Clinical Applications of State Alcohol Biomarkers: An update

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Abstract

Alcohol Use Disorder (AUD) is one of the most prevalent causes of global burden of disease.It is associated with significantphysical and mental health problems as well associal and economic consequences.Alcohol consumption is considered to be one of the most important preventable risk factors for adverse health outcomes, hence proper assessment is must for alcohol use disorder. The assessment pattern of alcohol use based on self-report of patients may not be always reliable due to recall bias and minimization highlighting the need of accurate biomarkers.In this narrative review we discuss about the different state biomarkers of alcohol, their properties and their utility in different settings during patient care. Despite many limitations traditional state markers are still useful and we recommend clinicians tofamiliarize themselves to use them as additional outcome measures in clinical interventions for AUDs and associated medical complications.

KEYWORDS:
Alcohol Use Disorder, State biomarkers, Direct biomarkers, Indirect biomarkers

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INTRODUCTION

Alcohol Use Disorder is one of the most prevalent mental health problems causing significant global burden of disease. Mean lifetime prevalence of alcohol use in all countries combined is 80% which ranges from 3.8% to 97.1% in different countries and WHO regions.1Excessive alcohol use is associated with several leading causes of death among adults including heart disease, cancer, unintentional injury, and liver disease. Excessive alcohol use is an also a leading preventable cause of premature death. There has been increase in rates of deaths due to fully alcohol-attributable causes like alcoholic liver diseases in past decade, at a global level.2Hence there is importance of assessment for proper management in order to minimize the burden of disease.In medical settings, alcohol use disordersare routinely assessed by detail clinical interview of the patients and collateral information from informants. Various standard assessment scales have been developed and validated for screening of alcohol consumption,assessment of drinking pattern and diagnosis of alcohol use disorders likeCAGE questionnaire, Michigan Alcohol Screening Test (MAST), Alcohol Use Disorders Identification Test (AUDIT)and Alcohol,Smoking and Substance Involvement Screening Test) (ASSIST).3,4,5,6The assessment pattern of alcohol usebased on self-report of patientswhether interview based or standard questionnaires are not reliable due to recall bias and minimization.7Also there is stigma attached that prevents a person from revealing his alcohol use. Other factors like social desirability bias, poor episodic memory, cognitive decline and minimization prevents from getting a reliable history.Hence there is a strong need of biological markers for accurate assessments. Biomarkers are the biochemical substances in the body that can indicate the presence or progress of a condition, or any genetic predisposition towards it.8Apotential biomarker of alcohol consumption will not rely on self-reporting or become vulnerable to falsification due to inaccurate recall or reluctance of individuals to give genuine reports of their drinking pattern. Thus, it can provide clinicians with an additional source of objective information about alcohol consumption and add credibility to research dealing with efficacy of clinical interventions for AUDs.

There are two kinds of alcohol biomarkers: state markers and trait markers.State markers of alcohol use give information about an individual's drinking pattern, includ-

ing chronic heavy drinking, a recent binge or even just a few drinks. On the other hand, trait markers for alcohol use reveal about a person's inherited risk of abusing alcohol and help to identify people with a genetic predisposition to alcohol abuse and alcoholism.9,10Various biochemicalparameters such as Mean Cell Volume (MCV), serum LFT parameters: Aspartate aminotransferase (AST) and Alanine transaminase (ALT) and Gamma-Glutamyltransferase (GGT or γGT)serve purpose as traditional state markers of alcohol use. However, their values may be affected by multiple factors related to patient characteristics (age, gender, obesity) and medical conditions (e.g. co-morbid liver disease). Carbohydrate-deficient transferrin (CDT) is a relatively more useful state marker which is not affected by underlying liver condition.The accuracy of the traditionalbiomarkers also depends on sample handling, storage, quality assurance of laboratory procedures, methods and cut off levels for quantification and interpretation of results.The shortcomings in traditional biomarkers have also lead to development of new laboratory tests, formulation of algorithms to combine results on multiple measures, and more extensive applications of newer in treatment and research.9 Emerging biomarkers such as ethanol metabolites [Ethyl glucuronide (EtG), Ethyl sulfate (EtS), Phosphatidyl ethanol (PEth), blood acetaldehyde adductsFatty acid ethyl esters (FAEE's)], hexosaminidase, sialic acid, and urine serotonin metabolites (5-HTOL/5-HIAA ratio)are being researched and they are sensitive and specific.These markers are also detectable in other body sources such asurine (hexosaminidase,EtG, EtS acetaldehyde adducts, 5-HTOL/5-HIAA), saliva (sialic acid)and hair (EtG).

