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ORIGINAL ARTICLE

A Novel and Simple Method for a Differentiation of Alcohol Types

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SUMMARY

Background: **Alcohol poisoning is a significant global problem that has become an epidemic. The determination of the alcohol type is hereby essential as it may affect the course of the treatment; however, there is no routine laboratory diagnostic method for alcohol types other than for ethanol. In this study, we aimed to define a simple method for alcohol type differentiation by utilizing a combination of breathalyzer and spectrophotometrically measured serum ethanol results.**
Methods: **A breathalyzer and spectrophotometry were used to measure four different types of alcohol: ethanol, isopropanol, methanol, and ethylene glycol. To conduct serum alcohol analysis, four serum pools were created, each containing a different type of alcohol. The pools were analyzed using the spectrophotometric method with an enzymatic ethanol test kit. An experiment was conducted to measure the different types of alcohol using impregnated cotton and a balloon, simulating a breathalyzer test. An algorithm was created based on the measurements.**
Results: **Based on the results, the substance consumed could be methanol or isopropanol if the breathalyzer test indicates a positive reading and if the blood ethanol measurement is negative. If both the breathalyzer and the blood measurements are negative, the substance in question may be ethylene glycol.**
Conclusions: **This simple method may determine methanol or isopropanol intake. This straightforward and innovative approach could assist healthcare professionals in different fields with diagnosing alcohol intoxication and, more precisely, help reducing related morbidity and mortality.**
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INTRODUCTION

The consumption of homemade or distilled alcohol produced in unsanitary conditions has increased in Turkey. Experts say the rise in the price of ethanol drove illegal producers to substitute it with methanol, fueling the string of deaths from the lethal substance by doing so. Even a small amount of methanol can be poisonous and can end in death [1,2]. In potentially intoxicated individuals, breath alcohol analyzers are used by law enforcement and healthcare personnel to estimate the eth-

anol concentration [3]. Alcohol poisoning (ethanol, methanol, or ethylene glycol) is challenging in clinical practice. The prevalence of admissions to the emergency department due to alcohol poisoning has been reported to be 8.8 per million in the USA and 25 per million in our country [4,5]. Common characteristics of alcohol poisoning are high anion gap metabolic acidosis and increased osmolality. While these compounds cause increased serum osmolality, their accumulating metabolites cause an increased anion gap [6]. Ethanol and isopropanol are commonly ingested and cause gastrointestinal irritation; they do not produce metabolic acidosis. On the other hand, methanol and ethylene glycol are toxic alcohols, because they cause severe physiologic morbidity [6].

Ethanol: A standard alcoholic beverage contains about fifteen grams of ethanol. It is available in various forms and may be found in high concentrations in many household products such as mouthwash, colognes, perfumes, and as a diluent or medication solvent. The bright colors and flavors of these products may appeal to children and cause severe intoxication if ingested, especially if mistaken for harmless candies or beverages. Ethanol depresses the central nervous system (CNS). It enhances the inhibitory neurotransmitter γ-aminobutyric acid receptors and blocks excitatory N-methyl-d-aspartic acid receptors. Modulating these systems leads to developing tolerance, dependence, and withdrawal syndrome when ethanol intake ceases in independent individuals.

Because of the phenomenon of tolerance, blood ethanol levels correlate poorly with the degree of intoxication. Ethanol ingestion is the most common cause of an osmolar gap (OG) in serum electrolyte analysis. It may be associated with mild metabolic acidosis, but a significant anion gap metabolic acidosis suggests the presence of lactic acidosis, ketoacidosis, or methanol or ethylene glycol toxicity [7].

Isopropanol: Isopropanol, also known as isopropyl alcohol and 2-propanol, has a molecular weight of 60.10 and is a colorless, volatile liquid with a bitter, burning taste and an aromatic odor. It is found in many readily available, inexpensive household products, such as rubbing alcohol. Isopropanol is widely used in industry as a solvent and disinfectant and is a component of various skin and hair products, cleaners, detergents, paint thinners, and deicers. Ketosis and an OG without acidosis are the hallmarks of isopropanol toxicity. The primary metabolite, acetone, does not cause eye, kidney, cardiac, or metabolic toxicity, although high acetone levels may contribute to CNS depression. Acetone is eliminated primarily by the kidneys, with some excretion through the lungs. The primary clinical toxicities of isopropanol are CNS depression, caused by the parent compound and acetone, and gastric irritation from isopropanol. Serum isopropanol and acetone levels may be assessed, although isopropanol levels may not be readily available from hospital laboratories. Isopropanol levels of 50 milligrams/dL (8 mmol/L) are often related to an intoxication in individuals not habituated to ethanol. Still, alcoholic individuals may be considerably more resistant to the CNS effects of isopropanol [6].

