also the VH glucose level was determined were assessed in detail.

Of the 17 victims of EG poisoning, 14 were males and 3 females. The median (range) age was 47 (20–77) years. Of the cases, 6 were suicides, 5 accidents and in 6 cases the intent remained undetermined.

The median (range) PM blood concentration was 1.6 (0–6.0) g/L for EG and 1.9 (0.94–2.3) g/L for GA. The median (range) PM urine concentration was 12 (0.61–22) g/L for EG and 6.3 (2.6–11) g/L for GA. In one case EG and GA were measured in AM blood in addition to PM blood (Table 1). Formic acid was not detected in any of the 17 cases.

In all 17 cases glucose was detected in VH above the limit of quantification (LOQ) of the method (0.35 mmol/L). In four cases VH glucose was above the reference value in our laboratory (7 mmol/L). The median (range) glucose concentration in VH was 5.2 mmol/L (0.52–19.5 mmol/L). As illustrated in Fig. 1, there was no correlation between the EG or GA blood concentrations and the glucose concentration measured in VH.

In cases 5–17, BHB was determined in VH in addition to glucose, and it was below the LOQ (0.5 mmol/L) in all cases. Before the introduction of the BHB analysis in our laboratory in 2016, total ketone bodies converted to acetone were measured for the same purpose. Of the cases 1–4, where BHB was not analysed, the value for ketone bodies was below the LOQ (0.5 mmol/l) in all but one case (Case 4) in which the value was slightly elevated (1.8 mmol/L). Lactate was below the reference value (35 mmol/L) in all cases.

In one of the studied cases (Case 15) the deceased was reported to have suffered from diabetes mellitus type 2 (DM2). In all the other cases there was no mention of previous diabetes of any kind.

A low ethanol concentration was detected in two cases: *Case 1* (blood 0.20 g/kg, urine negative) and *Case 11* (blood negative, urine 0.27 g/kg). In addition, acetone was detected *Case 13* (blood 0.2 g/kg, urine 0.4 g/kg) and in *Case 15* (blood 0.2 g/kg, urine 0.3 g/kg). Methanol was not detected in any of the cases.

To assess VH glucose concentration in a larger sample of PM material, the following control data was considered. In 2016–2021, VH glucose was measured in a total of 11 539 PM cases. Of these cases, glucose was above the LOQ (0.35 mmol/L) in 36% (N = 4202) and above the reference value (7 mmol/l) in only 7.0% (N = 811).

Of the 4202 cases in which glucose was above the LOQ, BHB was normal (<0.5 mmol/l) in 79% (N = 3 307) indicating that these individuals had not suffered from ketoacidosis before death.

Of the cases in which glucose was ≥ 7 mmol/L and BHB < 0.5 mmol/L (N = 438), the proportion of deaths related to diabetes, alcoholism or severe obesity was 57% (N = 248).

Table 1
Demographic information and the concentration of ethylene glycol, glycolic acid, glucose and lactate in the studied cases (N = 17).

			Ethylene glycol concentration (g/L)			Glycolic acid concentration (g/L)			Glucose (mmol/L)	Lactate (mmol/L)
No	Gender	Age	PM blood	PM urine	AM blood	PM blood	PM urine	AM blood	Vitreous humour	Vitreous humour
1	Male	67	5.3	21		1.9	5.2		8.6	29
2	Male	72	0.7	5		1.5	8.2		5.2	25
3	Male	36	0.77	5.4		1.5	7.4		5.8	32
4	Male	45	1.6	11		1.4	7.0		3.2	30
5	Male	47	6	17		2.3	6.1		2.4	16
6	Male	77	0.12	*n.a.*	0.14	0.94	*n.a.*	0.97	1.9	26
7	Male	56	2.6	12		2.3	5.9		4.5	15
8	Male	25	2.2	8.3		1.9	7.1		2.8	26
9	Male	45	0	1.8		1.5	6.0		6.5	30
10	Male	48	1.5	20		1.9	11		11	21
11	Female	20	0.39	0.61		1.5	2.6		19	21
12	Male	27	1.4	12		1.6	8.2		0.52	24
13	Female	46	4.4	11		2.3	6.2		20	25
14	Male	47	2.3	12		2.0	4.5		4.3	20
15	Male	55	1.7	15		1.3	6.3		6.7	29
16	Male	76	5.6	22		2.0	5.1		5.2	20
17	Female	64	1.1	6.8		2.0	6.7		6.7	19