Theutility of these biomarkers depend upon their psychometric properties and laboratory/clinical settings in which they are measured.Combining biomarkers with performance of standard scales such as CAGE, Quantity Frequency Index (Q. F. Index) Questionnaire, Michigan Alcohol Screening Test (MAST)and the Alcohol Use Disorders Identification Test (AUDIT) can provide a more elaborate information about diagnosis of alcohol use disorders as well as drinking pattern rather than relying on self-report or biomarker alone depending upon the demand of the setting.

Properties of Alcohol biomarkers

There are several properties of alcohol biomarkers which decide their utility in particular clinical settings.The time for which the marker remains positive for alcohol consumption in body sources depends upon its half-life and is useful predictor of duration of drinking. Furtherhigh sensitivity of the biomarker (ability to accurately identify those persons who have consumed alcohol) will help to pick up alcohol drinking and high specificity of the biomarker (ability of a test to accurately identify those persons who have not consumed alcohol) leads to a low false positive rate. The laboratory tests for biomarkers also should be preferably non-invasive, easy-to-perform, inexpensive, rapidand reproducible in laboratories worldwide.

MCV:It is elevated in chronic heavy drinking with lower sensitivity in males and higher sensitivity in females. The normal values for MCV are 87 ± 7 fl.11Since the life-span of a red blood cell is about three months,it may take several months for changes in drinking to be reflected in MCV levels. Hence, they cannot be used to detect and monitor early change in alcohol use.[12,13]

*GGT or γGT:*It is raised in chronic heavy users and more likely in more than 30 years of age. However, it is not a very sensitive marker as only 30 to 50 % of excessive alcohol users in the general population have significant rise in GGT levels. Values more than 54 U/l for both genders are considered abnormally elevated. GGT levels are also affected byvarious other factors like gender, smoking status, GI diseases (hepatic, biliary and pancreatic diseases),use of medications (e.g., hormones, anticonvulsants) and this increases the likelihood of false-positive results etc.[9,14]

*AST and ALT:*These are markers of heavy alcohol consumption and underlying liver disease. Ratio of AST to ALT signifies heavy alcohol consumption and a very high level of these enzymes with higher ratios of AST to ALT (AST/ALT>2) may reflectunderlying alcohol related liver damage rather than heavy drinking alone.[8,12,15]

*CDT:*This measures desialylated isoforms of transferrin in body fluids. The most alcohol-specific isoforms are asialotransferrin and disialotransferrin detected from serum. They have sensitivity almost equal to GGT and are less affected by the effects of liver disease. Serum CDT levels are elevated when daily ethanol consumption increases beyond 40 to 80 grams with duration for a duration of2 to 3 weeks. Recent investigations using CDT quantify it as a percent of total serum transferrin, rather than total CDT to correct for individual variations in transferrin levels. Laboratory test results of >2.5% suggest heavy drinking. Other than excessive drinking, end-stage liver disease, biliary cirrhosis, and a rare genetic variability will elevate CDT.[16]