Methanol: Methanol, the basic alcohol (CH3OH, molecular weight 32.05), is a colorless, volatile liquid with a distinctive "alcohol" odor. Methanol is used to synthesize other chemicals, and may be found in automotive windscreen cleaning solutions, solid fuel for stoves and chafing dishes, model airplane fuel, carburetor cleaner, gas line antifreeze, photocopying fluid, and solvents. Trivial amounts are found in fruits and vegetables, aspartame-containing products, and fermented spirits [8] – ingesting contaminants found within illicitly distilled liquor results in adverse health effects. Illicitly distilled liquor is also known as moonshine, bootleg, white lightning, corn liquor, or hooch [7]. Absorption of methanol occurs orally, through the skin, and through inhalation – absorption after oral administration is rapid, with a mean absorption half-life of 5 minutes [6]. While methanol itself has a low toxicity, it is metabolized in the liver at a rate 8 of 85 mg/L, 1 hour, 1 to toxic formaldehyde by the enzyme alcohol dehydrogenase (ADH), and within only several minutes to formic acid, which is directly correlated with increased morbidity and mortality [9]. The process results in metabolic and lactic acidosis [10] Consequently, multiple organ systems are affected. Severe metabolic acidosis, seizure, coma, and death may be observed [8].

Ethylene glycol: Ethylene glycol (CH2CH2(OH)2, molecular weight 62.07) is a colorless, odorless, sweet-tasting liquid. Like methanol, ethylene glycol itself has a mild toxicity (it is a stronger inebriant than both methanol and ethanol, and it causes gastric irritation), and it is the hepatic oxidation of ethylene glycol that creates the toxic metabolites responsible for metabolic acidosis and end-organ damage. The liver metabolizes about 80% of an ingested dose, whereas the other 20% is excreted unchanged in the urine [8].

Breath alcohol analyzers are used by law enforcement personnel to estimate the blood ethanol concentration of suspected intoxicated persons. Individuals with increased breath ethanol concentrations can be incarcerated, sent home, or brought to the emergency department (ED) to evaluate ethanol intoxication, associated illness, or injury [3].

On the other hand, the best laboratory test for diagnosing methanol or ethylene glycol poisoning is measuring the specific serum level of the alcohol [8].

Differentiation of alcohol types in a hospital setting is a clinical challenge. In this study, we aimed to develop a novel, rapid, and straightforward method for alcohol type determination by using a combination of breathalyzer tests and serum ethanol concentrations.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of Başakşehir Çam and Sakura City Hospital and con-

ducted in Turkey according to the Helsinki Charter (KAEK/2021.09.214).

A negative plasma pool was created from samples that were obtained from individuals who did not consume any alcohol. The pooled serum was divided into five equal parts. Using commercially obtained alcohols, five subgroups were formed separately for ethanol (ethanol absolute EMPLURA, Merck, Burlington, MA), isopropanol (isopropanol (2-Propanol) EMSURE® ACS, ISO, Reagent Merck, Burlington, MA), methanol (methanol EMSURE® ACS, ISO, Reagent Merck, Burlington, MA), and ethylene glycol (ethylene glycol EMSURE® ACS, ISO, Reagent Merck, Burlington, MA.). The first group was the alcohol-negative group. Alcohol derivatives were added to the other samples, so the final concentration was at the specified level of 50 mg/dL, 100 mg/dL, 200 mg/dL, and 400 mg/dL. Alcohol measurement was performed by a standard breathalyzer test used in a hospital setting. The study design is demonstrated in Table 2.

To carry out this task, a device was established. To replicate the natural process of exhaling, a sterile cotton swab was used to apply alcohol to the mouthpiece of the breathalyzer. Then, a balloon attached to the mouthpiece was filled with air by an air compressor similar to a standard lung capacity (~ 5 L). The air was transferred to the breathalyzer, mimicking a human lung. The procedure was performed 20 times for each type of alcohol, and the results were recorded as (+) or (-). See the device in Figure 1.

