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Review Article

Public Health Section

Biomarker Signatures to Monitor Alcohol Consumption and Induced Organ Damage

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ABSTRACT

The difficulty to differentiate long duration alcoholic behaviours is a major obstacle in the diagnosis and its treatment. Biomarkers in alcoholism are indicative of recent alcohol consumption or alcohol-induced organ damage. They are broadly divided into two; state markers, which are tools indicative of acute or chronic alcohol consumption, and trait markers, which are markers indicative of a genetic predisposition responsible to develop alcohol dependence. This review aimed to sensitise the practitioners on different alcohol state markers available now-a-days. An electronic search in Google Scholar, MEDLINE, and PubMed was conducted by using following keywords: Alcohol biomarkers, State markers, Trait markers, Alcohol consumption test. Studies on alcohol biomarkers published in English language were included in this review. Reviews and studies with free access to only abstract have been excluded. The state markers mostly used to identify chronic alcohol exposure are the Gamma-Glutamyltransferase (GGT), Aspartate and Alanine Aminotransferase (AST and ALT) which are routine serum liver function panels and Mean Corpuscular Volume (MCV) which is a haematological marker. The available non-conventional state biomarkers are Phosphatidylethanol (PEth), Fatty Acid Ethyl Esters (FAEE) and 5-Hydroxytryptophol (5-HTOL). The novel state markers which have been developed in recent research context are still awaiting validation and possible introduction to commercial settings are Plasma Sialic Acid Index of Apolipoprotein J (SIJ), Total Serum Sialic Acid (TSA), Acetaldehyde, Acetaldehyde adducts, anti-adduct antibodies and β-Hexosaminidase. Conventional alcohol biomarkers are routinely used in clinical practice. Non-conventional biomarkers seem to be promising for its estimation. Novel biomarkers are at various stages of research and development.

Keywords: Alcohol consumption test, State markers, Trait markers

INTRODUCTION

The difficulty to differentiate between the long-duration drinking behaviours is an important challenge in the treatment of alcoholism, both in its diagnosis and prognosis [1]. Hence it is important to establish the development of specific diagnostic tools in the identification of patients with excessive drinking patterns and to evaluate the degree of abstinence. Researches in this area identified and proposed the use of several clinical tools as efficient alcohol biomarkers of alcoholism with direct reflection to the proportion of individual alcohol intake [2].

Biomarkers in alcoholism are indicative of recent alcohol consumption or alcohol-induced organ damage. They are grouped into state markers and trait markers. State markers give the clinicians an objective understanding about the recent (acute or chronic) alcoholic behaviour and trait markers about the genetic predisposition to develop dependence on alcohol after chronic exposure. State markers are both direct and indirect biochemical tools which helps the practitioner in analysing the degree of alcohol abuse by estimating the levels of alcohol consumption, metabolic analysis, and by evaluating extent of the development of alcohol-induced organ damage. Direct biomarkers are based on ethanol metabolism or its products in the body. Acute or chronic alcoholism induce changes in enzymes or cells in response to alcohol consumption. They are the indirect biomarkers [3]. These have several applications such as screening tools, diagnostic tools and for the diagnosis of pre-symptomatic individuals [4].

This systemic review was conducted to sensitise the practitioners on different alcohol state markers available. The findings are presented as conventional state markers, non-conventional state markers and the compounds that can serve as novel state markers.

LITERATURE SEARCH

The literature search was performed in the MEDLINE and Google Scholar database for the direct and indirect alcohol state biomarkers, covering the period 1960 to 2019.

Inclusion criteria: The studies that fall under alcoholism, biomarkers and state markers were included in the study. Full length articles (original articles and review papers) published in English language were only included in the review. The inclusion criteria based on Population, Intervention, Comparison and Outcomes (PICOS) were: alcohol abuse; biomarkers; articles with a parallel design; relative uses of alcohol biomarkers in diagnosis and prognosis outcomes; full text articles in English.

Exclusion criteria: The articles with irrelevant titles, contents, duplicates and in-vitro studies were excluded from this review. Case control and cohort studies were excluded from the study. Reviews and studies with free access to only abstract were also excluded.

DISCUSSION

The biochemical state markers are grouped into conventional, non-conventional and novel markers [1,3], based on their usefulness, diagnostic characters as significant biomarkers for alcoholism and also on the degree emergence, prevalence and validation in the clinical set-up on a global index.