*Direct biomarkers:*These areanalytes of alcohol metabolism

and can be measured in sources other than blood (urine, hair) for a longer period than the time alcohol remains in the body. Some of the recent markers are Ethyl glucuronide (EtG) and Ethyl sulfate (EtS) and Phosphatidyl ethanol (PEth). EtG and EtS, measured in urine are highly sensitive to even low-level exposure to alcohol and may remain detectable in urine for 1 to 2 days. Extraneous exposures to alcohol such as that present in many daily use products canalso result in false positive results which can provide misleading information. However, this property of EtG makes it useful to monitor abstinence settings where detection of even low alcohol consumption is important.A combination of EtG and EtS has potentially increased sensitivity as they are formed via different metabolic pathways.[17] A test for PEth is more useful because of its persistence in blood as long as three weeks after even only a few days of moderately heavy drinking (about four drinks per day).PEth appears to be a more sensitive and specific indicator of alcohol consumption than traditional alcohol markers, such as CDT, GGT, and MCV.[18] Newer markers such as urinary Derivative of Serotonin expressed as elevated ratio of 5-HTOL to 5-HIAA (due to shift of metabolism towards 5-HTOL) may be indicative of alcohol consumption over the last 24 hours.[19] Other markers like fatty acid ethyl esters, sialic acid, acetaldehyde adducts, N-Acetyl B-Hexosaminidase etc. are at various stages of development and not commercially available for routine clinical practice other than for research purpose.[9, 10]

Among the traditional biomarkers, the highest sensitivities are obtained with the CDT and GGT tests, ranging from 65% to 73%. AST, ALT, and MCV have significantly lower sensitivities of 50%, 35%, and 52%, respectively.CDT show the greatest specificity of 92%, whereasGGT hasthe lowest specificity at 75%.[20] A multi-centric study (WHO/ISBRA Collaborative Project) across the globe found that CDT and GGT had comparable performance with AST performing slightly less well. Also, CDT is a slightly but significantly better marker of high-risk consumption in men. The values of CDT and GGT are influenced by body mass index, sex, age, and smoking status.14To improve sensitivity, tests can measure CDT as a percentage of total transferrin (%CDT) which excludes the trisialotransferrin isoform from the measurement. This leads to improved accuracy and better correlation with self-reported alcohol use rather than an absolute value of CDT,GGT or AST.[21]

In one of the earliest studies in South India, GGT was elevated (above lab, normal range) among 47% of inpatients admitted for management of alcoholism (based on Research Diagnostic Criteria) and 3% of controls, AST was elevated among 60% of alcohol users and 40% of controls and ALT was high among 35% of alcoholics and 16% of controls. Values of AST, ALT, GGT and serum bilirubin elevated at admission showed significant decline after one month's abstinence. The results showed that in this sample of patient, these tests together were more specific (false +ve 20%) than sensitive (true +ve 47%).[22] 20% of the controls were misclassified. Only 3% of them had elevated GGT, though 35-40% had elevated SGOT or SGPT.Another study from Indiawhich employed AUDIT for screening of patients with problem drinking, %CDT had the highest sensitivity (84%) and specificity (92%), GGT had lower sensitivity and specificity (64% and 72% respectively) and MCV had the least (48% and 52% respectively).[23] In China, itwas found that the CDT values are raised graduallywith increasing daily mean alcohol intake, and this trend becomes statistically significant for daily alcohol intake > 45 grams of alcohol.[24]

It has been found that combination use of both GGT and CDT has superior utility than either of them alone.CDT-GGT values combined into a mathematical algorithm GGT–CDT = {0.8 × ln (GT) + 1.3 × ln (%CDT)}has a higher sensitivity (90%) compared to CDT or GGT alone (60 to 70%).GGT–CDT is also known to be unaffected by underlying Liver disease compared to GGT, MCV, AST and ALT which change as a function of liver status. [25,26]

Overall, evidence shows that CDT and GGT are superior to other biochemical measures, demonstrating comparable sensitivities, with CDT showing greater specificity and % CDT performing better than CDT. Among newer tests, EtG is useful to detect recent drinking whereas PEth can identify severity and pattern of drinking and also correlates with long term self-reported alcohol use as measured by AUDIT.

Comparisonand combination of Biomarkerswith self-report

A study in Indiarecommended that there is good correlation between biomarkers and self-report in both community and hospital settings. However, based on sensitivity and specificity, laboratorytests preferably in combination (MCV and GGT) were more useful in diagnosing, monitoring and follow-up assessment of patients with alcoholism whereasquestionnaires (Q.F. Index and MAST) were more useful in community.[27] Combining self-report and raditional biochemical parameters (mainly LFTs) may not always havea favourable outcome. One study highlighted that all