This study was conducted with NAM-07 breathalyzers (ARMAS Electronics, Türkiye) equipped with a new-generation electrochemical sensor. According to the user guide, it has high sensitivity and specificity due to its new-generation sensor technology. It is used both in healthcare facilities and in traffic controls. The automatic measurement mode is usually used in individuals who can blow into the breathalyzer properly. Manual and passive modes can be used in individuals who cannot blow properly or in those with altered mental status.

Technical features of the breathalyzer are as follows: Sensor: New generation electrochemical. Mouthpiece: Disposable (packed one by one for hygiene). Measurement mode: Automatic, manual, and passive. Measurement range: 0.00 - 5.00‰ range. Sensitivity: 0.00 - 1.00‰, range ± 0.05‰. For the value between 0.00 - 1.00, ± 5%. Standard deviation: < 0.008‰. Preparation period: Lower than 8 seconds after the device is turned on. Resulting time: 20 seconds after sampling. Temperature: -10°C and +50°C.

To create a serum pool, four containers were filled with serum and four types of alcohol were added to each container. Samples were taken from the pools and were analyzed for ethanol twenty times, using the enzymatic method. The results of serum obtained from the pool and the breathalyzer (as (+) or (-)) were compared, and an algorithm was created. Measurements were made 20 times from the pools formed at the levels determined according to the type of alcohol, both by alcoholmeter and by enzymatic spectrophotometric method.

RESULTS

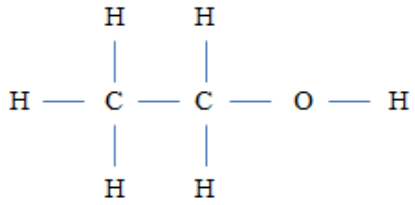
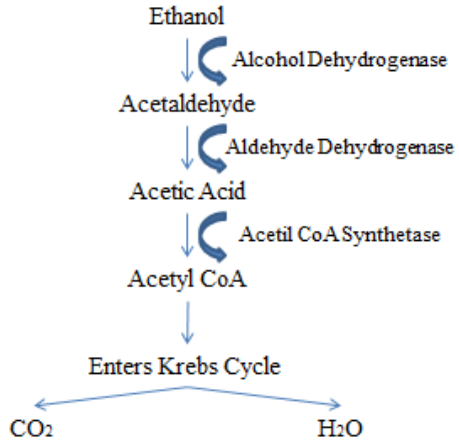
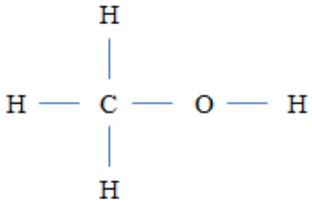
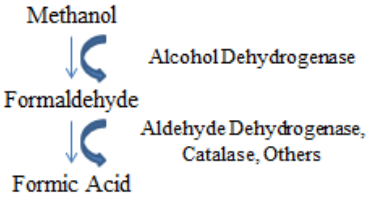
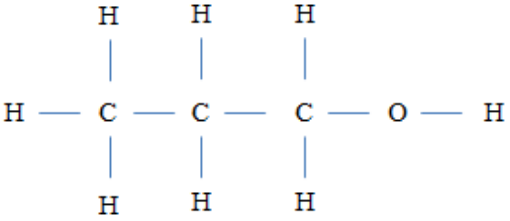
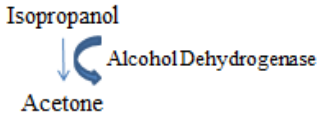
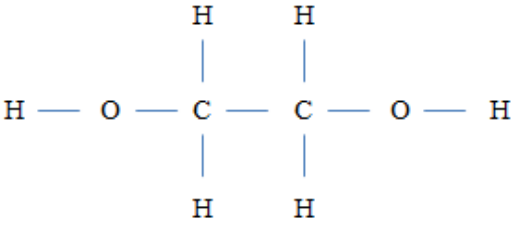
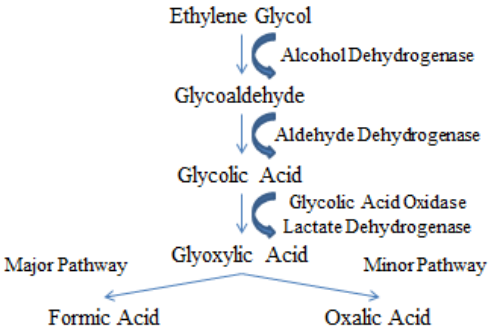
Initially, our study was designed to measure alcohol levels quantitatively. Despite the enzymatic method providing consistent results when grouped by different levels of ethanol, the breathalyzer results did not indicate a correlation with the alcohol levels. Based on this observation, it was decided to evaluate the results qualitatively. Table 3 displays the summarized measurements of ethanol, methanol, isopropanol, and ethylene glycol results from both breathalyzer and serum tests.

