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Biomarkers in the treatment of alcohol use disorders

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Abstract

Several biochemical measurements to objectively assess patients' current or past alcohol use are available. The blood tests used traditionally as markers of excessive drinking are the liver enzymes, gamma glutamyltransferase (GGT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and the red blood cell volume (mean corpuscular volume; MCV). However none of these currently available biomarkers are ideal. Several more experimental markers hold promise for measuring acute alcohol consumption and relapse. These include certain alcohol byproducts such as acetaldehyde, ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEE) as well as two measures of sialic acid, a carbohydrate that appears to be altered in alcoholics. Some progress has been made in finding markers that predict people's genetic predisposition to alcoholism such as genetic differences in several neurotransmitters including beta endorphin and gamma aminobutryic acid (GABA).

Chisty SJS, Das D. Biomarkers in the treatment of alcohol use disorders. Dysphrenia. 2012;3(1):21-31.

Keywords: Alcohol drinking. State markers. Trait markers.

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Received on 28 December 2011. Accepted on 30 December 2011.

Introduction

Alcoholism represents a serious health issue with major socioeconomic consequences. Physicians are likely to identify only 20-50% of patients with alcoholism who are attending for medical care.[1,2] The diagnosis is often based on the patient's self-reporting of alcohol consumption which is unreliable[3,4] and requires a high degree of clinical suspicion. To treat people with alcoholism adequately, clinicians need tools that can properly assess not only the extent of the patients' recent and heavy past drinking activity but also any family history of drinking problems (i.e. genetic predisposition to alcohol abuse and alcoholism) they may have. Biochemical substances in the body that can indicate the presence or progression of a condition or any genetic predisposition towards it are called biomarkers. There are two kinds of biomarkers: state markers and trait markers. State markers indicate people with recent drinking patterns including whether they have a history of heavy drinking or recent binge or even just a few drinks. Trait markers reveal something about a person's inheritance of abusing alcohol.

State markers

Currently used state markers

For many years clinicians have had access to a group of biomarkers that indicate a person's alcohol intake. Several of these reflect the activity of certain liver enzymes: serum gamma glutamyltransferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and carbohydrate deficient transferrin (CDT), a protein that has received much attention in recent years. Another marker beta hexosaminidase (β Hex) indicates that liver cells as well as other cells have been breaking down carbohydrates which are found in great numbers in alcohol.[5] Clinicians also have used red blood cell volume, known as mean corpuscular volume (MCV), as a biomarker of alcohol intake.

Gamma glutamyltransferase

Brief description and history

GGT is one of the longest established biochemical tests for excessive alcohol consumption.[6] GGT is a glycoprotein enzyme situated on the cell membrane in several tissues. It is possibly involved in reabsorption of glutathione from the glomerular filtrate and in protection against oxidative stress via maintenance of intracellular glutathione levels.[7] Clinically it has been used as a measure of liver function or damage but it is also found in the kidney, brain, spleen, pancreas and heart.[8] This is one reason why increases in GGT are not specific for excessive alcohol consumption.

Association with alcohol consumption

Serum levels of GGT rise in response to alcohol consumption to a variable extent. The response varies between individuals and within individuals according to the phase in their drinking history. GGT levels typically correlate only moderately with alcohol consumption (r=0.30-0.40 in men, 0.15-0.30 in women)[9] and there is some unpredictability about which drinkers will respond to excessive drinking with an elevation in GGT. GGT

does not respond to a single dose of alcohol unless the person has previously been an excessive drinker.[10] GGT levels respond to even low levels of habitual drinking[9] but generally sustained excessive drinking is needed to raise a significant proportion of drinkers' levels above laboratory reference ranges. In experiments with volunteers, 60g ethanol daily for three weeks produced no more than a 15% increase in enzyme levels[11] while five weeks produced almost a doubling of mean levels in young volunteers from 27U/l to 52U/l.[12] Regular drinking is more likely to increase levels than episodic drinking[13] and intensity of drinking (i.e. number of drinks per drinking day) appears to be important. GGT increases more rapidly with resumption of alcohol consumption in those with a history of excessive drinking and particularly if there has been a past raised GGT.[14] While GGT typically begins to fall within the first week of cessation of excessive drinking, the rate of decrease is variable, particularly in the presence of background hepatic impairment.

