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REVIEW ARTICLE

Alcohol Biomarkers and their Relevance in Detection of Alcohol Consumption in Clinical Settings

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Abstract

Alcohol use disorder is a growing public health concern worldwide. Accurate assessment is an important step towards effective management of the patient suffering from alcohol use disorder. Self-report often lacks accuracy and may be misleading in a hesitant patient. The use of biochemical laboratory measures such as alcohol biomarkers, often helps in obtaining independent estimations of alcohol use. The traditional biomarkers (such as Aspartate Aminotransferase, Alanine Aminotransferase or Mean Corpuscular Volume) have lower specificity as number of other comorbid disease conditions can independently increase their levels. Additionally, they increase after prolonged exposure to alcohol, and recede to baseline levels after several weeks to months of alcohol abstinence. The recently detected advanced biomarkers (such as 5-Hydroxytrptophol, Ethyl Glucuronide and Fatty Acid Ethyl Esters) are direct products of ethanol and are relatively unaffected by other disease conditions. These biomarkers can be detected soon after a moderate-heavy bout of alcohol use and persist in the body fluids for a shorter period of time. Such biomarkers can help in detection of shorter period of episodic alcohol use also. For better treatment and good outcome, clinicians must therefore be aware of the various advantages and limitations to make optimal use of each biomarkers used in detection of alcohol. This review provides an insight about the different alcohol biomarkers, their advantages and disadvantages in the clinical aspects, and a brief overview of the variety of future biomarkers for detection of alcohol use and drinking patterns.

Keywords

Alcohol dependence, Screening for alcohol use, Alcohol-biomarkers, Combination of alcohol biomarkers

Introduction

Alcoholism ranks as one of the leading threats to the health and safety of people worldwide [1]. According to World Health Organisation (WHO), 2010, 38.3% of the world is reported to consume alcohol regularly. 3.3 million deaths every year result from harmful use of alcohol [2]. The Global Status report released by WHO, 2010, also revealed around 30% of Indian Population are involved in drinking with over 11% indulge in binge drinking [2]. At national level, most doctors and health agencies have reported alcoholism as one of the leading causes of liver cirrhosis and failure.

Assessment - The First Step in Effective Management

The first, and perhaps one of the most important, step in effective management of alcohol use disorder is initial assessment of the patient. The assessment relies heavily on the clinician accurately eliciting details of alcohol use from the patient. Additionally, several standardized questionnaires have been developed to improve the validity and reliability of assessments. These include CAGE questionnaire [3], Michigan Alcoholism Screening Test, Alcohol Use Disorders Identification Test (AUDIT) [4], and the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) [5], which have been developed for screening purpose. The validation of these questionaries' varies, and also their accuracy varies in different population [6]. However, all such queries, including the questionnaires, rely mostly upon patient's self-report. Despite its widespread use,

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self-reports can lack accuracy and reliability at times. The problems are not due to issues of honesty of patient's report alone; patients can err on the report of quantity of alcohol consumed, as they do not measure or count their drinks during consumption, and hence provide a guesstimate [6]. Family members can help in providing information on the duration of alcohol consumed, and complications due to alcohol use. However, they often fail to provide details of quantity of alcohol consumed by the patients.

Laboratory Based Measures

Various laboratory-based estimates can play a key role in improving the accuracy of alcohol use. One set of estimates can be to assess the extent of alcohol-induced damage to different organs. These may include assessment of liver damage by measuring serum bilirubin, liver enzymes, or renal function tests to assess any kidney damage [7]. However, these estimates do not provide measures of alcohol use. A more accurate way to assess alcohol use is analysis of alcohol biomarkers: specific biological pointer of alcohol consumption measured in tissues, cells, body fluids, detecting any changes in the patient's health [8]. They are efficient tools for clinicians providing a proper assessment to patient's recent and past drinking activities [7]. It helps the clinicians with information like patient's recent drinking pattern; history of heavy drinking habits as well as whether the drinking was heavy or moderate.

This article presents an update on the alcohol biomarkers and their relevance in clinical settings.

