Alcoholism: Common and Oxidative Damage Biomarkers

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Abstract

For many years it has been assumed that moderate alcohol intake might provide health benefits to humans, while alcohol abuse generally produces some systemic complications, even when physical and mental dependence on alcohol has not developed. Many studies have focused on distinct molecules that might serve as useful early or late biomarkers in individuals that consume important amounts of alcohol. Indeed, the diversity of molecules postulated as biomarkers is quite wide and this is the direct reflection of the broad range of mechanisms involved in the impact that alcohol has on human health, either as a result of acute or chronic intake, the latter being a cause death. In this review, we have considered most of the molecules that have been followed-up in individuals who drink large amounts of alcohol, as well as some of those that have been less intensely studied, and we present a pair of oxidative biomarkers that could be used to evaluate early stage alcoholism.

Keywords: Alcoholism; Oxidative damage; Biomarkers

Introduction

Alcoholism represents one of the most serious socioeconomic and health problems worldwide. The American Medical Association defines alcoholism as “an illness characterized by significant impairment that is directly associated with persistent and excessive intake of alcohol”, which also results in the deterioration of social behavior and/or a physical illness, with the concomitant development of other adverse effects. Children and adolescents are more vulnerable to the harmful effects of alcohol than adults, although it is considered that this may reflect the fact that they have no context or reference point to regulate their drinking and they are building their own range of tolerance [1].

The important difference between alcohol abuse and alcoholism has often been highlighted. Accordingly, alcoholism indicates a physical and mental dependence on alcohol, which leads to regular or periodic, heavy and uncontrolled alcohol consumption [2]. By contrast, the individual who is considered to abuse alcohol has experienced severe sequels of their habit in the past year, and they may develop alcoholic liver disease and its systemic complications without developing the physical and mental dependence on alcohol [3]. Lamentably, in alcohol-dependent individuals most pathological processes precede the onset of clinical symptoms and unfortunately, many individuals with a history of chronic alcohol abuse die before reaching the clinical stage. However, if the problems provoked by alcohol are recognized at an early stage, a physician may be able to prevent their further development and progression. Therefore, it is essential to characterize the early events leading to alcoholic diseases and to define markers that may aid in the implementation of early preventive measures [4]. In addition, it is important to first identify the markers of long-term alcoholism (either early or late) that can be detected before medical symptoms appear.

In this review, we aim to consider the molecules more commonly associated with these alcohol induced changes, as well as some of those less commonly considered. In addition, we focus on a pair of oxidative molecules that could be used as markers of the early stage of alcoholism, in general gaining a global idea of the potential early and late biomarkers of alcohol consumption.

Alcohol metabolism

Alcohol is generally accepted to be a compound toxic to cells or tissues that is readily metabolized to acetaldehyde by alcohol dehydrogenase (ADH). A variety of ADH isoforms have been identified, present largely in the liver, and they require a vitamin-related co-factor, nicotinamide adenine dinucleotide (NAD+), to accept reducing equivalents (hydrogen atoms and electrons) from the alcohol. Circulating levels of acetaldehyde are low under normal conditions and much of the acetaldehyde produced by the oxidation of alcohol is in turn oxidized in the liver to acetate. This leaves the liver and circulates to peripheral tissues where it is converted to a key Acetyl CoA.

In the liver, ethanol may also be metabolized through two additional pathways, that involving cytochrome P450 2E1 (CYP2E1) or catalase [5]. CYP2E1 plays an important role in ethanol oxidation in the brain (Figure 1).
Alcohol elimination rate

The Alcohol Elimination Rate (AER) is a measure that is conditioned by several factors, some of which are associated with the characteristics of the individual while others are more specifically modulated by the environment. As indicated above, ADH is the most important enzyme in alcohol metabolism and it directly influences the AER. With respect to the age of individuals, studies realized on animal models have indicated that the sub-maximal expression of ADH in young animals leads to a low AER [11]. Whereas the AER is thought to be higher in individuals that express the beta3 class I ADH isoform than in those who express the beta1 isoform. Moreover, alcohol metabolism is higher in the fed nutritional state than in the fasted state, presumably due to the higher ADH levels and the elevated capacity of substrate shuttle mechanisms to transport reducing equivalents into the mitochondria. Indeed, food intake may increase liver blood flow and it is known that the sugar fructose enhances alcohol metabolism by providing substrates that help to convert NADH to NAD⁺, as well as by enhancing mitochondrial oxygen uptake. The increased AER associated with food was similar for meals with different compositions, and there appears to be no difference between carbohydrate, fat and protein on the rate of alcohol metabolism [11].