Screening

GGT is limited as a tool in screening by its relatively poor sensitivity. Only 30-50%[15,16] of excessive drinkers in the general community or family practice settings has elevated levels although sometimes the proportion is less than ten percent.[17,18] In these settings specificity varies from 40% up to nearly 90%. Sensitivity is similar in psychiatric inpatients (36%, specificity 87%).[19] In residential alcoholism treatment units, sensitivity may be high (50-90%) with reasonable specificity (65-90%)[20-24] but in this setting a screening test is not needed because the sensitivity and specificity of GGT vary by setting and performance may be enhanced when alcohol dependent 'cases' are compared against light- or nondrinking controls. A wide range of reference for GGT has been used to define an abnormal test ranging from zero to 35U/l to zero to 80U/l.

Prognostic value

In addition to detecting current pathology, GGT levels have been reported to be predictive of future morbidity and mortality. Pregnant women who have elevated GGT levels are more likely to deliver a baby with foetal alcohol syndrome[24] although sensitivity in predicting this condition is only 50% (for a specificity of 80%) in pregnant women drinking more than 100g of alcohol per week.[25] It is not yet well understood why GGT acts as a marker and predictor of nonhepatic complications of alcohol use. It is possible that it is because it reflects a pattern of drinking that is more regular, higher quantity and/or intensity and of longer duration. This theory is supported by the fact that raised GGT levels also predict social consequence of drinking[24,26] including drunk driving.[26] Alternatively it has been proposed that a raised GGT may be a marker for susceptibility to the physical complications of drinking.

Monitoring treatment success

GGT is used regularly both clinically and in research[27,28] to monitor response to treatment. Typically a reduction in GGT levels will be apparent from the first week of stopping drinking and will be marked by the end of the first month.[29] The early reduction can help confirm a diagnosis of excessive drinking. GGT levels typically fall halfway towards normal over five to 17 days of abstinence.[30] The fall towards normal takes longer in dependent drinkers with a reported half life of 26 days.[31] In those with hepatic damage (particularly cirrhosis) it may be still longer (range 11-54 days)[32] or may be incomplete. Even when a drinker has a GGT within the reference range at baseline, following the individual's changes in levels may be useful.[28,33] GGT levels are likely to increase 20-30% above the baseline in dependent drinkers who relapse (e.g. 50g+ ethanol on two+ consecutive days).[28,34,35] Nondrinking controls had no significant increase in GGT. The rise in GGT with resumption of heavy drinking in dependent people is by no means universal. In a recent US study of 344 dependent drinkers in treatment, a rise in GGT of 30% above baseline occured in only 29-32% of patients who relapsed (two days or more of drinking five or more drinks per day) and rose by 30% without evidence of a relapse in eight to 11% of patients.[28]

Factors that affect results

GGT levels tend to increase with age up to age 65 years independent of alcohol consumption and levels may then fall.[9] In parallel with this, GGT has been found to be of limited value in those aged less than 30 years even when they have alcohol dependence.[36] GGT becomes a more sensitive marker of alcohol use with increasing age but has been reported to perform less well in people aged over 60 in some[37,38] but not all studies.[39] GGT levels may be raised above the reference range in persons with obesity even in nondrinkers. Body mass index or better the waist-hip ratio has been reported to be a better predictor of GGT than alcohol.[40] Obesity and alcohol have additive harmful effects on the liver so this is an important clinical finding not just a false positive test result.[41,42] It is difficult to separate potential effects of race from influences of varying prevalence of viral illnesses and environmental and cultural influences. Nonetheless the prevalence of elevated GGT and aminotransferase levels has been found to be higher in South Asian excessive drinkers,[43,44] in those of African[45,46] or Mexican[45] descent and in Brazilian people.[47] A wide range of medications affect GGT particularly those that induce the microsomal enzymes. Any hepatic or biliary

condition may affect GGT including hepatic congestion in cardiac failure. Disorders of the other body sites where GGT is found can also affect levels (e.g. diabetes and pancreatitis).