Alcohol biomarkers

Gamma Glutamyl Transferase (GGT): Gamma Glutamyl Transferase is a membrane bound glycoprotein enzyme made up of both proteins and carbohydrates [9]. It aids digestion, found abundantly in liver cells and is involved in bile production [10,11]. GGT is commonly used biomarker for indicating alcohol-induced liver damage and has immense utility in primary health care [12,13].

Serum levels of GGT rise in response to alcohol consumption, varies between individuals and within individuals according to the phase in their drinking history [9,14]. A positive correlation between ethanol intake and serum GGT activity have been established in many studies. The measurement of serum GGT is limited as a primary tool due to its poor specificity and sensitivity [15]. The minimal alcohol consumption required for having an elevated GGT is about 74 g/week for men and 60 g/week for women [16]. GGT levels typically rises after heavy alcohol intake that has continued for several weeks, rather than episodic, heavy drinking [17,18]. The level of GGT generally returns to normal reference range in 2-6 weeks after abstinence, as half-life of GGT is 14-26 days (Table 1). Therefore, GGT levels is used as an indicator of chronic consumption of alcohol [19]. GGT never elevates with a single dose of alcohol unless the person has previously been an excessive drinker [20]. GGT increases more rapidly with resumption of alcohol consumption in those with a history of excessive drinking, particularly if there has been a past history of raised GGT [21]. Its clinical utility is limited due to high rate of false positive results as it gets elevated in non-alcoholic liver diseases such as biliary cirrhosis, obesity, pancreatitis, prostate-related diseases, diabetes, hypertension, hypertriglyceridemia, smoking and also with some medications (hormones and anticonvulsants) [9,20,22,23]. However, test for GGT being inexpensive, is included in Liver Function Test (LFT) panels [24].

Mean Corpuscular Volume (MCV) of red blood cells: MCV is a traditional non-protein alcoholic biomarker [25,26]. Regular alcohol drinking leads to increase in the size of RBC [27,28]. MCV increases with excessive alcohol intake after four to eight weeks and return to its normal size within two to four months (Table 1) [29].

MCV may increase among individuals reporting moderate levels of drinking (< 40 g/day) by 1-2 fl as compared to abstainers [30,31]. Population studies have shown that MCV levels are elevated in 4% of adults, of which 65% are likely related to alcohol consumption [32]. MCV lacks sensitivity when used individually and

Table 1: Sensitivity, specificity, drinking behaviour and window of assessment of alcohol biomarkers [7,8,23,65,73].

Biomarkers	Sample Source	Sensitivity%	Specificity%	Drinking Behaviour	Window of Assessment
GGT	Serum/Plasma	40-50	80-90	Chronic Heavy Drinking	2-3 weeks
MCV	Blood	60-90	30-75	Chronic Heavy Drinking	2-4 months
ALT/AST	Serum/Plasma	15-69	50-95	Chronic Heavy Drinking	2-3 weeks
CDT	Serum/Plasma	80-90	85-95	Heavy alcohol use	2-3 weeks
5-HTOL	Urine	n/a**	n/a**	Recent Use	5-20 hours
PEth	Blood	80-90	90-95	Heavy alcohol use*	2-4 weeks
FAEE	Serum	> 75	> 75	Recent Use	2-3 days
FAEE	Hair	100	90	Chronic Heavy Drinking	Several Months depending upon hair length
EtG	Urine	73-75	55-60	Recent Use	2-5 days
EtG	Hair	70-90	80-95	Chronic Heavy Drinking	Several Months depending upon hair length

*= more than 60 grams per day (4-5 standard drinks); **n/a = data not available.

has limited specificity, as false positive test can be seen in cigarette smokers, liver diseases, vitamin B12 or folic acid deficiency, thyroid disease, various haematological diseases, or in anaemia [9,30,31].