DISCUSSION

Alcohol consumption or abuse causes 3 million deaths per year, worldwide. This proportion represents 5.3% of all deaths. Alcohol abuse is the reason for more than 200 illnesses and injuries. It is estimated that 5,1% of all diseases and injuries are related to alcohol. Alcohol consumption causes morbidity and mortality in the relatively younger population. It is the cause of 13.5% of deaths in 20 - 39 year-age groups [11]. In the era of the COVID-19 pandemic, it is known that people tend to consume excessive alcohol to fight against stressful situations [12].

Methanol poisoning epidemics result from the consumption of unofficially produced alcoholic beverages. Lately, these epidemics have been observed in many countries, such as Cambodia, the Czech Republic, Ecuador, Estonia, Indonesia, Kenya, Libya, Nicaragua, Norway, Pakistan, Turkey, and Uganda. These epidemics cause 20 to 800 victims. In some instances, the case-fatality rate may rise to 30% [13]. In Iran, there were outbreaks of methanol poisoning that caused a significant number of illnesses and deaths [14]. The most recent and most crucial epidemic was experienced during the COVID-19 pandemic. The pandemic has affected Iran mostly between February 19th, 2020, and April 27th, 2020. In this period, 90,481 confirmed cases and 5,710 confirmed deaths were determined. Due to misinformation suggesting that alcohol could neutralize SARS-CoV-2, there has been a rise in illnesses and deaths related to methanol consumption. Between February and April 2020, over 5000 poisonings and 500 confirmed deaths occurred. Also, in some cities, it was announced that fatalities due to methanol poisoning were more prevalent than deaths due to COVID-19. Unlike previous epidemics, the methanol poisoning epidemic results from the understanding that disinfectant and alcohol consumption prevents a COVID-19 infection [14,15]. Alcohol poisoning has emerged as a public health problem due to the pandemic. This study proposes a rapid method for determining the alcohol type in alcohol poisoning; a prominent cause of morbidity and mortality.

Table 1. Formulations and metabolisms of alcohol types.

	Alcohol formula	Alcohol metabolism
Ethanol		
Methanol		
Isopropanol		
Ethylene glycol		

Our results revealed that if the breathalyzer is (+) and serum ethanol measurement is (-), the substance taken is either isopropanol or methanol. If both the breathalyzer and serum measurement is (-), then the substance is ethylene glycol.

Healthcare facilities and law officers commonly use breathalyzers to identify ethanol intake [3]. When a breathalyzer is used alone, it gives (+) ethanol, methanol, and isopropanol results. This may result in delays in the treatment of methanol intoxication. If both are (+), the alcohol consumed is ethanol.

In a case report by Gümüş et al., a patient with alcohol poisoning, who was administered an ethyl alcohol infusion for an antidote therapy and subsequently died, was presented. An autopsy was performed and initial blood samples were taken on admission to the hospital.

Table 2. Study design and preparation process of the sample pools.