The aminotransferases

Aspartate aminotransferase and alanine aminotransferase

Brief description and history

AST (previously known as SGOT, serum glutamicoxaloacetic transaminase) and ALT (also known as SGPT, serum glutamicpyruvic transaminase) are sensitive indicators of liver cell injury.[48] They are hepatocellular enzymes involved in amino acid metabolism. ALT is found predominantly in the cytosol whereas AST activity is highest in the mitochondria. While present in the greatest concentration in the liver, AST is also present in heart, muscle, kidney, brain, pancreas, lung, leucocytes and erythrocytes. Because of this, it has limited specificity for alcohol use. Because ALT is found predominantly in the liver it is affected less by nonhepatic insults.[48]

Association with alcohol consumption

Like GGT, aminotransferases are not increased by a single episode of excessive drinking.[49] In eight young healthy male volunteers, consumption of 60g ethanol per day for five weeks produced only slight rises in aminotransferases from the baseline and none were elevated above 30U/l. Probably a longer duration of drinking (and/or increased age of volunteers) would be more likely to result in increased levels. While AST values correlate highly with GGT values (r=0.61-0.68), they do not correlate as highly with alcohol consumption (r=0.24-0.34).[47]

Screening

The aminotransferases are less sensitive than GGT in detection of excessive alcohol consumption. Typically less than half the subjects entering an alcoholism treatment unit have aminotransferases above the reference range and in some samples the prevalence of positive results may be very low (e.g. 3/114).[50]

Prognostic value

As with GGT elevation, AST levels have been found to be predictive of morbidity. In an Australian study, AST results above the 80th percentile (32U/l) for an emergency department population were predictive of development of liver disease, gastrointestinal bleeding or trauma over the ensuing three-year period.[51] This association was independent of alcohol intake, age and sex.

Monitoring treatment progress

An increase of 40% or more in AST level and 20% or more in ALT value has been reported to be suggestive of relapse to drinking in alcohol dependent men (sensitivity and specificity >90% for AST, ≥80% for ALT).[35] This was true even if the marker remained within the reference range.

Factors which affect aminotransferase levels

As with GGT, the aminotransferases are relatively insensitive to alcohol use in those aged less than 30 years.[37,40] They may also be insensitive in elderly drinkers (>70 years).[38] Like GGT, the aminotransferases can increase with obesity or weight gain[40,52,53] and obesity may have a stronger correlation with ALT than alcohol.[40] As with GGT, aminotransferase levels have been reported to be higher in South Asian[43,44] and in Brazilian[47] excessive drinkers and in those of African[45,46] or Mexican[45] descent but it is difficult to be sure if this is due to genetic or environmental influences. Almost any medication can raise aminotransferase levels including antibiotics, antiepileptics, statins and nonsteroidal antiinflammatory agents.[48,54] Factors affecting muscle (e.g. strenuous exercise, muscle disorders) can also affect AST levels.[48] In a study of 2240 elderly subjects' coffee consumption was inversely associated with ALT levels and was a better predictor of ALT than was drinking of alcohol.[55] Coffee consumption is also inversely associated with AST.[56]

Serum mitochondrial aspartate aminotransferase

Serum AST consists of two isoenzymes: mitochondrial AST (mAST) and cytosolic AST (cAST). In serum samples of normal healthy individuals cAST makes up >90% of the total activity but when excessive alcohol consumption selectively injures mitochondria in the liver, mAST is preferentially released. It is measured by an immunochemical technique in which specific antibodies precipitate cAST, leaving mAST to be measured using standard AST methods. A sensitivity of approximately 90% in alcoholic patients has been reported although the specificity is low (in one study 79% of patients with nonalcoholic liver disease had elevated mAST). The specificity was improved to 82% by using the mAST:total AST (mAST:tAST) ratio (cut-off seven percent) but with the consequence of lower sensitivity (range 52-100%). The mAST:tAST ratio returns to normal levels within a few weeks of abstinence. Like most other markers, both mAST and the mAST:tAST ratio have lower sensitivity (30-40%) in screening for hazardous drinking in the community and primary care. The mAST:tAST ratio can be particularly useful in a liver unit in helping to distinguish alcohol induced from nonalcohol related liver damage.[57]