This classification is based on the regularity of these tests, traditionally used or not and feasibility of these markers in clinical use. This is explained as follows:

- The conventional state markers include routine laboratory biochemical tests which directly points to the chronic alcohol consumption and hence, considered as the gold standards.
- Non-conventional state markers are indirect biochemical investigations that help the practitioner in analysing the degree of alcohol abuse by estimating the levels of alcohol consumption, metabolic analysis, and by evaluating extent of the development of alcohol-induced organ damage.
- Novel state markers are yet to be commercially available, but a few of them are quite promising. The cost of equipment required for its detection and also the cost per test is high and this delays the use of these investigations in routine clinical use.

Conventional State Markers

Detecting the presence of ethanol in breath and various body fluids like serum and urine is considered as the gold standard and known to be the direct indicators of alcohol consumption. But as the time window for the ethanol detection in the body is too short, these detection methods are valid only for very recent alcohol consumption. As a result, other direct and indirect methods are used in the routine clinical setup for alcohol detection [5,6]. These includes conventional biochemical liver function tests such as Gamma-Glutamyltransferase (GGT) and Aspartate and Alanine Aminotransferase (AST and ALT) in serum which indicate non-specific liver dysfunction [7,8] and the mean corpuscular volume of erythrocytes in blood (MCV) [Table/Fig-1] [9].

Conventional markers		Sensitivity	Specificity	Test accuracy	Time between consumption and reliable test result
Ethanol [5]		70%	98%	98%	6-12 hrs
GGT [5]		37-95%	18-93%	44%	<2 to 3 wk
AST [5]		25-60%	47-68%	44%	<1 to 4 wk
ALT [5]		15-40%	50-57%	44%	<1 to 4 wk
MCV [5]		40-50%	80-90%	44%	<1 to 4 mnth
CDT [5]		46-90%	70-100%	77%	<1 to 2 wk
EtG [5]/ EtS [5]	In blood	89%	100%	70-85%	<45 min to 36 hrs
	In urine	89%	99%	70-85%	<60 min to 5 days
	In hair	75%	96%	70-85%	1 wk to several months

[Table/Fig-1]: Conventional markers.
GGT: Gamma-glutamyltransferase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; MCV: Mean corpuscular volume; CDT: Carbohydrate-deficient transferrin; EtG: Ethyl glucuronide; EtS: Ethyl sulphate

Ethanol: Although the time window of ethanol detection in the breath and body fluids is too narrow, the relatively simple and cost-effective method of the detection procedure makes it more reliable for field applications like in hospital emergency situations, in traffic control purposes and in various workplace settings. Ethanol detection can be done by simple hand-held breath analysers for expelled breath and dip-stick method for saliva and other body fluids. As the traces of ethanol rapidly eliminates from the body, the detection is reliable only for very recent alcohol intake [5,6]. These drawbacks made the necessity to establish stable methods for the detection of alcohol, and since then, more and more tools which directly and indirectly mark the alcohol existence is being developed until now. 5-hydroxytryptophol or ethyl glucuronide are examples for such stable compounds with comparably larger time window and provides more sensitive methods to detect recent drinking [7].

Gamma-Glutamyltransferase (GGT): GGT is the most important and sensitive liver enzyme clinically used for the detection of general liver and bile duct problems. Its normal value in the blood ranges from 8-38 IU/L, which gets elevated when any obstruction occurs in any of the bile ducts. Bile duct carries bile from the liver to the intestines. Any interference in the bile duct passage caused by tumors or stones or by either alcohol induced or non-specific liver damage, GGT levels elevates. The increase in rise is proportional to the magnitude of damage. Although the enzyme particularly indicating the general liver damages, other illnesses like digestive system diseases (pancreatitis) and prostate diseases too causing an increase in its levels, thus affecting its specificity and sensitivity towards alcohol induced liver damage, i.e., sensitivity only ranges from 30-50% in a general population [8].

Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT): The enzyme AST, formerly known as serum glutamic-oxaloacetic transaminase (SGOT) is primarily present in its higher concentrations in liver, and in various other tissues like muscles, heart, kidney, brain, and red blood cells in lower amounts. AST levels increase six hours after tissue damage. The normal values of the enzyme are 10-40 IU/L for men and 9-32 IU/L for women. Just like GGT an elevated value indicates a higher than normal amount of this enzyme and which in turn designate liver injury [8,9].

The ALT, formerly known as serum glutamic-pyruvic transaminase (SGPT), is also a liver enzyme with a critical role in the metabolism and functioning of liver cells. When any damage or inflammation is occurred in the liver tissue, this enzyme gets released into the bloodstream and serum ALT levels gets elevated over its normal ranges (which is 29-33 IU/L for men and 19-25 IU/L for women). Measuring the ALT values in serum plays an important part in the screening for liver disease [10].