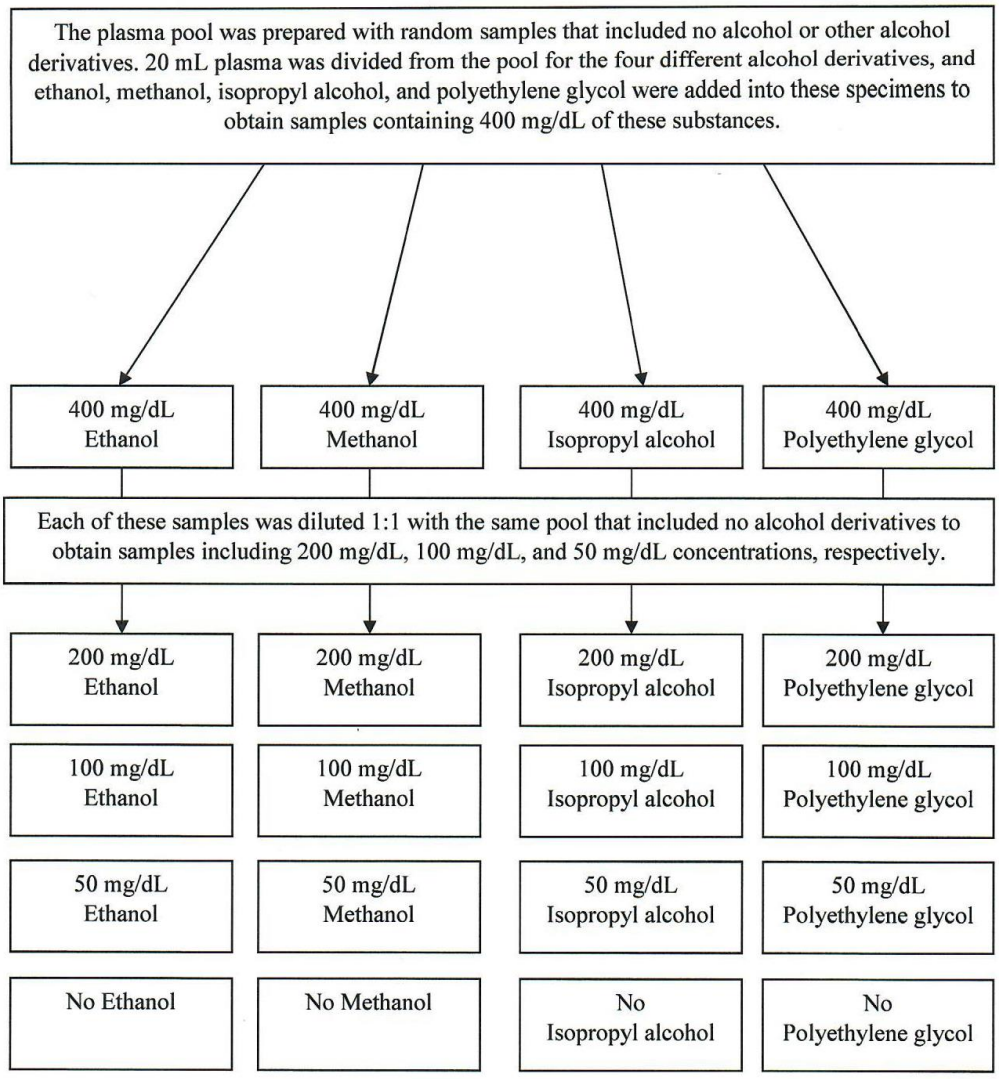


Table 3. Results for ethanol, methanol, isopropanol, and ethylene glycol measurements with both breathalyzer and serum results.

	Ethanol	Methanol	Isopropanol	Ethylene glycol
Breathalyzer measurement result	+	+	+	-
Serum ethanol measurement result	+	-	-	-

Ethanol intake may be determined if the breathalyzer result is (+) and if serum samples reveal a (+) ethanol measurement. If the breathalyzer is (+) and serum ethanol measurement is (-), then the substance taken is either isopropanol or methanol. The substance may be ethylene glycol if both breathalyzer and serum measurements are (-).