Mean corpuscular volume

Brief description and history

The mean volume of the red blood cell (MCV) has been recognised for many years as increasing with excessive alcohol consumption.[58] In alcohol excess, the majority of cases of macrocytosis occurs in the presence of normal folate levels[58,59] and without anaemia and do not respond to folate treatment.[58] The cause of macrocytosis is complex. Ethanol appears to have a direct marrow toxic effect causing reduced marrow cellularity and vacuolisation of red cell precursors similar to that seen in choramphenicol toxicity.[60] In 30% of dependent drinkers with increased MCV there will be some reduction in folate levels.[58] This may be due to dietary deficiency, impaired absorption or increased excretion[60,61] but in only 17% of cases of alcoholic cirrhosis will there be actual folate deficiency (serum folate <2.5ng/ml).[59] Ethanol also appears to have a specific antifolate action.[60] A variety of other red cell changes may occur in association with alcohol dependence such as sideroblastic anaemia and particularly in alcoholic cirrhosis, occurence of spur cells and stomatocytes.[50,58,60] MCV levels may also become elevated with liver disease of any cause due to altered synthesis or increased destruction (haemolysis) of red cells in the congested spleen.[59] Furthermore occult bleeding is common in alcohol dependence. Either haemolysis or bleeding results in increased numbers of young red cells (reticulocytes) with larger cell volumes.

Association with alcohol consumption

As the life span of a red blood cell is 120 days, it may take several months for changes in drinking to be reflected in MCV levels.[62] Sustained and regular excessive drinking appears to be needed to result in elevated MCV levels in the absence of folate deficiency, liver disease or bleeding. There are no experimental studies demonstrating an increase in MCV with administration of alcohol in healthy volunteers. Regularity of drinking is important. Meerkerk *et al*.[13] demonstrated that no irregular excessive drinkers (60g+ per occasion) in a family practice setting had increased MCV while 33% of those drinking 20 times or more per month did. In alcohol dependence, MCV levels may continue to rise upon ceassation of drinking.[29] This may be due in part to increased numbers of reticulocytes as the marrow begins to recover.[60]

Screening

MCV has limited value as a single marker in screening because of its poor sensitivity typically below 50%. In one general practice setting MCV detected less than 20% of excessive drinkers. On the other hand MCV is more specific than GGT in most populations. In Meerkerk *et al*.'s[13] general practice study MCV had specificities of more than 90%. In medical inpatients sensitivity tends to be higher but specificity lower (sensitivities of 52-75% for specificities of 85-74%).[63,64] Despite these limitations MCV may be the best of the traditional markers in screening for excessive drinking in women.

Prognostic value

MCV has been found to be significantly higher in women who have miscarriages than matched controls[65] and is highly specific but poorly sensitive in predicting occurence of foetal alcohol syndrome.[66,67] It is hard to ascertain whether these effects are independent of the effects explained by alcohol consumption.

Monitoring treatment progress

Because of its slow response to changes in drinking, MCV is generally unsuitable as a marker of short term progress.[29,68] It has been proposed that MCV can be useful in reflecting earlier drinking. However in the first week of treatment there can be alterations to MCV. Interestingly in some cases of cirrhosis, MCV may begin to fall even after one week's abstinence[59] perhaps pointing to improvement in more rapidly reversible factors such as red cell destruction. In other cases MCV may rise in the first week as erythropoeisis increases.[60]

Factors which affect performance

As with the liver enzymes, MCV may have a poor sensitivity in those aged less than 30.[69] It becomes more sensitive with increasing age throughout most of adulthood[39,40] although may be of limited value in detecting excessive drinking in the elderly. In one study of 162 medical inpatients aged 65-99 years, MCV detected less than 20% of excessive drinkers.[70] In contrast in another study of medical inpatients and elderly people living in the community, sensitivity and specificity in elderly patients were both in the 60s.[38] The reason for these differences is not clear. Several authors have reported MCV to be more sensitive in women than in men.[40,71]

