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Review Article

Detection of alcohol use: Guidance of direct biomarker phosphatidylethanol

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Abstract

Acknowledging the fact that alcohol is an important source of fatalities in traffic, the amount of alcohol consumed and the exact time of the consumption could enlighten forensic cases and guide the justice system correctly. However, determining the alcohol use is a difficult problem due to alcohol metabolism in individuals and parameters such as sex, age, amount of alcohol in the drink, satiety, should be taken into account which can be challenging for amount of alcohol interpretation. Considering that blood alcohol concentration (BAC) may not be reliable, alternative metabolic products of alcohol has arisen after alcohol consumption. One of the most interesting alcohol biomarker phosphatidylethanol (PEth) has caught attention due to its long half-life and not being affected from sex, liver diseases or age in addition to that it is only synthesized under the presence of ethanol. PEth is synthesized in cell-membranes and not being a single molecule, its homologues should be considered when determining the amount of alcohol intake. Although the homologues of PEth could be isolated from whole blood, less invasive dried blood spots (DBS) also provides reliable information. The analysis of PEth is performed in LC-MS/MS which is highly sensitive and specific. For forensic applications, direct alcohol biomarker PEth may be useful for distinguishing the alcohol use and helpful for justice system. This review focuses on studies about PEth biomarker, its applications and limitations conducted from 2010 to 2019.

Keywords: Direct biomarker PEth, LC-MS/MS, alcohol

INTRODUCTION

According to the report published from OECD, alcohol-related traffic accidents in Europe is approximately 12.9% [1]. Alcohol is an easily accessible legal drug and used in social environments. According to World Health Organization, in 2016, 3 million people died worldwide due to alcohol-based reasons [2]. Giving the importance of alcohol in terms of forensic sciences, determining the amount of alcohol consumed become essential. Although it is hard to detect the ethanol in biological matrices after 10-12 hours after consumption [3], other biomarkers produced in the body might be more convenient for detection of alcohol. There are indirect and direct alcohol biomarkers to identify alcohol consuming for helping the justice. Among indirect biomarkers aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), mean corpuscular volume (MCV) and carbohydrate deficient transferrin (CDT%) provide information with indirect response of body after the ethanol consumption [4]. Indirect biomarkers generally used for supportive evidence of alcohol use due to their low sensitivity and specificity [4]. To obtain reliable information for alcohol use, direct biomarkers which are ethyl glucuronide (EtG), ethly sulfate (EtS), fatty acid ethyl esters (FAEE) and phosphatidylethanol (PEth), are relied upon due to their power of being ethanol metabolites [5]. However, there are some limitations in every biomarker. For instance, EtG and EtS are the most common used biomarkers for clinical studies and forensic laboratories, although their detection time in both urine and serum is short [3]. FAEEs are generally used

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for chronic alcohol determination from hair [4] and alcohol misuse of the mother from meconium [6].

PEth as a Biomarker

PEth is the name of different phospholipids found in the outer layer of the cell membranes. Not representing a single molecule, PEth is synthesized from one of the basic homologues forming the outer cell membrane phosphatidylcholine (PC). PC is an interesting substance that is affected by the nutrients intake and when water is present in the cell, with the help of phospholipase D (PLD) enzyme, it is converted to phosphatidic acid (PA) and choline. However, if there is an alcohol intake, PLD enzyme has more affinity to ethanol than water, PC forms PEth and choline which makes the reaction highly specific [3,4,7,8].

PEth homologues can be detected in various parts of the body including brain, liver, and kidney. Although it is found in some parts of the body, antemortem forensic cases require different matrices for detection of PEth. The fact that PEth is successfully isolated from erythrocytes in whole blood, it is routinely analyzed in some countries for determining alcohol misuse. In whole blood, the most abundant homologue of PEth is 16:0/18:1. This homologue made up 37% of all PEth and it is formed by PC 16:0/18:1, which the left side represents the number of carbon atoms and the right side shows the number of double bonds in the homologues. Among forty homologues, other PEthsbe considered while deciding the amount of alcohol intake are 16:0/18:2, 16:0/20:4, 18:1/18:1from high percentage to low, respectively [7-9].