However, given that first pass metabolism occurs in the stomach, it is possible that intake of a similar dose of alcohol may produce a higher blood ethanol concentration in females than males. Conversely, men and women exhibit a similar AER when this rate is calculated in terms of gr/hr or gr/dm³ liver volume. Moreover, liver size may at least partially explain the ethnic and gender differences in AERs [12].

Biochemical parameters and biomarkers

Despite efforts to study the molecules that could serve as early markers in individuals with an important consumption of alcohol, none have yet been identified. Nevertheless, much information has been generated regarding the biochemical and physiological parameters that are modified at these stages. For such, molecules to be considered and used as biomarkers, ideally they should display high sensitivity and specificity, discriminating between safe and hazardous drinking. Importantly, such markers should not be elevated by non-alcohol-induced organ damage and they should be non-invasive [13].

Blood, urine or breath alcohol analyses provide information as to whether the patient has alcohol in their system, yet such approaches provide no information as to the severity of alcohol abuse [14]. Moreover, the detection time is limited to hours until the alcohol consumed is metabolized to acetaldehyde. At present, no single value can differentiate between chronic and acute abuse [15].

Below, we shall consider what are thought to be the most common potential biomarkers, as well as other non-conventional and oxidative biomarkers that could be used to determine chronic and sub-chronic exposure to ethanol (Table 1).
Conventional Biomarkers

Biomarkers have mainly been used to screen patients for possible problems of alcohol use and the conventional biomarkers considered to date will be discussed here (Figure 2).

![Figure 2: Conventional Biomarkers of Alcohol: Ethylenglycol (EtG), carbohydrate deficient transferrine (CDT), mean erythrocyte corpuscular volume (MCV), aspartate transferase (AST), alanine transferase (ALT) and Gamma-glutamyl transferase (GGT).](image)

Blood ethanol

Blood ethanol is a widely accepted marker for recent ethanol consumption. Levels exceeding 1.5% with no evidence of intoxication, or of 3% at any time, are indicative of the ethanol tolerance typically found in alcohol abusers and alcohol-dependent patients. This parameter is useful for emergency clinics, although the rapid elimination of ethanol from the blood nearly always makes it impossible to assess anything other than recent ethanol ingestion in this way: within 6–8 h [16].

γ-Glutamyl transferase (GGT)

Gamma-glutamyl transferase (GGT) is a glycoprotein found at the membrane of cells in several tissues. This enzyme catalyzes the first step in the degradation of GSH, and it plays important roles in glutathione homeostasis and mercapturic acid metabolism. In individuals that consume excessive alcohol, the release of GGT from the cell membrane may be enhanced [17,18] and indeed, serum-GGT has been widely used as an indicator of liver dysfunction and a marker of alcohol intake [19]. Thus, GGT represents a sensitive and inexpensive, age-dependent marker, although its specificity is diminished by obesity, diabetes, non-alcoholic liver disease, pancreatitis, hyperlipidemia, cardiac insufficiency, severe trauma, medication, nephrotic syndrome and renal rejection [17].

Mean corpuscular volume

Red blood cell size (MCV, mean corpuscular volume) is often used in screening procedures to detect alcohol abuse [18]. An increased MCV follows chronic heavy drinking and it is correlated with both the amount and frequency of alcohol ingestion. However, it may take up to a month of drinking more than 60 g of alcohol daily to raise the MCV above the reference range [14,16]. The principal mechanism by which alcohol causes an increase in the MCV may be through a direct toxic effect of alcohol on erythrocytes and through folic acid deficiency rather than hepatic damage [20].

Formation of Carbohydrate-Deficient transferrin (CDT)

Transferrin is a liver protein that participates in iron transport and that is synthesized and secreted by the liver, with a half-life of 7–10 days. This marker is a desialylated transferrin variant that appears in the serum [21]. Alcohol abuse hampers the glycosylation of this protein such that they have significantly higher amounts of carbohydrate deficient forms of transferrin. A rise in CDT occurs after a daily consumption of 60–80 g of alcohol for at least one week and this can be normalized slowly during abstinence [22]. Serum CDT levels may be influenced by other elements unrelated to alcohol use like anorexia nervosa and pregnancy, and indeed, false positives have been associated with genetic factors, iron levels, age and liver disease [23].