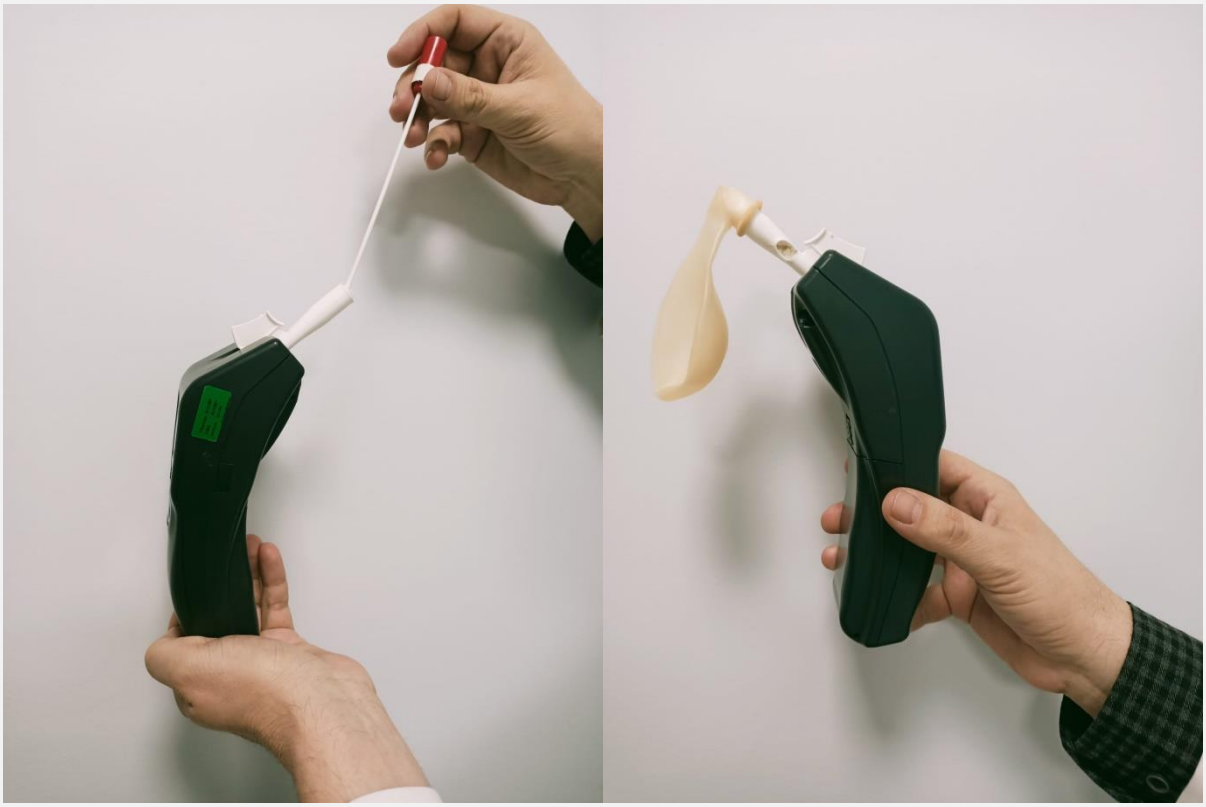


Figure 1. Figure demonstrating the experimental measurement method of alcohol types with a breathalyzer.

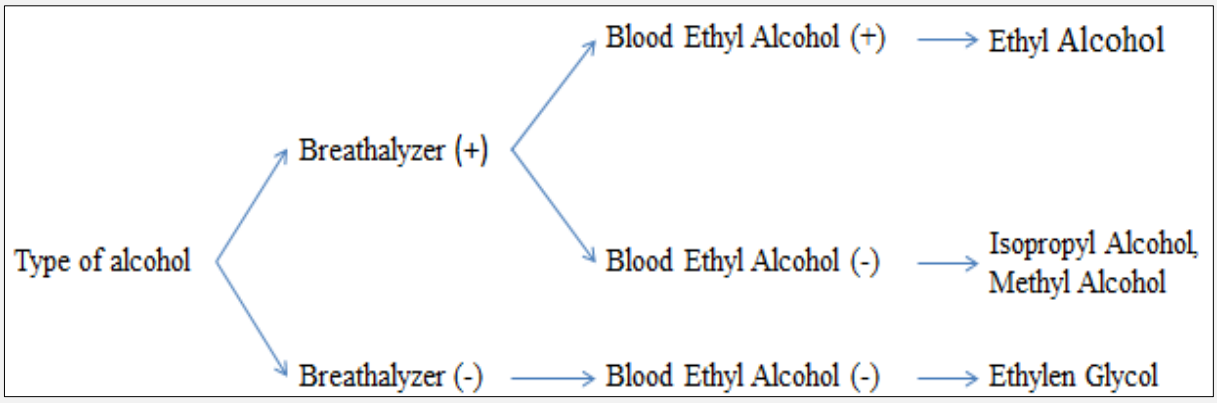


Figure 2. An algorithm for alcohol type determination by using a combination of breathalyzer and serum ethanol measurement.