Out of these two enzymes, ALT is considered as more specific marker for alcohol-induced liver damages because it is found mainly in the liver, whereas AST is also found in various organs other than liver such as heart, muscle, kidney, brain, etc. However, when in comparison with GGT, these tests are having a major restraint of lesser sensitivity and lesser accuracy for ages under 30 or over 70 [9,10].

Mean Corpuscular Volume (MCV): This test measures the average size of the Red Blood Cells (RBC). Elevated values for MCV (macrocytosis) can be the result of excess alcohol intake, as well as associated dietary deficiency or impaired absorption. As the half-life of the RBCs is about 120 days, a high MCV level continued in the bloodstream for several months even after a person stops drinking. As a result, it is considered as an effective biomarker of excessive alcohol use, like GGT [11]. MCV is routinely estimated by automated hematology analysers and normal reference range is 80-95 fL. However, specificity of the test is low when in comparison with GGT [11].

Carbohydrate-Deficient Transferrin (CDT): CDT is a kind of glycoprotein transferrin. Transferrin is a blood plasma protein which carries iron to the bone marrow, liver, and spleen and contains four to six sialic acid molecules in its structure [12]. Excess alcohol intake disrupts the attachment between transferrin to sialic acid as well as to other molecules. Heavy drinking correlates with proportionate increases in CDT. This increased levels in the bloodstream is an efficient biomarker of alcohol [13,14]. The reference value indicative of heavy drinking is >1.7%. But relatively higher rate of false negatives are there. Combining both CDT and GGT tests, shows an increase in sensitivity [15].

Ethyl Glucuronide (EtG) and Ethyl Sulphate (EtS): Ethyl glucuronide (EtG) and Ethyl Sulfate (EtS) are breakdown products of ethanol and are predominantly found in urine. Apart from urine they are also present in blood, hair and other body tissues in relatively lower levels [16]. After heavy alcohol intake, EtG persists in blood for 36 hours and in urine for 3-5 days, and the EtS is present in urine for up to 16-27 hours, which is longer than that of ethanol. Thus, EtG is an effective marker for detecting drinking relapses. EtG measurements in hair have also demonstrated as a relatively sensitive and specific marker of excessive alcohol intake. The reference range of EtG is 25-100 ng/mL [17].

Non-conventional State Markers

These tests are newly introduced and less frequently used to monitor alcohol related distress. These indirect biochemical investigations help the practitioner to analyse the degree of alcohol abuse by estimating the levels of alcohol consumption, metabolic analysis, and by evaluating extent of the development of alcohol-induced organ damage. Fatty Acid Ethyl Esters (FAEE), Phosphatidylethanol (PEth) and 5-Hydroxytryptophol (5-HTOL) are the most popular non-conventional biomarkers [Table/Fig-2] [18,19].

Fatty Acid Ethyl Esters (FAEE): FAEE is one of the breakdown products of alcohol and found normally in the liver, pancreas, and adipose tissues up to 24 hours after alcohol consumption. As FAEE cannot be excreted, FAEE gets accumulated in the hair over the

Non-conventional markers	Sensitivity	Specificity	Test accuracy	Time between consumption and reliable test result
FAEE in hair [5]	90-97%	75-90%	98%	24 hrs in serum Up to 2 yrs in hair
PEth [5]	88-100%	48-89%	99%	<1 wk to 1 month
5-HTOL [19]	50-78%	82-97%	88%	5 to 24 hrs

[Table/Fig-2]: Non-conventional markers.
FAEE: Fatty acid ethyl esters; PEth: Phosphatidylethanol; 5-HTOL: 5-Hydroxytryptophol

extent of excessive drinking [20]. It's long-lasting presence in the human hair for up to several years (10 months to 2 years) makes the FAEE hair testing as one of the crucial breakthroughs in the biomarker testing panel for long-term alcohol consumption and in evaluating abstinence. So the differences in the range of values of FAEE helps the practitioner to distinguish between occasional, social and heavy drinkers with a more specific and sensitive approach [20,21]. As the reference range of detection limits between 0.005 and 0.009 ng/mg, the test is considered as the most sensitive biomarker above all the other conventional and non-conventional markers of alcoholism. When comparing the FAEE and EtG hair tests together, FAEE provides a better output for the alcohol detection without being hit by the false positive interferences of ethanol traces from hair cosmetics, as this cannot be differentiated in EtG [22].