Serum Amino Transferases (AST, ALT): Aspartate Aminotransferase (SGOT, Serum Glutamic Oxalo-Acetic Transaminase) and Alanine Aminotransferase (SGPT, Serum Glutamic Pyruvic Transaminase) are building blocks of proteins as they help to metabolize amino acids [33]. ALT is found predominantly in the cytosol, whereas AST activity is highest in the mitochondria [34]. They are good indicators of liver disease when interpreted together [35].

Enhanced aminotransferase levels in alcohol dependent patients reflect liver damage [36]. However, the levels of these enzymes remain elevated in patients abstinent to alcohol with chronic liver disease [37]. Like GGT, aminotransferases are not increased by a single episode of excessive drinking [38].

ALT is more specific to alcohol induced liver cell injury compared to AST which is also found in heart, muscle, kidney and brain cells [39]. Any injury or disease that can increase the level of cellular injury or death in these organs will cause an elevation of AST [8]. ALT levels can also increase in extrahepatic conditions such as type 2 diabetes, metabolic syndrome, and insulin resistance [40,41]. Typically, less than 50% subjects entering treatment for alcohol use disorder have aminotransferases above the reference range [42].

Carbohydrate-Deficient Transferrin (CDT): Transferrin is a glycoprotein that transports iron in the body. Normal individual's transferrin contains four to six Sialic acid (carbohydrate) molecules. Alcohol consumption interferes with the ability of sialic acids to attach to transferrin and causes a deficiency of sialic acid content in transferrin, hence the name carbohydrate-deficient transferrin [8].

CDT is raised when the daily alcohol consumption is greater than 60 grams for two to three weeks. The elevated CDT levels due to heavy drinking also normalize after abstinence in two to four weeks [19]. CDT showed 100% specificity and 91% sensitivity in healthy individuals after 60 g of daily consumption ethanol during a 10-day period [43]. Hence, CDT is a sensitive marker to detect relapse in alcohol dependent people (Table 1) [43,44]. Serum CDT can differentiate between heavy drinkers and non-drinkers, and between heavy drinkers and social drinkers ($p < 0.0005$ for both), but not between social drinkers and non-drinkers ($p = 0.063$) [45]. CDT lacks sufficient sensitivity to detect binge drinking [46] but is highly specific for measuring alcohol consumption, showing low rates of false positive.

Another disadvantage with the CDT marker is that there is a relatively high rate of false negative results: Studies have reported that some patients with heavy drinking history did not show elevated levels of CDT [47]. However, CDT is not influenced by any liver diseases [48]. Hence, CDT positivity, unlike other biomarkers such as GGT or aminotransferases, is independent of liver damage. With high specificity, CDT shows better performance than other traditional biomarkers such as GGT, ALT, AST, MCV [46].

5-Hydroxytrptophol (5-HTOL): Another biomarker that focuses on recent moderate-to-high drinking levels is 5-Hydroxytrptophol (5-HTOL) [49]. Serotonin (known as 5-hydroxytryptamine, 5-HT) is a monoamine neurotransmitter which forms an intermediate aldehyde, 5-hydroxyindole-3-acetaldehyde (5-HIAL) by the action of monoamine oxidase (MAO). This intermediate is oxidized by an aldehyde dehydrogenase to 5-Hydroxyindoleacetic Acid (5-HIAA). However, in the presence of alcohol, the intermediate is reduced to 5-Hydroxytrptophol (5-HTOL) by alcohol dehydrogenase [50]. To improve the accuracy in routine clinical use, 5-HTOL is reported as a ratio to 5-hydroxyindoleacetic acid (5-HIAA) instead of creatinine, which can compensate for urine dilution and accounts for dietary source of serotonin [51].