Blood samples taken during the autopsy were sent to an advanced center for alcohol gas chromatography analysis. As a result, ethyl alcohol was not determined in the initial samples by the enzymatic method. After gas chromatography, the samples revealed 343 mg/dL methyl alcohol and 518 µg/mL formic acid in the blood samples. Following two days of ethyl alcohol administration, the blood samples revealed 73 mg/dL ethyl alcohol and 44 mg/dL methyl alcohol after gas chromatography. This report confirmed that while the enzymatic method successfully determines ethyl alcohol, it fails in the methyl alcohol determination. In our study, we composed a serum pool and measured ethyl alcohol, methyl alcohol, isopropyl alcohol, and ethylene glycol levels using the enzymatic method. Similarly, only ethyl alcohol could be measured by an enzymatic method [16].

Directly measuring methanol in blood by benchtop liquid or gas chromatography (GC) is accepted as the "gold standard" for diagnosing methanol poisoning. However, this is laborious, expensive, and typically performed in specialized laboratories, delaying diagnosis for several hours to days [9]. According to our results, if the breathalyzer is (+) and serum ethanol measurement is (-), the substance taken is either isopropanol or methanol.

Early in ingestion, Ethylene Glycol contributes to significant OG, but as metabolites start forming, the OG disappears and the anion gap increases. This indicates that OG, as a measure of the severity of poisoning, is only valuable early in an intoxication The presence of calcium oxalate crystals, which may appear in the monohydrate form as prisms or dumbbell-shaped and in the dihydrate form as tent-shaped or octahedral shapes under light examination of urine through a microscope, can assist in diagnosis due to the presence of sodium fluorescein in antifreeze. Other laboratory abnormalities include hypocalcemia causing QT prolongation on electrocardiogram and microscopic hematuria, low bicarbonate, leukocytosis, and increased protein in the cerebrospinal fluid [4]. According to our results, ethylene glycol may be considered when both breathalyzer and serum measurements are (-). Ethylene glycol cannot be detected by a breathalyzer because of the much higher boiling point and the lower vapor pressure of ethylene glycol than of pure water, as is typical with most binary mixtures of volatile liquids, unlike other types of alcohol [14].

Isopropanol poisoning can be diagnosed in patients with normal acid-base parameters, hyperosmolarity, and positive urine and blood nitroprusside reactions. Hyperosmolarity is the most common laboratory abnormality associated with isopropanol poisoning [6]. Our results revealed that if the breathalyzer is (+) and the blood ethanol measurement is (-), the substance taken is either isopropanol or methanol.

A report indicates that a 47-year-old man was found heavily intoxicated in a park. A breathalyzer (Intoxilyzer 5000EN) analysis revealed 0,288 g/210 L alcohol. In his detailed anamnesis, it was understood that he drank antifreeze with an alcohol content of 99%. After 2 or 3 hours, the serum and urine analysis for ethyl alcohol and other substances was negative. The serum methanol concentration was 589 mg/dL. This was a unique case in which methanol was reported as ethanol. According to this report, breathalyzers may falsely report methanol as ethanol, and diagnosis and treatment may be delayed, resulting in methanol poisoning [3].

In concordance, our results revealed that breathalyzers measure methanol along with ethanol, which means that breathalyzers are not specific for ethanol but also measure other types of volatile alcohols.

Limitations of the study

Our study also has some limitations. The method suggested in this study may be inadequate when a patient consumes a mixture of different types of alcohol.

CONCLUSION

It is known that methanol may cause interference; a falsely increased breath ethanol level in humans may be determined when breathalyzers are used alone. However, our results revealed that when breathalyzer and serum ethanol measurements are used in combination, a novel and simple method for alcohol type differentiation may be established. When combined with the patient's history and clinical findings, our technique may help clinicians to develop a more accurate diagnosis.

Acknowledgment:

This work was not supported by any institution or organization.

Ethical Approval:

This study was approved by the Ethics Committee of Başakşehir Çam and Sakura City Hospital and was conducted in Turkey according to the Helsinki Charter (KAEK/2021.09.214).

Declaration of Interest:

The authors state that they have no conflicts of interest concerning the publication of this paper.

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