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World J Gastroentero, 2001;7(3):297 - 302
World Journal of Gastroenterology
 ISSN 1007 - 9327

Reviews

Detection of alcoholic liver disease

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Subject headings liver diseases, alcoholic/diagnosis; biological markers

Gordon H. Detection of alcoholic liver disease. ***World J Gastroenterol***, 2001;7(3):297-302

INTRODUCTION

Alcohol has been used in society over centuries and all over the world for its mood lifting properties and taste. It is probably, however, the commonest drug of abuse world wide and unfortunately causes considerable morbidity, mortality and social disruption. In 1990 the cost to the USA was more than $100 billion and 100 000 lives[1].

The relationship between alcohol and mankind is well documented from the earliest times. Wine-making equipment was found in the remains of an early neolithic village in Northern Iran dated about 5 000 BC[2]. In the Bible Noah 'planted a vineyard: and he drank of the wine, and was drunken'[3].

Beer was first produced in Ancient Mesopotamia, Egypt and Greece. There is evidence that alcohol was used for religious purposes and recreationally in Egypt around 3 000. The Ancient Greeks worshipped the God of wine, Dionysius, and seem to have been the first to develop large-scale wine fermentation and production, with export to other countries. The Romans in turn worshipped Bacchus, their God of wine, and were significant wine producers, planting vineyards across Europe. It is not just wine production that has survived through the ages. Barley provided both bread and beer from the first agricultural communities, but mead, made from fermented honey, was the preferred choice for most of Western Europe until Tudor times. Beer, brewed with hops, was introduced later from Germany.

Alcohol has long been an accepted part of human daily life, and throughout the centuries there has been evidence that, for both men and women, consumption gradually increased. This is due, at least in part, to the boiling of the water in the brewing process. An alcoholic beverage was wellknown as an adjunct in the treatment of cholera. For example, in the seventeenth century the water supply for Nottingham, Great Britain, came from the sewage laden river Leen, but there was a plentiful supply of ale houses (about one for every eighty people) thus ensuring some relatively clean fluid to drink[4].

However, there are long-term side effects and problems with excessive drinking. In 1726 the Royal College of Physicians in England issued a statement to the House of Commons asking for an increase in taxes on spirits to act as a disincentive to this 'great and growing evil'[5]. Concern for the effects of alcohol misuse continued and at the start of the twentieth century the British Prime Minister, Lloyd George, addressed factory workers during the first World War with 'we are fighting Germany, Austria, and drink; and, as far as I can see, the greatest of these three deadly foes is drink'[6]. As a result licensing laws were introduced in Britain limiting the hours during which alcohol could be served, and these are currently still in place[7].

Immoderate alcohol consumption may result in a broad spectrum of medical, psychiatric and social problems. These in turn are an expensive burden on any health service and society at large. However alcohol is also widely available and enjoyable so there have been attempts to identify levels of drinking at which alcohol related damage occurs.

LEVELS OF ALCOHOL RELATED DAMAGE

Harmful drinking is alcohol consumption that is causing actual physical or psychological harm[8]. Alcohol dependency refers to those individuals who 'have a compulsion to drink...the same amount each day...and suffer withdrawal symptoms on stopping' (Diagnostic and Statistical Manual of Mental Disorders, DSM III)[9]. This is the most obvious immediately attributable disorder directly related to alcohol misuse.

Regular excessive alcohol consumption is known to cause a wide range of diseases and disorders. Alcohol permeates every system in the body as it is water soluble. Every system in the body is therefore liable to alcohol induced damage, and the spectrum of deaths attributed to alcohol misuse reflects this. The commonest causes of death in the general population in the UK are cardiovascular causes (44.5%), cancer (28.6%) and then accidents (12.6%). These remain the three commonest causes for alcohol misusers, but accidents constitute a much larger proportion (44.1%) in this group[10]. The overall mortality in

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Received 2001-03-20 **Accepted** 2001-04-15

individuals misusing alcohol is three and a half times that for the general population.

Alcoholic liver disease forms the largest component of gastrointestinal causes of alcohol related mortality. The first histologically identifiable change seen in alcoholic liver disease is fatty liver. It is usually asymptomatic and can develop within days of heavy drinking[1]. Histologically, droplets of triglyceride can be seen within the hepatocytes. This can progress to alcoholic hepatitis, severe fibrosis and finally cirrhosis.