5-HTOL level remains increased in the urine for several hours even with ethanol being no longer measurable in body fluids or breathe [51-53]. 5-HTOL is detected for up to 24 hours after last drink; the detection window of the 5-HTOL/5-HIAA ratio in urine is approximately 5-15 hours longer than that of ethanol in urine. Therefore 5-HTOL is considered as a 24 hours marker of alcohol use [49,54]. This alcohol biomarker has a specificity of almost 100% and sensitivity of around 77% at a cut off value of 15 pmol/nmol for consuming 50 g or more of ethanol (Table 1). 5-HTOL displays high sensitivity and specificity and is uninfluenced by age, gender, liver diseases, or medications other than disulfiram [49]. Consumption of 50 g or more increases the 5-HTOL/5-HIAA ratio in urine significantly, with higher ratios indicative of more ethanol consumption, in a dose dependent manner. 5-HTOL appears uninfluenced by age, gender, liver diseases, or medications other than an aldehyde dehydrogenase inhibitor such as disulfiram which causes an abnormal rise in 5HTOL/5HIAA ratio [49].

Phosphatidylethanol (PEth): PEth is a specific metabolite of ethanol. Phosphatidyl choline is hydrolysed by phospholipase-D to form phosphatidic acid. In the presence of alcohol, PEth is formed at the expense of phosphatidic acid through transphosphatidylation of phospholipase-D [39].

Peth can be detected in blood after consumption of a minimum of approximately 1000 gram of alcohol with an assessment window of 1-2 weeks [55-57]. It requires about 15 days of abstinence for PEth to return to normal values and has a half-life of approximately seven days in blood in alcohol users but can vary considerably between three to nine days [58]. PEth has higher

sensitivity and specificity compared to other traditional markers and helps to detect even low/moderate drinking (Table 1) [56].

Peth is better than CDT to detect relapse especially with quantity of alcohol consumption not being high enough for CDT to become elevated. PEth, not being influenced by any liver disease, is useful in monitoring heavy drinkers with hepatic pathology [59,60]. PEth proved better and stable for assay results of dry blood spot cards, suggesting improving potential of PEth for routine applications [61]. Thus, PEth tests can monitor alcohol consumption and can help identify early signs of harmful alcohol consumption.

Fatty Acid Ethyl Esters (FAEE): Fatty Acid Ethyl Esters (FAEE) are breakdown products of non-oxidative pathway of alcohol metabolism, formed by esterification of endogenous free fatty acids and ethanol by specific and non-specific enzymes in blood and several tissues [8,62]. FAEE, formed from several fatty acids and ethanol, is basically a combination of various esters. With the alcohol component being glycerol, several monoglycerides, diglycerides or triglycerides are formed [63]. Although approximately 15-20 fatty acid ethyl ester can be detected in a single specimen, frequently the sum of the concentrations of four fatty acid ethyl esters (ethyl oleate, ethyl palmitate, ethyl myristate and ethyl stearate) is commonly used [8,62,64].

Recent studies demonstrate that FAEEs are sensitive and specific markers for distinguishing social drinkers from heavy or alcohol-dependent drinkers [65]. FAEE levels have been detected from blood 24 hours after last drink, with blood ethanol level increased for only 8 hours (Table 1) [66]. Further, FAEE levels showed elevation after ethanol consumption for at least 99 hours in heavy drinkers [67]. Also, serum concentrations of Ethyl Oleate in chronic alcohol users is observed to be higher than in binge drinkers, thus distinguish between binge drinkers and alcohol dependent persons [68]. Recent studies have measured FAEE in hair observing that it can be detected in hair for months [69,70].

FAEE have a long half-life in adipose, hence it may be useful for forensic applications as well because adipose tissue samples are readily obtainable. Refaai, et al. analysed FAEE concentrations and speciation in solid organs and tissues as markers of pre-mortem ethanol intake. They concluded that the mass of FAEE in liver and adipose tissue can serve as post-mortem markers of pre-mortem ethanol intake when blood samples cannot be obtained [71].

Ethyl Glucuronide (EtG): EtG (ethyl β-D-6-glucuronide) is a direct PHASE II metabolite of ethanol [72]. This minor non-oxidative metabolite of alcohol forms in the liver after alcohol consumption [73] when ethanol reacts with glucuronic acid in the presence of UDP-glucuronosyltransferase (UDP-GT) enzyme, leading to the formation of ethyl β-D-6-glucuronide (EtG) [74].