Serum beta hexosaminidase

β Hex is an acid lysosomal glycosidase. Increased serum and urine levels have been reported in alcoholic patients and in healthy volunteers after consumption of >60g of alcohol per day for at least ten days with sensitivities of 70-90%; this is better than GGT and other established markers. However like CDT, β Hex appears not to be as effective in identifying less excessive but still harmful levels of drinking in unselected populations. In alcoholics β Hex levels fall rapidly (seven to ten days) to normal following abstinence. The β Hex isoform in particular is highly indicative of alcohol abuse. Although high specificities (approximately 90%) have been reported

for β Hex, serum levels of β Hex have been noted to be increased in hypertension, diabetes mellitus, cirrhosis, pregnancy, in users of the oral contraceptive pill, cerebral infarction and myocardial infarction. One of the major potential strengths of β Hex is that it can be measured using standard and inexpensive laboratory techniques (spectrophotometre and urometry). Thus serum β Hex is a sensitive, easily measured, inexpensive test for excessive alcohol consumption but like CDT it does not perform well in unselected populations; moreover there are conditions other than alcohol intake that may cause it to be elevated.[57]

Carbohydrate deficient transferrin

CDT is a version of the glycoprotein transferrin, a molecule responsible for carrying iron within the bloodstream. Many versions of transferrin normally are found in healthy people but studies indicate that heavy drinkers have higher amounts of the CDT version than nondrinkers.[72] Alcoholic subjects consuming 50-80g of alcohol per day for at least a week will show increased serum levels.[73] During abstinence CDT normalises with a half life of 15 days[73,74] and it thus remains elevated for several weeks. If drinking resumes, lower levels of alcohol intake can lead to a rapid re-elevation.[74] CDT has been widely used by clinicians in recent years to screen for heavy alcohol consumption. Although it appears to be a highly specific measure of alcohol consumption, showing low rates of false positives, CDT is difficult to measure accurately. Another disadvantage with the CDT marker is that there is a relatively high rate of false negative results: some patients who drink heavily do not also find that, in general, women tend to have higher CDT levels than men, regardless of their drinking history.[72] What causes this gender difference is not clear. False positives can occur with nonalcoholic liver disease (primary biliary cirrhosis, chronic active hepatitis, chronic hepatitis C and hepatocellular carcinoma); CDT is useless as a screening test for alcohol abuse; a recent meta analysis of 110 clinical studies showed it to be no better than GGT in this respect.[75] In an attempt to compensate for the low sensitivity the CDT:total transferrin ratio has been proposed as a better marker.[76] Despite the disadvantages of the CDT marker, it remains a very well characterised biomarker for heavy alcohol intake.

Combinations of markers

Attempts have been made to improve the sensitivity of single laboratory markers by combining them but although some of the combinations have shown enhanced sensitivity (e.g. CDT plus GGT, CDT plus MCV), none has been widely accepted. Sophisticated mathematical treatment of results from multiple laboratory tests has also been proposed but the large number of test parameters required makes the approach impractical and in any case increased sensitivity invariably decreases specificity. Use of two or three different established markers appears to be optimal.[57]

Newer state markers under study

Several new markers for assessing alcohol intake and alcohol abuse are at various stages of research and development including the plasma sialic acid index of apolipoprotein J (SIJ), total serum sialic acid (TSA), 5-hydroxytryptophol (5-HTOL) and various fatty acid ethyl esters (FAEEs). None of these tests are commercially available but some look promising as described in the following section.

Plasma sialic acid index of apolipoprotein J

Apolipoprotein J is a glycoprotein found in needed complexes (i.e. lipoproteins) that are responsible for transporting fats (i.e. lipids) in the blood. Research indicates that apolipoprotein J may help transfer fats such as cholesterol from one lipoprotein to another.[5] Like the molecule transferrin, apolipoprotein J contains sialic acid particles that may be reduced in number after alcohol consumption. Apolipoprotein J has more than four times sialic acid chains than transferrin, making it easier to measure changes in sialic acid content caused by heavy alcohol consumption. More study is needed but preliminary findings show promise for SIJ as a highly specific and easy-to-measure marker.