self-reported heavy drinking could be corroborated with collateral information but GGT values were elevated among only 39.7% of those who admitted to heavy drinking. It was argued that in clinical trials using self-selected research volunteers, biochemical tests and collateral informant reports do not add sufficiently to self-report measurement accuracy to warrant their routine use.[28] In this regards, the performance of biochemical parameters have also been compared with standard scales used by the clinicians for assessment of drinking pattern and diagnosis of spectrum of alcohol us disorders. Overall,self-reporting with AUDIT has been found to significantly correlate with % CDT both for men and women ($p<0.0001$).[29] A combination of both self-report and biochemical parameters could be more useful to identify potential users of alcohol. A study conducted during routine health examinations combined the use of AUDIT, GGT and CDT and found that by using only the AUDIT (without biomarker tests), half of the drinkers could only be identified. However, using only CDT and GGT (without the AUDIT),almost one third of the positive cases would have been missed.[30] Another study suggested that combination of use of at least two other abnormal biological markers (MCV,AST,ALT,GGT) along with AUDIT improved detection of alcohol withdrawal.[31]

It has also been argued that CDT and AUDIT identify patients with different drinking patterns. CDT identifies heavy drinking only in the past couple of weeks up to roughly one month as its half-life is about 10 days. On the other hand, the AUDIT questionnaire picks up information about the usual quantity and frequency of drinking, the general drinking behaviour and prior alcohol-related problems in the last one year. Hence, a high correlation might not be expected between the AUDIT and CDT results. The clinical performance of screening tests can be significantly improved by combining self-report and biomarker measures.[32] Regarding EtG or EtS, they may not correlate with long-term biomarkers such as % CDT, GGT or the AUDIT but they may be useful in emergency department to detect recent drinking even in cases of negative ethanol test and to confirm abstinence from alcohol. This sensitive and specific short-term biomarker provides valuable additional information about recent individual drinking habits and alcohol hangover.[33] PEth in whole blood and dried blood spots can significantly distinguish between binge drinkers, moderate drinkers and abstainers better than MCV or GGT. Further, it has significant correlations with self-reported alcohol use as measured by AUDIT scores.[34]

Biomarkers in different settings

The state alcohol biomarkers are helpful in identification of problem drinking and the severity of alcohol use, assessment of alcohol-related medical conditions such as the extent of medical complications such as alcohol-related liver. Such markers can also be used to provide feedback to patients about drinking pattern and motivate to cut down or refrain from drinking. A comparison of baseline and follow up test values can be used to monitor change in alcohol use and verify self-reported treatment outcomes after clinical interventions for alcohol use disorders. Additionally, alcohol biomarkers are also being used in occupational, public health, medico-legal and research settings.

A. Primary care: It is known that, as many as 20% of primary care patients drink at levels that are harmful to their health.[35] As harmful/hazardous and dependent alcohol use can cause or aggravate numerousmedical complications, biomarkers for heavy alcohol are useful to yieldclinically relevant information in primary care patients. Early detection of alcohol use problem using biomarkers or chronic heavy drinking like GGT and CDT could be potentially beneficial to initiate physician advice and counselingto lower long term alcohol use in such patients.[36] This can be also useful to prevent and control risk factors of chronic illness-such as type 2 Diabetes and hypertension in primary care patients(e.g. GGT).[37] Ongoing research on the association between alcohol biomarkers and specific medical conditions has provided substantial evidence that the combination of CDT, GGT, and self-report questionnaires (e.g., the AUDIT) can serve as risk indicators for alcohol-sensitive medical diagnoses. Unfortunately, preliminary findings indicate that physicians have little knowledge of current biomarker research as applied to primary health care. Use ofthe biomarkers in routine clinical practice could improve the quality of medical care by early identification, and treatment of AUDs and alcohol sensitive medical problems and monitoring response to treatment to AUDs and other associated medical conditions. (e.g.persisting high BP due to continuousheavy-drinking).[5]