EtG is a sensitive marker of alcohol consumption that can be detected for an extended time period after the complete elimination of alcohol from the body providing a strong indication of recent drinking. EtG can be detected in body fluids shortly after its intake and dose-dependently up to 80 hours after the complete elimination of alcohol from the body [24,72]. With chronic alcohol consumption, EtG peaks 2 to 3.5 hours later in blood than ethanol and remains in blood up to 36 hours [33,72]. It is capable of detecting relapse in patients thus enabling the therapist to intervene at an early stage of relapse [75]. Measurable concentrations of EtG (> 0.1 mg/L) are detectable in serum for more than 10 hours, whereas ethanol is detectable for over 8 hrs [54,75].

In urine, EtG can be detected up to 13-20 hours with small quantity (~0.1 g/kg body weight) of ethanol intake (Table 1) [54]. However, after heavy consumption it can be detected for up to three to five days [24,54]. EtG concentration in urine peaked approxiamately four hours after ethanol intake. EtG cut-off of 100 ng/mL is most likely to detect heavy drinking for up to five days. Cutoffs of ≥ 500 ng/mL are likely to only detect heavy drinking during the previous day [74]. Also, EtG was demonstrated to become concentrated in the urine to reach much higher levels than in blood. Urinary EtG has much longer detection time compared with blood (range 14-24 h) making urinary EtG a more sensitive biomarker of recent drinking [54]. However, the absolute concentration of EtG in urine after a given dose of ethanol may vary considerably between, and also within, individuals, as it is influenced by several factors besides the amount of alcohol consumed, such as urine dilution and time of voiding [75].

EtG can also be detected in hair that can help in evaluation of chronic ethanol use over several months from a single sample [64,76,77]. Hair analysis provides a long detection window and potential establishment of longer-term drinking history. As a long-term biomarker, hair EtG is highly advantageous due to its ability to provide consumption trend for several months from a single non-invasive sampling. Also, in the absence of self-reports from patients, segmental hair analysis would provide a proportional relationship between EtG concentration in hair and considerable progress in the alcohol consumption monitoring [78,79].

EtG concentrations can be affected by age, male gender, tetrahydrocannabinol (THC) use, kidney disease, creatinine and total grams of ethanol consumed in the last month. Male gender and kidney disease were associated with decreases in urine EtG concentration, whereas THC use was associated with an increase [76]. Slightest incidental exposure to alcohol (such as cooking wines, flavouring extracts, over-the-counter cold medications) may result in positive urinary EtG. Additionally, consumption of 'non-alcoholic' drinks, use

Table 2: Key findings of combination biomarkers as reported by various studies.