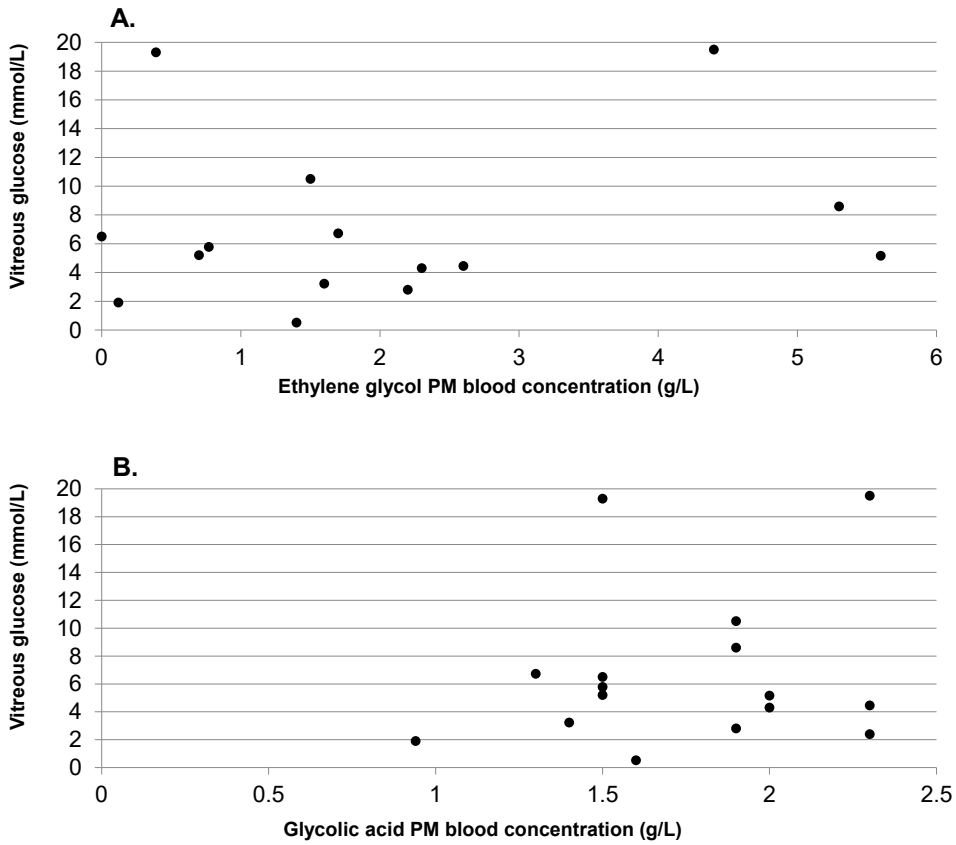

Fig. 1. Glucose concentration in vitreous humour plotted against (A) the ethylene glycol blood concentration and in (B) the glycolic blood concentration in each of the studied cases (N = 17).

4. Discussion

We investigated all cases in Finland over a period of 6 years and 3 months in which EG was detected in PM toxicology and in which VH glucose concentrations were determined.

The median EG and GA blood and urine concentrations were at the same level or higher than those reported earlier by our group [27]. Our results confirm the earlier observation that there seems to be an approximate threshold of 1.5 g/L for the concentration of GA in fatal EG poisonings [27]. In another study reporting PM concentrations for EG and GA, no median values had been calculated but the wide range of concentrations (EG 0.058–7.79 g/L and GA 0.81–1.77 g/L in PM blood) was similar to those seen in our study [8].

Although VH glucose was detected in all EG poisonings (mean: 5.2 mmol/L; range 0.52–19.5 mmol/L), this does not necessarily mean that all these cases were characterized by ante-mortem (AM) hyperglycaemia. Although PM VH glucose is the most appropriate marker of AM hyperglycaemia, because PM glucose is more stable in VH than in blood, no consensus exists on cut-off values for normal PM VH glucose concentrations.

On one side, Belsey and Flanagan suggest that any detectable glucose in VH collected at autopsy indicates AM hyperglycaemia and that normal AM glucose levels are not usually detectable in VH after death [28]. These statements are based on studies that suggest that glucose concentration in VH is usually about half of the concentration in the plasma of living individuals and that VH glucose concentration decreases by about 3 mmol/L in the early PM period [29]. The drop in glucose concentration after death is extensively explained and experimentally demonstrated in the study by Zilg et al [29].