Phosphatidylethanol (PEth): PEth are group of phospholipids formed by the action of phospholipase D on ethanol. Since the PEth is having a detection window greater than that of ethanol, its estimation in blood is considered as a definitive biomarker of chronic excessive alcohol abuse over 3-4 weeks [22]. Blood sample collected by single finger prick is enough for the test measurement either by High-Performance Liquid Chromatography (HPLC) or by Liquid Chromatography Tandem Mass Spectrometry (LCMS-MS). The lower limit of detection using the HPLC is 0.25 µmol/L and by the LCMS-MS in µmol/L is 0.005. Since minute amount of sample collected by the single prick method cannot detect the small traces of ethanol present in the entire bloodstream, PEth is considered to be suitable for a detection scenario with minimum ethanol intake of 1000 g ethanol in three weeks, with a daily consumption of at least 50 g. This less sensitivity towards the small amounts of ethanol makes this method a less sensitive when compared with EtG and EtS [22].

5-Hydroxytryptophol (5-HTOL): 5-HTOL is a serotonin metabolite, produced in the body as the result of heavy consumption of alcohol. The ethanol together with acetaldehyde (which is a breakdown product of alcohol) alters the serotonin metabolism resulting in the production of 5-HTOL. Rise in the 5-HTOL levels is directly proportional to the amount of alcohol consumed, which can be detected in urine for approximately up to 5-15 hrs after alcohol consumption by the Gas Chromatography-Mass Spectrometry (GC-MS) or Liquid Chromatography and Mass Spectrometry (LC-MS) techniques [23]. Thus it is considered as a 24-hour biomarker of alcoholism and can be well utilised in forensic toxicology.

Novel State Markers under Research and Validation

Despite of the existing biomarker tools explained in the previous sections, there are several newly emerging tools (markers) for detection and assessment of alcohol intake and evaluating the alcohol abuse. These newly emerging biomarkers are at various stages of research and development [Table/Fig-3]. These investigations are yet to be commercially available, but a few of them appear promising [19].

Total Serum Sialic Acid (TSA): With the well-known fact that the alcohol consumption directly correlates with the sialic acid contents in the body, various researches now reveals that, alcohol-dependent heavy drinkers (both men and women) had developed

Novel markers under research	Sensitivity	Specificity	Test accuracy	Time between consumption and reliable test result
TSA [19]	37-95%	70-85%	High	<1 to 2 wk
Acetaldehyde [1]	91%	100%	High	3 hrs to 3 wk
Acetaldehyde Adducts [1]	91%	100%	High	1 to 3 wk
Anti-Adduct Antibodies [1]	91%	100%	High	1 wk to 120 days
β-Hexosaminidase [19]	84-98%	84-98 %	86%	1 to 4 wk
Cholesteryl ester transfer protein [1]	8-50%	82%	High	<1 to 4 wk
Circulating cytokines [1]	32-50%	96.1%	High	3 hrs to 1 mnth

[Table/Fig-3]: Novel state markers.

remarkably high levels of TSA than that of social drinkers. When differentiation analysis is carried out for both TSA and CDT, the sensitivity and specificity of this test is found to be almost similar. Since the TSA levels has longer time window than CDT and GGT, and takes a remarkable time to diminish during abstinence, the TSA test might not be suitable for relapse measurement in treatment and rehabilitation purposes [19,24].

Plasma Sialic Acid Index of Apolipoprotein J (SIJ): Apolipoprotein J also known as clusterin is a component of plasma high-density lipoproteins. Apolipoprotein J is structurally similar to transferrin molecule in a way that it contains sialic acid in it, and this sialic acid content (Apo J Sialic acid) provides a tool in alcohol detection and treatment. After alcohol consumption, there is a fall in the levels of Apo J Sialic acid. Since the Apolipoprotein J molecule contains four times more sialic acid chains than that of transferrin molecule, the test is revealed to be more sensitive than CDT. Test employs an Automated HPLC System for the procedure [24].

Acetaldehyde, Acetaldehyde Adducts, and Anti-Adduct Antibodies: Acetaldehyde is one of the first set of compounds produced by the ethanol oxidation. So as the alcohol is being consumed, free acetaldehyde levels in the body elevates, which lasts for a time window of 3 hours to 3 weeks. Acetaldehyde reacts with hemoglobin produces Hemoglobin-bound Acetaldehyde (HAA) which in turn gets accumulated in the red blood cells. Intake of even a single high dose of alcohol (2 g/kg) may cause its accumulation, whereas MCV and GGT show no sensitivity at these minute concentrations. Detection methods for both free and bound acetaldehydes is being developed and the whole blood-associated acetaldehyde assay (WBAA) sets an example [22,24]. WBAA seem to be an extremely sensitive, specific, and precise marker of alcoholism, both in its diagnosis and prognosis [24].