Transaminases

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are enzymes that transform α-keto acids into amino acids. ALT is mainly present in hepatic tissue, while AST (also known as serum glutamic oxaloacetic transaminase) is found predominantly in the liver but it is also found in considerable amounts in other tissues like heart and muscle. The ratio of AST to ALT in serum may aid the diagnosis of some liver diseases and while in most patients with acute liver injury the ratio is 1 or less, in alcoholic hepatitis it is generally about 2. Predictably, the sensitivity of both these enzymes in the context of alcohol abuse is low and varies greatly [24], such that these enzymes are apparently not good biomarkers.

HDL

Alcohol intake may raise plasma high density lipoprotein (HDL) levels, either by altering the synthesis of HDL or by affecting the enzymes and proteins that influence HDL metabolism. In addition, alcohol may also alter HDL levels by increasing the plasma concentrations of apolipoproteins AI and AII, the main components of HDL [25]. Since HDL may increase as a result of drinking relatively low amounts of alcohol (≤5 drinks per day), it has been used to detect early phase drinking problems in individuals, as well as in the follow-up of patients with no significant liver injury. In lipid profiles, alcohol abusers also frequently show increased concentrations of serum triglycerides and free fatty acid ethyl ester following recent bouts of heavy drinking [26].

Acetaldehyde

Acetaldehyde is a product of oxidative ethanol metabolism and since it is a reactive molecule, forming Schiff bases with amines, it readily binds to proteins, leading to an irreversible reaction that give rise to an acetaldehyde-protein adduct. The concentration of acetaldehyde after alcohol intake is highly variable, with a lifetime of approximately 3 h. Two approaches have been adopted to detect acetaldehyde as a marker of alcohol intake. The first involves acetaldehyde detection in its free form, or reversibly bound to plasma proteins or blood cells. This acetaldehyde is liberated from the blood and it can be measured by gas or liquid chromatography [27]. The second approach involves the use of an immunoassay to detect...
epitopes derived from acetaldehyde on proteins in the plasma. Another option is to directly measure the levels of anti-adduct IgAs as biomarkers of alcohol intake. This approach may reflect the close relationship between marker levels and the actual amounts of recent ethanol ingestion. Indeed, the specificity 88-94% and sensitivity of 65-73% confirm the potential of this approach [28].

**Ethyl glucuronide (EtG)**

Ethyl glucuronide (EtG) is a non-volatile, water-soluble, direct metabolite of ethanol. EtG forms in the liver when alcohol reacts with glucuronic acid, a substance that participates in the detoxification of drugs by turning them into water-soluble compounds that can be easily removed from the body [29]. EtG is only detected if alcohol is consumed and it remains present in urine well after the disappearance of ethanol itself. EtG appears to be a highly sensitive, specific and reliable marker of recent alcohol intake (up to 5 days). Moreover, EtG has also been shown to be present in other tissues and in hair, which may have additional diagnostic implications [18]. EtG has a half-life of approximately 2-3 hours [30], and it can be measured by GC/MS, LM/MS, LC/MS-MS and ELISA. However, all these techniques are currently rather laborious and technically demanding for routine laboratories.

**Non-Conventional Biomarkers**

Non-conventional biomarkers enable alcohol consumption to be assessed in a chronic and sub-chronic setting. Some characteristics of these non-conventional biomarkers can be seen schematically in Figure 3.

![Figure 3: Non-Conventional Biomarkers of Alcohol: Hexosamidase, phosphatidylenol (Peth), 5-Hydroxytryptophol (5HTOL), Fatty acid ethyl ester (FAEE).](image)

**Phosphatidylethanol (Peth)**

Phosphatidylethanol Peth is also a specific metabolite of ethanol that is synthesized by phospholipase D in the presence of ethanol. Therefore, the diagnostic specificity of Peth as a biomarker of alcohol is theoretically 100%. However, this marker only reacts to a single dose greater than 60 g of alcohol and thus, single doses of 32–47 g alcohol will not be detected by this marker [31]. The half-life of Peth is about 4 days and Peth may remain in circulation for more than 2 weeks, possibly being detected for up to 3 weeks in heavy consumers. This marker is sensitive to storage and to avoid in vitro formation of Peth in blood, samples must be frozen at –80 °C [32].

