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Serum alcohol concentrations in trauma patients determined by immunoassay versus gas chromatography

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Abstract

There has been an extensive discussion in the forensic toxicology community regarding the effect of trauma on the enzymatic method for ethanol analysis. There is a paucity of information in the literature that addresses this question. This study was designed to compare the Dade Behring Dimension® enzymatic method with the reliable gas chromatographic method. The blood samples collected at the same time from trauma patients were analyzed by both methods. The result of the study shows no significant quantitative difference between the enzymatic and gas liquid chromatographic (GLC) methods. Also, the enzymatic method did not show any false positive ethanol in cases where the GLC method showed a negative finding.

Keywords: Enzymatic; Gas liquid chromatography (GLC); Trauma; Ethanol (alcohol); Blood alcohol concentration (BAC); LDH; Lactate

1. Introduction

Currently, most forensic laboratories determine blood alcohol concentrations (BAC) using gas liquid chromatography (GLC). However, in patients with traumatic injury, analysis of blood for BAC may be performed in hospital laboratories. In such laboratories, BAC analysis is performed mostly by enzymatic methods. Some publications have stated that traumatic injury may result in increased serum lactate and lactate dehydrogenase (LDH). These increases are claimed to interfere with enzymatic analysis of blood alcohol producing false positive or elevated BAC [4]. These claims may have been true with enzymatic methods before 1996 [4–6]. Currently, enzymatic methods of analysis require pre-precipitation of blood proteins in the sample before analysis [1], thus eliminating any possible interference due to presence of high serum LDH. This study targets an answer to the question ''Does trauma result in elevation of positive BAC, or does it lead to false positive BAC in a blood sample that has no alcohol?'' In an attempt to pursue an answer to this important question, blood samples were collected from patients with traumatic injury. The same samples were analyzed by two methods; the highly dependable method of GLC, and the enzymatic method. The enzymatic method used in this study was the Dimension® clinical chemistry system, Ethyl Alcohol Flex™ reagent cartridge [1].

2. Methodology

2.1. Sample collection and preparation

Blood samples were obtained from a local hospital. All samples were collected from trauma patients after using non-alcoholic antiseptic solution to disinfect the site (e.g. Povidone iodine). The blood was stored under refrigeration until the time of analysis. A 0.1 ml serum or plasma sample was used for GLC analysis. Enzymatic method utilized 1.0 ml of serum or plasma. Sample preparation was performed

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according to the package insert instructions [1] and according to the method previously published [2] for the enzymatic and the GLC methods, respectively. Each sample was analyzed in triplicate and in duplicate by the enzymatic and GLC methods, respectively. In both methods, a blank, control and standards were analyzed at the same time with the patients' samples [3].

2.2. Instrumentation

The instructions in the Dimension® clinical chemistry system were followed and the analysis was performed on the Dimension® system as described [1].

Gas chromatographic parameters were the same as previously published [3].

2.3. Results

Detector response was linear at ethanol concentrations of 0–300 mg/dl for both enzymatic and GLC methods. Samples with ethanol concentrations higher than 300 mg/dl were first diluted, then analyzed. Blood alcohol concentrations ranged from 0 to 460 mg/dl. Forty-three out of 67 samples analyzed were negative by both methods. None of the negative samples showed false positive by the enzymatic method. This is in contrast to what was previously reported [4]. Twenty-four samples were positive and the percent difference between BACs obtained by the enzymatic versus the GLC method ranged from −10 to +22% (Table 1). Out of the 24 positive samples, 10 samples (41.7%) showed the same or lower BAC values by the enzymatic method when compared with the GLC analysis. Only three samples (12.5%) showed more than 14% difference between the two methods.

Table 1
Serum/plasma BACs from trauma patients

Serum/plasma BAC by enzymatic method	Reference laboratory serum/plasma BAC by GLC	Difference (%) (enzymatic vs. GLC)
22	17	+22
43	36	+16
92	92	0
111	110	0
143	141	+1.39
151	169	−10.65
165	173	−4.62
173	154	+10.98
175	191	−8.38
191	182	+4.71
196	182	+7.14
201	197	+1.99
240	207	+13.75
249	238	+4.42
274	230	+16.06
279	268	+3.94
280	282	−0.71
284	318	−10.69
291	287	+1.37
297	354	−16.10
298	311	−4.18
400	427	−6.32
417	388	+6.95
460	448	+2.61

3. Discussion

Some publications have stated that traumatic injury is associated with an increase in serum lactate dehydrogenase and lactate concentration [4–6]. As a result of these increases, it is claimed that enzymatic methods for BAC analysis, especially the Syva EMIT® method (as it existed) would yield false positive ethanol in negative samples [4]. Elevation of LDH was observed only in postmortem samples and in patients with end-stage liver and kidney failure [4]. Other conditions that cause elevation of serum LDH include acute pancreatitis, megaloblastic and hemolytic anemia, muscle damage, neoplastic states and myocardial infarction [7]. False positive ethanol results were also observed in postmortem infant plasma at LDH concentration of approximately 2800 IU/l or greater, with BAC results of less than 10 mg/dl [5]. For this reason, many forensic and clinical laboratories use this BAC value as a cutoff concentration. Currently, many practitioners do not choose to test for LDH. This is because, clinically, LDH is nonspecific and isoenzyme measurement is not routinely available. Also, measurement of LDH does not confer any additional information about skeletal muscle or hepatic disease that is provided by more specific enzyme assays used for these purposes, e.g. creatine kinase (CK) for muscles and alanine aminotransferase (ALT) for liver [7]. In addition, postmortem determination of LDH in the blood is of little value [8]. Published data showed no consistent correlation between traumatic injury and serum LDH concentration [9,10]. Levy et al. [11] reported that EMIT-II Plus® ethyl alcohol assay does not produce false positive results in postmortem, and clinical blood samples [11] even in the presence of elevated blood LDH and lactate. The EMIT-II Plus® ethyl alcohol assay effectively eliminates LDH activity from patient samples by protein precipitation. In 1994, Thompson et al. showed that the older EMIT® assay was producing false positive results in postmortem cases and in two clinical cases, one with end-stage renal disease and the second with myocardial infarction [12]. However, the protein-free ultra filtrate showed no false positive with both the GLC and EMIT® methods [12]. In a study by Jartani and Poklis [13], new EMIT-ETS® Plus ethyl alcohol assay showed acceptable reproducibility and recovery for the determination of ethanol in clinical serum and urine specimens. Results obtained by EMIT-ETS® Plus correlated well with those obtained by GLC. In other publications, correlation coefficients of 0.990 and 0.994

were obtained. When the results obtained by head-space gas chromatography were compared with the enzymatic methods, Vitros® [15] and Axsym® [16], respectively.

The blood samples used in this study were collected from living human trauma patients. There was no significant difference in BAC values obtained by the enzymatic and GLC methods. None of the samples analyzed were postmortem. None of the negative samples for ethanol by GLC showed a false positive ethanol by the enzymatic method. As a matter of fact, in 10 out of 24 cases (41.6%) BAC values obtained by enzymatic method were lower or equal to the GLC method. With the enzymatic method, only three out of 24 samples had BAC values more than 14% higher than those obtained by the GLC method.

In this study, blood and LDH and lactate levels were not available. We hope that we will include these values in a future study. In conclusion, this study shows that in living patients with trauma, there were no false positives or significant increase in BAC as a result of using the enzymatic method of analysis as compared to the most reliable GLC. This study should put to rest the false, unsubstantiated notion that trauma is associated with an increase in LDH and lactate levels which leads to an elevated BAC when analyzed by an enzymatic method [14,16].

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