Total serum sialic acid

Because of sialic acid's clear potential as a highly specific marker for alcohol use, researchers have begun to study the potential of measuring total sialic acid levels in patients' blood rather than looking at the difference in sialic acid chains only on glycoproteins such as transferrin and apolipoprotein J. Early studies[5] demonstrate that, compared with social drinkers of both genders, both male and female alcoholics had elevated amounts of TSA. The test for TSA has similar sensitivity and specificity to the test for CDT for measuring alcohol consumption. However because TSA levels take longer than either CDT or GGT to decrease during periods of abstinence, the TSA test might not be as useful for treatment programs assessing patients for relapse.

5-hydroxytryptophol

Alcohol and its primary breakdown product, acetaldehyde, affect the metabolism of serotonin so that the body produces more 5-HTOL when people consume alcohol than when they do not drink. The body disposes of 5-HTOL via the urine where it can be detected for approximately five to 15 hours longer than standard alcohol measurements which can detect alcohol in the urine for a little over an hour for each drink consumed.[77] Because of its ability to detect people's alcohol use for up to 24 hours after they have been

drinking, 5-HTOL is considered a 24-hour biomarker for heavy alcohol consumption. Although the marker requires more study, preliminary work indicates that it is both sensitive and specific for detecting recent heavy alcohol consumption.[77] Testing for 5-HTOL may prove especially useful in forensic toxicology. Emergency room clinicians may find it helps detect people who consumed large amounts of alcohol before preparation for surgery and treatment professionals may be able to use this test to monitor the care of people involved in treatment maintenance medication disulfiram which people in these settings may be taking, also can lead to increased 5-HTOL levels. In addition research has shown that the ratio of 5-HTOL to another serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA) or 5-hydroxyindole-3-acetic acid, is a useful indication of previous drinking.[78]

Fatty acid ethyl esters

Along with acetaldehyde, the body also produces FAEE when it breaks down alcohol. FAEE is measured as a combination of four separate molecules and is found in the liver, pancreas and fat (i.e. adipose) tissues up to 24 hours after alcohol consumption. FAEE is a sensitive and specific marker for distinguishing social drinkers from heavy or alcohol dependent drinkers.[79,80] Because it also is found in human hair, some researchers suggest using FAEE in hair as a marker for chronic heavy alcohol consumption.[79] The body cannot flush FAEE out of hair, so the compound builds up over a long period of chronic drinking. FAEE measured in liver and adipose tissue also has been used as a postmortem marker of alcohol consumption.[80] Such a measure is needed because current measures such as blood alcohol levels can be artificially high as a result of alcohol formation in the body after death. So far FAEE looks promising. Preliminary studies[80] show that when measured in adipose tissue, FAEE is useful as a biomarker up to 12 hours after death in alcohol treated animals; when measured in animal liver tissue, FAEE is useful up to 24 hours after alcohol treatment. Further study is required to fully explore FAEE's sensitivity and specificity.

Ethyl glucuronide

Ethyl glucuronide (EtG) is another minor metabolite of alcohol that forms in the liver when alcohol reacts with glucuronic acid, a substance which works to detoxify drugs by turning them into water soluble compounds that can be easily removed from the body. EtG can be detected in the blood for up to 36 hours and in the urine for up to five days after heavy alcohol use. In addition to blood and urine, EtG is detectable in other body fluids, hair and body tissues[81] although no apparent correlation has been found between alcohol consumption and the presence of EtG in hair. When people test positive for EtG, it is likely that they consumed alcohol recently even if there is no alcohol left in their bodies. This makes EtG especially useful for detecting drinking relapses. Measuring EtG levels is difficult however. A rather sophisticated instrument, the mass spectrometre, is required for an accurate reading of EtG from urine. And so far attempts to produce a measure for urine based EtG using simpler techniques or to measure EtG in other body fluids or hair have yielded less than satisfactory results.[81]