**5-Hydroxytryptophol**

The serotonin metabolite 5-Hydroxytryptophol (5-HTOL) is a normal constituent of urine and after alcohol consumption, there is a marked increase in 5-HTOL probably due to inhibition of ALDH or the increase in the NAD/NADH ratio. Indeed, the concentration of the normal serotonin metabolite 5-hydroxytryptophol-3-acetic acid (5-HIAA) decreases correspondingly [24]. This marker seems to have a high diagnostic accuracy to detect recent alcohol consumption and for monitoring relapses. Accordingly, the 5-HTOL:5-HIAA ratio was found to have 100% sensitivity 4 h after consumption of a moderate dose of ethanol, although the reliability of this marker decreases fairly rapidly after 7 h [33,34]. Moreover, the HPLC-based methods currently used to determine the 5-HTOL:5-HIAA ratio are difficult to translate into routine clinical practice.

**Serum β-hexosaminidase**

β-Hexosaminidase (HEX) is a lysosomal enzyme that is involved in the metabolism of carbohydrates and gangliosides in the liver. In both serum and urine, β-HEX has long been known to be a very sensitive biomarker for chronic alcohol use. Its activity in serum reflects excessive alcohol consumption (more than 60 g of alcohol daily for more than 6.5 days [35]. Moreover, one of the major potential strengths of β-HEX is that it can be measured using standard and inexpensive laboratory techniques (spectrophotometry and fluorimetry). However, elevated serum β-HEX also occurs in patients with hypertension, diabetes, cirrhosis, myocardial infarction, in pregnancy and after oral contraceptive use. Nevertheless, β-HEX may be a useful marker to discriminate between alcoholics and abstemious individuals or social drinkers [36].

**Fatty acid ethyl esters (FAEEs)**

Fatty acid ethyl ester (FAEE) synthases catalyze the reaction between ethanol and a fatty acid to produce a fatty acyl ethyl ester. FAEEs may be toxic, inhibiting DNA and protein synthesis, and high concentrations can be detected in organs commonly damaged by alcohol abuse, in particular the pancreas and liver. In serum, FAEE levels remain elevated for up to 12–24 hours after alcohol consumption [37], although the number of false positive cases represents one of the disadvantages of FAEE as a biomarker. For example, such false negatives may be produced in hair specimens as a result of the indistinctive or daily use of alcohol based lotions or sprays. The principal methods to analyze FAEEs are laborious and time-consuming, including gas chromatography-mass spectrometry, which may prevent its routine use in clinical laboratories [38].

**Oxidative Biomarkers**

Alcohol consumption is associated with a number of changes in cell function and in the oxidant-antioxidant system. The liver is known to be the major site of ethanol oxidation and in the first oxidation step ethanol is metabolized to acetaldehyde mainly through oxidation catalyzed by ADH. Subsequently, this acetaldehyde is rapidly oxidized to acetate by liver acetaldehyde dehydrogenase [39].
Alcohol is also metabolized in non-liver tissues, such as by CYP2E1 and catalase enzyme in the brain. This causes oxidative damage by augmenting the levels of reactive oxygen species (ROS) [40], and through reduced glutathione and superoxide dismutase activity [41].

In addition, the metabolism of alcohol increases the levels of NADH, which provides electrons for the mitochondrial electron transport chain (ETC), leading to increased one-electron reduction of oxygen to superoxide [42]. Furthermore, formation of acetaldehyde by alcohol has been shown to cause mitochondrial damage, which may also lead to increased formation of superoxide within the ETC [43]. Finally, ethanol metabolism is not only involved directly in the production of ROS but also, it has been associated with establishing a setting favorable to oxidative stress (e.g., hypoxia and endotoxaemia: Figure 4) [44].

Figure 4: Oxidative Biomarkers of Alcohol. MDA, HNE, GSH/GSSG and acetaldehyde could represent relevant biomarkers, which are produced by oxidative ethanol metabolism.

Reduced glutathione (GSH)

The reduced glutathione (GSH) tripeptide is involved in many cellular functions and it plays a central role in antioxidant defense, especially in the liver. Chronic ethanol consumption selectively depletes GSH in the mitochondria due to the defective activity of this carrier, probably mediated by decreased fluidity of the mitochondrial inner membrane [54]. The endogenous glutathione-glutathione peroxidase system and catalase are important antioxidants, and they form part of the cytoprotective machinery in the hepatocyte mitochondria that is activated on exposure to ethanol [55]. Glutathione reductase and catalase activities increase on exposure to ethanol, and chronic alcoholism induces alterations in the GSH/GSSG ratio that might enhance the oxidative modification of liver mitochondrial proteins [56].