As being discovered at 1983 in rats and seen as a potential alcohol biomarker at 1997 [7], scientists have studied PEth homologues and their applicability for approximately 40 years. PEth is a powerful source for deciding chronic alcohol use and abstinence. In addition, it can guide to differentiate between moderate and low alcohol consumption. There has been studies for PEth and promising results have been found (Table 1).

Table 1. The summary of PEth studies

Biomarkers Studied	Study Matrices	Study Groups	Study Design	Results	Reference
MCV **ALT** **AST** **GGT** **CDT** **FAEE** **EtG** **EtS** **PEth**	Serum Whole Blood Hair Urine	Driving Under the Influence (DUI) participants	Examining the indirect and direct biomarkers, studying AUDIT, TLFB, DRINC, TRI as psychometric evaluation, The duration time of the study:8 months	PEth is the most strong indicator to correlate with BAC, other biomarkers and psychometric tests	[10]
PEth (16:0/18:1) **CDT** **GGT**	Whole blood	Healthy social-drinkers	Studying volunteers with 3 weeks of abstinence and requesting them to consume alcohol for 5 consecutive days to reach BAC with 1 g/kg by blood collection of 20 days	PEth can be detected as long as ethanol is present in the body and there is a concentration and half-time difference between alcoholics PEth values versus social drinkers, which is alcoholics have higher PEth values and shorter half-lives	[11]
PEth (16:0/18:1) **(16:0/18:2)** **BAC**	Whole blood	DUI cases	Determining cut-off values for PEth 16:0/18:1 and 16:0/18:2 by examining BAC levels of DUI cases with prolonged excessive drinking	Cases with BAC ≥ 1.6% levels accepted in the category of excessive drinking and the cut-off levels determined as PEth (16:0/18:1) ≥ 700 ng/mL and PEth (16:0/18:2) ≥ 300 ng/mL	[12]
PEth (16:0/18:1) **CDT** **GGT** **AST** **ALT**	Whole blood	Alcohol-dependent patients in a clinical trial to reduce alcohol consumption	Patients level of alcohol biomarkers examined and self-report of AUDIT including AUDIT-C tests	Although PEth is superior to CDT, both of which are the biomarkers correlate with self-report; however, no difference between sexes compared to PEth half-lives	[13]
PEth (16:0/18:1) **(16:0/18:2)** **EtG**	Whole blood Urine Hair	16 healthy volunteers	Single-dose of alcohol prepared to reach 1 g/kg BAC levels for two	PEth 16:0/18:2 was detected at a lower concentration than 16:0/18:1 and the latter	[16]

AUDIT: Alcohol Use Disorders Identification Test, TLFB: Alcohol Timeline Followback Method Assessment, DRINC: Drinkers Inventory of Consequences, TRI: Temptation and Restraint Inventor

CONCLUSION

In conclusion, phosphatidylethanol (PEth) has emerged as a promising alcohol biomarker due to its high specificity and sensitivity for detecting recent heavy alcohol consumption. PEth is formed in the body when alcohol is metabolized, and its levels in blood and other bodily fluids can be used to determine the extent and duration of alcohol consumption. Its reliability is based on the fact that PEth is produced only in the presence of ethanol and is not influenced by other factors such as food intake or non-alcoholic beverages.

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In the light of the results obtained from the previously mentioned articles, studies on PEth in 2020 and beyond show us that PEth shows promise as a biomarker [17-22].

PEth is advantageous over traditional alcohol biomarkers such as blood alcohol concentration (BAC) and liver function tests (LFTs) because it is less susceptible to short-term fluctuations and can detect alcohol use up to several weeks after consumption. Additionally, PEth testing is non-invasive and can be performed using a simple blood test, making it a more convenient option for both patients and healthcare providers. PEth has been shown to be useful in a variety of settings, including clinical practice, forensic toxicology, and alcohol research. Although PEth has limitations such as inter-individual variability and the need for specialized analytical equipment, its advantages make it a valuable tool for assessing alcohol consumption in both clinical and research settings.

Further research is needed to establish standardized cutoff levels for PEth and to determine its usefulness in detecting different patterns of alcohol consumption, such as binge drinking or chronic heavy drinking. Nevertheless, PEth is a promising alcohol biomarker that has the potential to improve the accuracy of alcohol assessment and to inform treatment decisions for individuals with alcohol use disorders.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

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Ethical approval

Ethics committee approval is not required.

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