β-Hexosaminidase: β-HEX is a lysosomal exoglycosidase which undergoes a remarkable change in its activity after excessive alcohol consumption (for more than 60 gm/day for 10 consecutive days), and as a result of this increased activity, an elevated level of β-HEX is occurred in the body fluids, especially in urine. β-HEX is considered as a biomarker of chronic alcohol intake. The major advantage of the test is that it employs an inexpensive kit method which is also easy to perform [1,24]. However, the method has a disadvantage of resulting in a false positive elevation of enzyme values, due to various liver disorders, diabetes, hypertension, pregnancy, infections, and use of certain medications thus affecting its specificity [1].

Cholesteryl Ester Transfer Protein (CETP): Cholesteryl ester transfer protein (CETP) is a plasma protein, which facilitates the redistribution of cholesteryl esters, triacylglycerols, and phospholipids in the plasma [1,24]. An increased alcohol intake affects the protein activity and thus lower the level of CTEP in the blood. These lowered levels inversely affect the cholesterol HDL levels. So, the comparing values of CTEP with that of conventional markers like MCV, GGT, AST, and ALT is of greater value in confirming its specificity [1].

Circulating cytokines: Circulating cytokines are proteins which play a critical role in both innate and adaptive immune response systems and controls body's altogether communication. It is known that alcohol consumption can affect and influence the immune system of an individual [1]. This notion of general awareness accelerates the chance or choice of applying the use of circulating cytokines as biological marker for alcohol abuse [1,25]. Studies revealed that most of them could be used as perfect tool for alcohol detection. Most promising among them includes tumour necrosis factor-α (TNF-α), interleukin (IL-1α, IL-1β, IL-6, IL-8, IL-12), and monocyte chemo attractant protein-1 (MCP-1). In alcohol dependent heavy drinkers, the levels of TNF-α, IL-1, and IL-6 is shown to be elevated [25]. But surprisingly, in abstainers even with alcohol induced liver cirrhosis, there is no difference in cytokine levels observed. This may indicate the possibility of using circulating cytokines as a potential marker for acute alcohol consumption [25,26].

Use of Alcohol State Bio-Markers in Clinical Practice

A study from Visakhapatnam showed that abstinence brought the AST/ALT levels to normalcy [1,25]. Percentage CDT as a biomarker of alcohol abuse was demonstrated in Chennai and it was found superior to GGT in terms of sensitivity and specificity [26,27]. Increased transaminase activity with AST greater than ALT, elevated MCV, GGT, and IgA/IgG ratio was studied and demonstrated as markers of chronic alcohol liver diseases. Also, the advantage of using ApoB and LDL/HDL ratio, AST, GGT to distinguish between alcohol dependent heavy drinkers from non-dependants was reported [26,28]. A case control study on 'the sensitivity and specificity of serum sialic acid as a biochemical marker in alcohol abuse' concluded that sialic acid can be used as a biochemical marker of alcoholism, where the comorbidities and the onset of secondary liver diseases challenge the use of conventional biomarkers [29]. An review from Kerala proves that a comparative assessment of conventional biomarkers (GGT, ALP, AST, ALT and MCV) provides an effective tool for assessing the severity of alcohol induced liver damage [30].

As a result of extensive research, there is a lot progression in the field of alcohol biomarker discovery, which successfully stimulated the development of numerous potential alcohol biomarkers. However, among all these panels of biomarker tools, very few of them are experimentally verified and validated for clinical utilisation. Also there still lies a huge need for relatively inexpensive methods of alcohol detection which should help the practitioners in their routine diagnostics procedures [30]. The requirement for costly equipment and expensive methods of detection limits the clinical application of some potential biomarkers like ethanol and serotonin metabolites, sialic acids, despite of their good diagnostic characteristics.

CONCLUSION(S)

The search for an ideal biomarker of alcoholism still continues. Conventional alcohol biomarkers such as ethanol, GGT, ALT, AST, CDT and MCV are known to be well-validated and routinely used in the clinical firm for the detection of acute/chronic excessive alcohol consumption and also indicatesliver dysfunction. Numerous potential alcohol biomarkers with good diagnostic characteristics have been discovered, but few are validated. Non-conventional biomarkers such as FAEE, PEth and 5-HTOL provide indirect ways to estimate the amounts of alcohol consumed. In this list PEth seems to be a promising biomarker for the alcoholism but the requirement of costly equipment necessary for their measurement acts as a potential hurdle for its clinical utilisation. Several noval markers such as β-HEX, acetaldehyde, acetaldehyde adduct, CETP, circulating cytokines are there for the biomarker assessment of alcohol intake and alcohol abuse. These investigations are yet to be commercially available, but a few of them like sialic acid transferrin and WBBA appear promising.

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