Authors	Biomarkers	Sample Source	Study Population	Key Findings
Bell, et al. [88]	GGT, CDT, AST, ALT and MCV	Serum/Plasma	400 Alcoholic patients observed for over 4 weeks	Highest sensitivities for CDT and GGT (65% to 73%). Lower sensitivity for AST, ALT, and MCV (50%, 35%, and 52%, respectively)
Doyle, et al. [89]	FAEE	Serum	Healthy subjects ingested a weight-adjusted amount of ethanol at a fixed rate	Positive over a period of 24 hour
Scouller, et al. [90]	CDT, GGT	Serum/Plasma	Meta-analysis of 110 clinical studies	CDT was little better than GGT in detecting high or intermediate-risk alcohol consumption in a large, multi-centre, predominantly community-based sample
Wurst, et al. [91]	Breath, urinary ethanol, urinary EtG, CDT, PEth, GGT, MCV	Breath/Serum/ Plasma/Urine/ Whole blood	Forensic psychiatry inpatient (committed a substance-related offence).	Ethyl glucuronide is capable of detecting alcohol consumption in cases where neither traditional biological state markers of alcohol intake nor clinical impression gave an indication
Borucki, et al. [68]	FAEE	Serum	Heavy drinkers	Remains elevated at least up to 44 hours
Borucki, et al. [92]	Serum FAEE, Urinary EtG, 5-HTOL/5-HIAA in Urine	Serum/Urine	Sixteen (14 male, 2 female) heavy alcohol drinkers	FAEE declined until 15 hours and 5-HTOL/5-HIAA declined after 29 hours, however EtG concentration showed 100% sensitivity for 39 hours
Chrostek, et al. [44]	CDT, MCV, AST, ALT, GGT and Sialic Acid (SA)	Serum/Plasma	Subjects recently abstinent from alcohol consumption	CDT appeared to have higher sensitivity however the sensitivity decreased for all studied alcohol markers when the period of abstinence was longer than one week
Høiseth, et al. [54]	EtG	Urine	Ten male volunteers consumed ethanol at a fixed dose of 0.5 g/kg body weight in a fasted state	Detected up to 13-20 hours with small quantity
Halender, et al. [93]	EtG	Urine	Alcoholic patients undergoing alcohol detoxification	Detected up to 40-90 hours (< 0.5 mg/g)
Morini, et al. [94]	Hair EtG and CDT	Serum/Hair	Subjects with alcoholic history	Superior sensitivity specificity of Hair EtG (then CDT)
Kharbouch, et al. [95]	Hair EtG, CDT, GGT, ATL, AST	Serum/Hair	Teetotallers, low-risk drinkers, at-risk drinkers, or heavy drinkers	Hair EtG diagnostic performance was significantly better
Hastedt, et al. [96]	CDT, MCV, GGT, ALT, AST, Hair EtG and Hair FAEE	Serum/Hair	Social drinkers, non-drinkers and alcoholics group	Hair FAEEs and Hair EtG offered a longer time frame of several months for detecting chronic excessive alcohol consumption than the traditional biomarkers
Alladio, et al. [97]	ALT, AST, CDT, GGT, MCV, EtG, FAEE	Blood/Hair	125 subjects including social and heavy drinkers	Hair FAEEs and Hair EtG offered detection of chronic alcohol consumption

of mouthwash (4 times/day for 3¼ days), and use of alcohol-based hand sanitizer can also result in positive urinary EtG [80,81]. Some urine samples may contain yeast that can convert urine glucose to alcohol and subsequently EtG, if stored at room temperature for more than 12 hours [82]. This is a concern especially among persons with diabetes who have high levels of glucose in the urine. False negative also can arise from urinary dilution or from ingestion of choral hydrate medication or from *E. coli* hydrolysis of EtG (in urinary tract infection) [83,84]. To counter this, it is recommended that either urinary creatinine be measured with a cut-off of 25 mg⁄dl to indicate dilution or EtG be expressed in ratio to creatinine [85-87].

With significant progress in laboratory assessment of biomarkers to estimate health risks related to excessive alcohol use, no single biomarker at present demonstrates 100% specificity and sensitivity. This can be overcome using combination panels, where tests are combined to increase the likelihood of an accurate diagnosis. (Table 2) summarizes the findings of some studies conducted across the world.

Conclusion

Alcohol biomarkers help a clinician to objectively ascertain the alcohol user's claim of the quantity, frequency and duration of alcohol use. The older biomarkers relied on the effect of alcohol on body systems such as liver (such as AST, ALT) or blood cells (such as MCV). These biomarkers have lower specificity as a number of disease conditions could also produce

raised levels. In addition, these biomarkers raise after prolonged exposure to alcohol, and take time to recede to baseline levels. Hence, detection of recent and short-term use of alcohol use was a challenge. Public health focus related to alcohol has also undergone change with time. Large-scale population studies have shown that even short-term use of heavy amounts of alcohol can lead to significant morbidity and mortality. This has also simultaneously led to quest for newer biomarkers that can detect recent alcohol use. The biomarkers such as 5-HTOL, urinary EtG and serum FAEE are direct products of ethanol and are relatively unaffected by disease conditions. They are detected soon after a moderate-heavy bout of alcohol use and are present in the body fluids for a shorter period of time. However, detection of biomarkers is not cheap, and hence, combining different biomarkers together offers the best solution to detect alcohol use.

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