The level of drinking that constitutes misuse and results in alcohol-related damage was first studied in France. The risk of developing significant liver injury increases with increasing alcohol intake above the threshold levels[11]. It has been suggested that the threshold level for developing liver injury, with or without cirrhosis, may be as low as 30 g per day of alcohol and that with increasing alcohol intake there is increasing risk[12]. Alternatively a study looking at a series of 400 male autopsies showed no significant features of alcoholic liver disease in those who it was estimated had drunk less than 40 g per day. Those drinking 40 g-80 g per day had an increased incidence of fatty liver and alcoholic hepatitis, while those drinking more than 80 g per day had an increased incidence of liver cirrhosis. The threshold for liver damage was seen to be 60 g per day, or 49 units per week in this study[13]. Cirrhosis may develop after only a minimal alcohol intake over a short period of time, or, despite drinking considerable amounts over a life time, never develop. Only 20% of chronic alcohol misusers progress to cirrhosis and the reasons for this have been postulated to be a combination of genetic and environmental factors, and are the subject of continued research.

One of the difficulties in establishing the level of alcohol intake required for liver damage to occur is that despite the common usage of alcohol, exact quantification of the amount drunk on an individual or population basis is difficult to estimate. In the UK, information on alcohol consumption comes from two main sources: Customs and Excise data and population surveys. Customs and Excise data have some confounding factors: they do not include 'home brew.

Population surveys consistently produce lower figures than the Excise data. The Office of Population Censuses and Surveys (OPCS) survey in 1987 estimated alcohol consumption to be 4.2 litres absolute alcohol per head per year, while Excise data estimated this at 7.4 litres per head per year[14]. Population surveys are difficult because, by their nature, they are based on several elements of subjectivity. There is a significant non-response rate which could be amongst the higher or problem drinkers. In general, individuals tend to underestimate their consumption to within, or close to, 'sensible limits'.

The UK Department of Health has suggested that 'sensible limits' for drinking are 21 units per week for men and 14 units per week for women. A unit contains 8g absolute alcohol and is approximately half a pint of beer, a glass of wine or a single measure of spirits. In December 1995 they changed this to 3-4 per day for men and 2-3 for women, in an attempt to reduce binge drinking.

The Royal Colleges of Physicians and Psychiatrists have collaborated to give indications of harmful levels of alcohol intake, and to demonstrate the relationship between alcohol intake and physical harm (Table 1). It was agreed that the threshold for definitely harmful drinking is 50 units per week for men and 35 units per week for women, while that for heavy or hazardous drinking is >35 units per week for men and >25 units per week for women[5].

Table 1 The royal college of physicians (UK) advice on 'safe' and at risk drinking[5]

	Men(units per week)	Women(units per week)
Low risk	21	14
Hazardous	22-49	15-35
Harmful	50^{+}	36^{+}

The problems related to alcohol misuse are preventable. There is a need for an effective screening method for the early detection of alcohol misuse so as to provide support services and then the monitoring of progress. To do this effectively there is a need for objective markers of alcohol misuse.

DETECTION OF ALCOHOL MISUSE

The early detection of alcohol misuse is vital, so that the physical and psychological damage can be limited and reversed where possible. Once those drinking at misuse levels have been identified they need to be monitored through treatment. The Royal College of Physicians (UK) recommend that 'Every person seen in general practice or in hospital should be asked about his or her alcohol intake as a matter of routine, along with questions about smoking and medication, and the answers recorded'[5].

HISTORY AND QUESTIONNAIRES

The history is the most important means for detecting alcohol misuse[15]. The history should cover current and past alcohol intake, and identify quantity and frequency of intake. Unfortunately, although self-report has been shown to be reliable and reproducible, it is subjective, and often is, intentionally or unintentionally, an underestimate.

Questionnaires have been used to try to improve

the identification of alcohol misuse. The CAGE questionnaire[16] consists of four questions; two or more positive answers warrant further investigation:

1. Have you ever felt that you should Cut down your drinking
2. Have people Annoyed you by criticising your drinking
3. Have you ever felt Guilty about your drinking
4. Have you ever had a drink first thing in the morning to steady your nerves or get rid of a hangover (an Eye opener).

This tends to be too sensitive, having a high false positive rate but, if combined with self-report, can detect 90% of alcohol misusers[17]. The MAST (Michigan Alcohol Screening Test) is best at detecting alcohol misusers who have had complications and has been modified to the MmMAST (Malmo modified Michigan Alcoholism Screening Test)[18]. The AUDIT (Alcohol Use Disorders Identification Test) was developed by the WHO collaborative group and was designed to detect early heavy drinking. It consists of ten questions to be used in primary care[19] and has been shown to have a sensitivity of 92% and specificity of 94% in detecting harmful or hazardous drinking[20]. The CAGE, MmMAST and AUDIT were compared in Occupational Health and detoxification clinic settings and compared with self-report for alcohol intake[21]. Over all the sensitivities for the CAGE and MmMAST were 100% and for the AUDIT was 91% amongst the alcohol-misusers, but the AUDIT had the best performance in the Occupational Health setting.