Catalase

Catalase is found in the cytosol and mitochondria, although it is mainly active in peroxisomes. This enzyme is very efficient in promoting the conversion of hydrogen peroxide to water and molecular oxygen. Catalase is capable of oxidizing ethanol in vitro in the presence of a hydrogen peroxide (H$_2$O$_2$)-generating system, such as the NADPH-oxidase enzyme complex or the enzyme xanthine. This is considered a minor pathway of alcohol oxidation, except in the fasting conditions [57]. Chronic alcohol consumption by rats results in increased H$_2$O$_2$ production in pericentral regions of the liver lobule and increased catalase activity [58]. Moreover, several studies have suggested that catalase may be involved in the metabolism of alcohol to acetaldehyde in the brain [46], which could in turn mediate some of the behavioral effects of alcohol.

Lipid peroxidation

Lipid peroxidation is a complex process whereby polyunsaturated fatty acids in the phospholipids of cellular membranes undergo a reaction with oxygen to yield lipid hydroperoxides (LOOH) [59]. This process results in the formation of compounds known as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE), both of which can form adducts with proteins [60]. MDA concentrations in alcoholics with or without cirrhosis are higher than in matched healthy controls [61] and therefore, MDA is considered as an excellent biomarker of lipid peroxidation.
bimarker to reveal and monitor oxidative stress triggered by alcohol consumption [62]. As mentioned earlier, ethanol metabolism by CYP2E1 and NADH oxidation by the ETC generate ROS, which drive lipid peroxidation. Lipid peroxidation products can be generated from the attack of ROS and iron on lipids or by CYP2E1 activity [63]. An increase in lipid peroxidation products has been detected in individuals with a 4-5 year history of alcohol drinking [10].

DNA damage

Chronic ethanol consumption leads to an increase in ROS and acetaldehyde, which have both been shown to induce DNA damage, including oxidative modifications, adducts and cross-linking [64]. 7,8-dihydro-8-oxo-2′-deoxyguanosine as the most representative product of modified DNA, while the primary acetaldehyde-derived DNA adduct is N-2-ethylidene-deoxyguanosine, which may be converted to N 2-ethyldeoxyguanosine (N 2-ethyl-dG)in vivo, a potential biomarker of acetaldehyde induced DNA damage in human alcoholics [65].

Endogenous DNA damage is constantly being produced and repaired in normal conditions, reflecting a low-steady state level of damage that is compatible with normal cellular function. Indeed, many studies have quantified the five basic types of DNA repair [66]. However, in conditions when DNA repair is deficient, endogenous damage is not repaired and accumulates over time, resulting in a variety of symptoms or diseases, such as liver cirrhosis in chronic stages or alcohol related neuronal loss in the brain. In the case of alcohol drinkers, it was assumed that oxidative process could only be observed with long-term and high doses of alcohol intake, however, excess alcohol consumption produces deleterious effects on many tissues in the body, even if this excess occurs in a short time window, such as a week. In this sense, we recently published a study where we observed biomarkers of oxidative damage in a group of young people who have been drinking alcohol for a relatively short period of time (4-5 years) [10].

Conclusions

In this review, we have focused on most of molecules that have been used as markers to identify individuals that consume an important amount of alcohol, each of which is are involved in some manner in the development of diverse signs and pathological processes that have an impact on the health of these individuals. However, the majority of work carried out both in vitro and in a clinical setting has concentrated on high consumption over long periods. Fewer studies have assessed biomarkers of early detection that could be present in individuals considered to be "social drinkers" or those that consume a moderate quantity of alcohol every so often. We believe that important changes exist in the cells of drinkers even within a short period after the onset of alcohol consumption, ergo, before any symptoms of organ or tissue damage can be perceived. Indeed, we reported that biomarkers that reflect oxidative damage and that were validated long ago for the determination of chronic stages of alcoholism could also be suitable to be used at earlier stages of consumption. Nevertheless, more studies should be conducted to identify and validate the use of these or other biomarkers in the early stages of alcoholism, which will have important benefits in clinical practice and with the goal of preventing a major deterioration of health. Finally, it is important to note that most studies analyzing molecules or biomarkers have been carried out on adults. However, we think that is important to realize such analysis in young people, due to the period of consumption and the future establishment of behaviors. More research is necessary to explore novel biomarkers or combinations of biomarkers known to reflect drinking patterns with high sensitivity and specificity.

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