B. Hospital settings: Problem drinking such as harmful/hazardous and dependent alcohol use is often associated with medical complications in the clinical course of patients with various medical and surgical issues like trauma victims or who are undergoing surgery and post-liver transplantation.[38,39] Among traditional biomarkers, CDT has been found to be an accurate marker for detecting patients at-risk for alcohol-related surgical complications, alcohol withdrawal,

an increased risk of complications, and a prolonged ICU stay after severe trauma.[40] A pre-op evaluation of patients planned for Upper GI surgery found that addition of CAGE questionnaires increased sensitivity of clinical routine evaluation (DSM-III-R) based diagnosis whereas additional screening with biomarkers (GGT,CDT) along with CAGE led to increase in sensitivity, which was highest (91%) when combination of all tests (routine evaluation, CAGE, GGT,CDT,) was used. The study suggested that patients should be seen more often, and additional diagnostic tools such as the CAGE, CDT, and GGT should be used before surgery to detect more alcoholic patients at risk for major complications.[41] EtG in urine and hair and blood PEth can be used for the selection and surveillance of patients within the liver transplant setting.[42] Newer biomarkers such as (FAEEs) in meconium, ethyl glucuronide (EtG) and ethylsulfate (EtS) in hair may also be measured for detection of gestational ethanol exposure among recently delivered babies.[43] Additionally, their combination with self-report and AUDIT has also been known to facilitate the diagnosis of foetal alcohol syndrome and foetal alcohol spectrum disorders by retrospective detection of alcohol consumption during pregnancy.[44] Further, PEthis known to have correlation with self-reported alcohol use among HIV-Infected patients initiating Antiretroviral Treatment in studies from Africa and Russia.[45]

C. Drug AddictionTreatment Setting: The evaluation of multiple traditional bio-chemical parameters and interpretation of their inter-relationship based on their properties is more likely to detect alcoholism and recovery following cessation of drinking. An earlier study from India found that AST,ALT, GGT were useful to confirm abstinence among alcohol dependent subjects after inpatient management of withdrawal. However, they had limited sensitivity and specificity as they are markers of chronic heavy drinking.[22] Upon serial tests among alcohol dependent patients, a parallel rise in AST≥40%,ALT≥20% and GGT≥40% at follow up compared to discharge values is useful to identify individuals who had resumed drinking over those who remained abstinent.[46] Biomarkers such as CDT and GGT, preferably in combination may be more useful in detecting relapse among traditional markers. A 30% decrease in either CDT or GGT is indicative of abstinence or significant reductions in alcohol consumption whereas a 30% increase might indicate relapse. However, relapse can be best identified by 30% increases in both CDT and GGT simultaneously.-Due to theirability to detect small amounts of alcohol, urinary EtG/EtS have better performance compared to CDT to monitor relapse in patients in addiction settings including community based alcohol treatment programs.[47] However, despite lower sensitivity and specificity to detect alcohol consumption, routine tests of AST, ALT among subjects in addiction programs can also identify subjects with co-morbid medical conditions (such as underlying liver diseases) that may affect short term (e.g. Benzodiazepine use for withdrawal) and long term (e.g. Disulfiram and Naltrexone) clinical management of alcohol use disorders. The traditional biomarkers seem to have limited utility to detect drinking patternamong subjects with co-morbid substance use disorders such as opioid dependence.[48] Among subjects with alcohol dependence taking treatment in addiction settings, EtG in urine has been found to closely correspond with self-report drinking to detect alcohol use for greater than 24 hours at 200 ng/mL cutoff level.[49] Among opioid dependent subjects stabilized on Methadone maintenance, few studies have combined self-report withnewer biomarkers such as5HTOL/5HIAA ratioin urine and Ethyl Glucuronide (EtG). [50, 51] Similarly, PEth has also been shown to have high specificity and correlation with self-reported alcohol use among young Injection Drug Users (IDUs).[52] Overall,incorporation of newer biomarkers seem to identify patients under treatment for drug addiction who denyor minimize alcohol usebut are otherwise in need of specific interventions for problem drinking.