This approach can be, in principle, appropriate when considering normal fasting blood glucose concentration in living (3.9–5.6 mmol/L), but it is less convincing when considering that in a random blood sugar test in living, blood glucose concentration up to 11.1 mmol/L can still be normal. Moreover, the PM decrease of VH glucose concentration vary among studies [30] and PM biochemistry reveals VH glucose concentrations > 0.35 mmol/L also in individuals with no diabetes or other known causes of hyperglycaemia.

In fact, in other studies most threshold VH glucose levels for hyperglycaemia are set at 6.9–10 mmol/L [13,26,29,31,32]. A recent review and *meta*-analysis suggest that, overall, VH glucose levels > 5.1 mmol/L are highly suggestive for a hyperglycemic status before death [30]. A certain consensus exists, on the fact that VH glucose concentrations > 10 mmol/L represent severe and life-threatening AM hyperglycaemia that may cause fatal diabetic coma or contribute to death [11,12,29,30,32].

In Finland, however, due to the relatively long PM interval between death and autopsy, VH glucose concentration may eventually decrease and be < 7 mmol/L even in cases of definite AM hyperglycaemia [26].

In our series, three EG poisonings have VH glucose concentration > 10 mmol/L, thus likely representing a situation of severe and potentially lethal AM hyperglycemia. In one additional case the VH glucose concentration was 8.6 mmol/L, therefore likely representing a definite case of AM hyperglycemia. Among the remaining 13 cases, VH glucose levels may have represented conditions of moderate and mild AM hyperglycemia with less pronounced clinical implications or, even, AM normoglycemia. In one of the studied cases (VH glucose concentration 6.7 mmol/L), there was information on a previously diagnosed DM2 but in that case the low VH BHB level indicated that diabetic or alcoholic ketoacidosis was unlikely.

PM detection of various concentrations of glucose in VH without elevated BHB was relatively common in our general autopsy population. Although in many of these cases, a disease (e.g., alcoholism, severe obesity, diabetes) or other causes of death could explain the detection of elevated VH glucose concentrations, in a consistent number of cases it

remained unexplained. High and moderately elevated VH glucose values in non-diabetics have been at times reported, in natural as well as in injury deaths, also in cases with strong AM physiological stress [26,29].

Based on the results of this study, the possibility of an EG poisoning should be kept in mind in cases with measurable/elevated PM VH glucose that cannot be otherwise explained. Since EG cannot be routinely analysed by the conventional headspace gas chromatographic method in combination with alcohol but requires a special method, unexplained high VH glucose concentration can support the decision to retrospectively perform toxicological analysis for EG poisonings when no background information on possible EG intake is available or microscopic investigation for oxalate crystals in kidney is not conclusive.

A case report describing hyperglycaemia in connection with EG poisoning in a clinical setting suggests two alternative explanations for the phenomenon: reduction of serum insulin level as the consequence of an EG induced transient pancreatitis, or insulin resistance caused by the acute renal failure that follows the intake of EG [21]. If appropriate treatment measures are started at an early stage, the prognosis of an acute EG poisoning is good. However, it is estimated that up to 22% of the acute EG poisonings lead to death despite of the treatment [33,34].

Therefore, accurate diagnosis and assessment of fatal EG poisonings is crucial not only from a public health perspective and for individuals' legal protection but also to better understanding these poisonings in clinical settings.

The study has some significant limitations. The time elapsed between ingestion of EG, and in- or out-of-hospital death was not available, although such information could have allowed a better assessment of EG and glucose concentrations and their potential clinical implications. We did not include in this survey PM histological findings of oxalate crystals in kidney that could have provided some clues on the interval between ingestion of EG and death and on the relation between PM laboratory and morphological findings. Data on the interval between death, retrieval of the body, and VH sampling were also not considered in this study. However, in this regard, as protracted PM interval causes a PM decrease of VH glucose, our results likely provide a conservative estimate of glucose concentration in EG poisonings rather than overemphasizing such association.

5. Conclusion

We expect that unexplained elevated VH glucose concentration could in some cases guide retrospective toxicology analysis for EG and disclose some EG poisonings with no background information on possible EG intake at the time of autopsy or with unconclusive microscopic investigations. Moreover, awareness of severe hyperglycaemia associated to EG poisoning can provide important clues for treatment of EG poisonings in a clinical setting.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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