Acetaldehyde

The first compound the body produces as it metabolises alcohol is acetaldehyde which exists on its own and also can bind to certain proteins including haemoglobin (a protein in red blood cells that carries oxygen). Researchers are able to measure concentrations of both free and bound acetaldehyde in blood samples using high performance liquid chromatography and fluorescence detection—known as the whole blood associated acetaldehyde assay (WBAA).[82] This assay is highly specific, extremely sensitive[83] and has excellent precision. The insurance testing industry has used WBAA for more than a decade to test for heavy alcohol consumption (Food and Drug Administration [FDA] approval for wider clinical use is pending). Its potential is even greater as a clinical tool to monitor people in alcoholism treatment programs because this test can provide a picture of alcohol use over time. This works because as a person continues to drink, haemoglobin bound acetaldehyde accumulates in red blood cells over their 120-day average life span and this buildup shows up as an increasing WBAA assay number. Levels of protein bound acetaldehyde remain high for approximately a month after alcohol consumption.[84] The ability of the WBAA assay to measure alcohol consumption patterns over time makes it unique among the biomarkers described here.

Salsolinol

This compound formed when the neurotransmitter dopamine reacts either with alcohol's byproduct acetaldehyde or with pyruvate (a metabolite of glucose that is used by cells for energy) shows some promise as a state marker for chronic alcohol consumption. However the usefulness of salsolinol may depend on how it is measured—whether for example in blood, urine or brain tissue. Salsolinol levels in urine have been found to decrease following acute alcohol consumption[85] and measuring salsolinol levels in the blood may provide a better indication of chronic alcohol consumption. A study by Haber and coworkers[85] showed that compared with nonalcoholics, alcoholics who have been abstinent for as little as one week have decreased salsolinol levels in lymphocytes. Studies of salsolinol levels in the brain in contrast found no difference in salsolinol levels between alcoholics and nonalcoholics.[86] This may indicate

problems of measuring salsolinol in the brain as well as inherent differences in salsolinol levels among different biological sources.

Proteomics

Researchers have begun to use proteomics, the systematic study of proteins that are matched to certain known genes, to search for biomarkers of alcohol consumption. Recently investigators used a powerful technique, surface enhanced laser desorption/ionisation–time of flight–mass spectrometry (SELDI-TOF-MS), to study serum samples from alcoholics who had consumed more than ten drinks a day for at least ten years.[87] The researchers examined the protein profile in the blood of these people upon admission to an alcoholism treatment program and again after abstinence—taking measures throughout the treatment program. They found measurable differences in the levels of two proteins, a fragment of the fibrinogen αE chain and a fragment of apoprotein A–II. Specifically patients had low levels of the proteins when they were drinking and significantly increased levels starting as soon as one week into the treatment programme. Nomura and colleagues[87] concluded that the two protein fragments have potential as markers of excessive alcohol consumption in heavy drinkers seeking treatment.

Trait markers

Biochemical markers are being developed to identify people with a genetic predisposition to alcohol abuse and alcoholism. Knowing who is at risk can help prevent alcohol problems altogether or enable a person to seek early treatment for developing problems or to experience better treatment outcomes. At a minimum, any useful trait marker should satisfy at least three criteria: it should be passed down from parents to children through the genes (i.e. be heritable), it should be associated with the disease in question in the general population and it should be independent of the status of the disease, meaning that it would be present whether the person displayed symptoms of the disease or was asymptomatic.[85] Biomarkers that meet these criteria and show low rates of false positive and false negative results will have excellent value in predicting the likelihood that a person will develop alcohol dependence. Much of the research in this area is preliminary but several markers including an enzyme and a group of neurotransmitters hint at its potential.

Adenylyl cyclase activity

A protein found in cell membranes, adenyl cyclase (AC) plays an important role in providing the cell with energy. Researchers became interested in AC activity as a potential trait marker when they discovered that AC activity is inherited and the enzyme is less active in the blood platelet cells of abstinent alcoholics than in nonalcoholics.[88] In addition AC activity increases when alcoholics begin drinking again suggesting that alcohol somehow stimulates AC activity. Unfortunately it appears that marijuana and other drug use also affect AC activity making it an imprecise marker for alcohol use specifically.[88] Researchers now are searching for possible differences between alcoholics and nonalcoholics in the structure of genes associated with AC activity.