D. Occupational, Medico-legal settings: Besides clinical settings, alcohol biomarkers are also useful to screen certain occupational groups for problem drinking. CDT can be a complementary test to the AUDIT in screening for alcohol use disorders among other specific occupational groups like transportation workersand migrant workers during routine health examination.[30, 31,32,] In one study among construction workers, elevated GGT and AST levels strongly related to early retirement and all-cause mortality.[53] In several European countries, drivers under the influence (DUI), suspected of chronic alcohol abuse are referred for medical and psychological examination. A study from Belgium (Recidivism of Alcohol-impaired Driving or the ROAD study) investigated CDT,AST,ALT,MCV,GGT levels among previously convicted drunk-driving offenders in the post-arrest period and observed them for 3 years. A logistic regression analysis revealed that ln(%CDT), ln(γGT) and ln(ALT) were the best biochemical predictors of recidivism of drunk-driving. Additionally, The ROAD index (which includes ln(%CDT), ln(GGT), ln(ALT) and the sex of the driver) could predict risk of relapse. [54]

E. Interventional studies: In addition to screening for heavy drinking, alcohol biomarkers are also useful for monitor

pre-post change in drinking behaviour after clinical interventions. Studies which test effectiveness of Psychological Interventions for reducing alcohol use in harmful/-hazardous pattern (such as Brief Intervention, Brief Counselling, Brief Advice, Physician advice) have found that feedback about elevated biochemical parameters can effectively reduce alcohol use.In one study, GGT feedback based intervention was found to be more effective than simple letter informed advice to restrict alcohol consumption. Follow up at 2, 4, and 6 years showed a significant reduction in sick absence and mortality among intervention group also accompanied by fall in serum GGT levels.[55] Another studyshowed that physician advice compared to no advice group resulted in significant reduction of alcohol consumption in hypertensive patients at weekly follow up for 18 months which was also accompanied by decrease in GGT and AST values.[56] In a multi-centric study, effectiveness of Brief Physician counselling was established by both self-report, corroboration from relatives and decrease in GGT values during follow up at 3 months, 1 year, and 2 years.[36] A pilot study among patients being treated for Type 2 diabetes and hypertensionrevealed that CDT feedback based brief clinician advice was effective as verified by fall in CDT levels significantly in the intervention group compared to control group patients.[37] The study showed that brief intervention, combined with feedback on %CDT levels can reduce alcohol use among primary care patients being treated for medical conditions such as Type 2 diabetes and hypertension. It appears that alcohol biomarkers can play an important role in corroborating patient self-reports and monitoring heavy drinking during and after brief alcohol interventions by general practitioners. In addition to monitoring per se, the role of biomarkers in providing patient feedback and accountability deserves further research attention. A review of role of biomarkers in interventional studies discussed that the application of biomarkers as inclusion criteria is generally not recommended in such studies. However, they may be useful to excludecertain subjects (e.g., liver disease leading to grossly deranged LFTs) and can also serve as secondary outcome variables. The relationship of outcome findings on biomarker and self- report measures also seems to be positive, but only moderate. Traditional biomarkers of drinking tend to be less sensitive than well standardizedand properly administered self-report measures. They do provide a useful, unique source of information on drinking status. In clinical research, it is suggested that certain design strategies should be incorporated into the application of biomarkers and critical information should be included in the research publication.[10] It is apparent that combination of newer biomarkers with more sensitive specificself-report measures of alcohol consumption will be ideal as outcome measures to monitor change in drinking pattern following clinical interventions.

Conclusion:

From the above review, it is evident that traditional biomarkers of alcohol use are useful to recognize pattern of drinking behavior and give personalized feedback to the patients despite limitations in their psychometric properties.Among traditional state markers,CDT is emerging as a more sensitive and specific alcohol biomarker with improvisation in laboratory testing methods and interpretation of results. Newer biomarkers such as direct metabolites of ethanolappear to be better than traditional biomarkers.However, they are still under research, development and commercially unavailable. Meanwhile, the parallel development of more sensitive and specific scales with both screening and diagnostic value across spectrum of alcohol use disorders is also good news for clinicians. In South Asian countries, until the use of newer biomarkers becomes clinically feasible, combining both self-report (based on information from patients and their informants) and clinically meaningful interpretation of available biomarkers, preferably in combination, should be encouraged during routine clinical practice for optimal evaluation of patients based on the clinical setting.Meanwhile, clinicians also need to familiarize themselves to use alcohol biomarkers as additional outcome measures in clinical interventionsfor AUDs and associated medical complications.

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