Gamma aminobutyric acid

The neurotransmitter gamma aminobutyric acid (GABA) is a chemical that acts on special docking molecules (i.e. receptors) in brain cells (i.e. neurons) for the GABA molecule. These molecules enable charged chlorine ions to enter and exit the cell thus controlling the chemical balance of the cells. Studies[89] find that people have different levels of GABA and these differences are inherited. In addition studies show that people who are alcohol dependent have lower levels of GABA than do nonalcohol dependent people. Thus at least in these preliminary studies GABA fulfills two of the three requirements of a trait marker for alcoholism.

Dopamine

Another neurotransmitter dopamine acts at the receptor level and is believed to be involved in the brain's reward system. A recent study[89] found that male alcoholics who had been abstinent for seven years showed a lower level of dopamine receptor activity compared with nonalcoholic men whereas a previous study[90] demonstrated that alcoholics, after a withdrawal period of four to seven days, showed an elevated response to dopamine indicating elevated receptor activity. Other studies examining levels of the major byproduct of dopamine metabolism, homovanillic acid, also have had contradictory results. Some investigators[84] found higher levels of homovanillic acid in alcoholics compared with nonalcoholics but other researchers[91] found lower levels for alcoholics. Because of these conflicting baseline findings, dopamine is not considered a good candidate trait marker at this time.

Beta endorphin

The neurotransmitter beta endorphin is an opioid produced by the pituitary gland. It works to activate neurons' opiate receptors and is thought to produce natural pain relief and a feeling of exhilaration. Studies find that alcoholics have lower levels of beta endorphin than nonalcoholics and that children of alcoholics have fewer opioid receptors than children of nonalcoholics.[55,92,93] These findings indicate that differences in beta endorphin levels are both specific to alcoholism and inherited, fulfilling two of the three requirements for a trait marker of alcoholism. Researchers still need to do much more work to establish beta endorphin as a true trait marker.

Serotonin

Preliminary research indicates that the neurotransmitter serotonin or other biochemical associated with serotonin show potential as trait markers for alcoholism. One such biochemical—the amino acid tryptophan which influences how much serotonin the brain produces—may be decreased in people consuming excess alcohol.[87,94] Another line of research examines the activity of the serotonin transporter which controls how much serotonin is available to cells. Research finds natural differences among people in serotonin transporter activity in blood platelets and these differences appear to be inherited. In addition alcoholics who have been abstinent for extended periods of time show higher serotonin transporter activity than nonalcoholics as do children of alcoholics compared with children of nonalcoholics.[95] These findings indicate that serotonin transporter activity in blood platelets has potential as a trait marker for alcoholism.

Conclusion

The search for ideal biomarkers of alcohol consumption (state) and for the genetic predisposition toward alcohol dependence (trait) continues. Although the state markers currently in use have value, their limitations and weaknesses make it desirable to develop more sensitive and specific markers. Alcohol consumption patterns, like most human behaviour, are complex. Clinicians often need to detect patterns of drinking other than the chronic, heavy drinking detected by GGT, AST, ALT and CDT. For example, they may need to know whether a person has done any amount of drinking recently or what type of drinking has occurred (e.g. heavy or social drinking). Therefore finding new biomarkers that measure many different aspects of alcohol consumption will vastly increase the clinician's ability to detect and treat alcohol abuse and dependence. In addition such biomarkers will help provide more precise definitions of alcohol consumption and alcohol use disorders not only in the clinic but in research where these terms currently are defined less precisely such as number of drinks consumed over a certain period of time. Finally more research is necessary before clinically useful trait markers of genetic predisposition to alcohol dependence are fully developed. The markers first must be validated clinically by testing people before they develop alcoholism and waiting to see how well the marker predicts later behaviour. As researchers further develop the markers described here and discover more biomarkers, their work should greatly improve clinicians' ability to objectively assess alcohol consumption as well as genetic predisposition to alcohol use disorders.

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