CLINICAL SIGNS

There are a number of cutaneous signs of chronic alcohol misuse but these may be found in those with no significant liver disease. They include spider naevi, telangiectasiae, palmar erythema, gynaecomastia and Dupuytren's contracture. The mechanisms by which these develop are unknown, and with the exception of Dupuytren's contractures, they may all regress with abstinence. There may be truncal obesity mimicking Cushing's Syndrome and parotid enlargement.

In the withdrawal phase of alcohol misuse there may well be sweating, tremor and tachycardia, all of which are difficult to distinguish from thyrotoxicosis.

BIOLOGICAL MARKERS OF ALCOHOL MISUSE

These are particularly valuable to screen or confirm a suspicion of alcohol misuse as they are objective, are useful serially for monitoring, and may be helpful in motivating the patient. These markers can be divided into those indicating recent alcohol drinking, and those of chronic alcohol misuse.

Markers of recent alcohol consumption

Ethanol is the most obvious confirmation of recent drinking and can be detected in breath, serum or urine. It is useful to validate self-report, if positive. Alcohol is eliminated at the rate of $1g \cdot kg^{-1} \cdot hr^{-1}$, usually in 4-6 hours from breath and blood, and within 8 hours from urine, although the elimination rate is affected by the chronicity of alcohol misuse. Most patients know this and adapt their habits so that their clinical value is limited.

Methanol is present in the body and in small amounts in alcoholic beverages as a congener. Both ethanol and methanol are metabolised via alcohol dehydrogenase. However alcohol dehydrogenase has a much higher affinity for ethanol, so this is preferentially metabolised. The level of methanol therefore accumulates during ethanol metabolism and does not start to fall until ethanol has been removed. In practice this is at least 2-6 hours after ethanol has ceased to be detectable[22]. It can be detected in blood or urine.

Serotonin metabolites The urinary metabolites of serotonin (5-hydroxytryptamine) are 5-hydroxytryptaphol (5-HTOL) and 5 hydroxy indole acetic acid (5-HIAA). These are natural substrates. Normally 5-hydroxytryptamine (5-HT) is metabolised predominantly to 5-HIAA by aldehyde dehydrogenase, but a small amount is metabolised to 5-HTOL by alcohol dehydrogenase. However after alcohol ingestion, alcohol is metabolised to acetaldehyde, which than inhibits aldehyde dehydrogenase. Therefore there is a shift towards 5-HTOL and an increase in the 5-HTOL:5-HIAA ratio. The increase is dose-dependent and can be detected 5-15 hours after the ethanol has been eliminated. In urine, methanol and serotonin metabolites can be detected up to 18 hours after drinking, long after the ethanol is cleared[22]. The sensitivity and specificity of the 5HTOL:5HIAA ratio is proportional to the alcohol intake above 200 $\mu mol \cdot L^{-1}$. This is however affected by serotonin containing foods, for example bananas, and disulfiram which both increase the 5HTOL level but not the 5HIAA level. This can be resolved by using the 5HTOL/creatinine ratio in addition to the 5HTOL/5HIAA ratio.

In summary, both methanol and an increase in the 5-HTOL:5HIAA ratio can be detected after ethanol has been metabolised but neither test is routinely available. Ethanol remains the most frequently used test, whether in breath, urine or

serum.

Markers of chronic misuse

The markers most commonly evaluated are those readily available as part of routine screening: erythrocyte mean corpuscular volume (MCV), serum aspartate aminotransferase and alanine aminotransferase (AST, ALT), and gamma glutamyl transferase (GGT).

Erythrocyte mean cell volume (MCV) is thought to be elevated as a result of direct toxicity by ethanol[24]. It becomes elevated after six weeks of alcohol misuse but, in view of the half life of the erythrocyte it remains elevated for up to three months and so has a limited use in monitoring alcohol intake. The sensitivity is higher in women (86.3%) than in men (63.0%)[24]. False positives are found in hypothyroidism, vitamin B_{12} and folate deficiency, non-alcoholic liver disease and in some patients who smoke[25].

Serum aspartate amino transaminase (AST) and serum alanine transaminase (ALT) are markers of liver damage as opposed to alcohol misuse. Both transaminases are found in hepatocytes but AST is also found in skeletal and myocardial cells. In alcohol related liver damage, the AST is elevated more than the ALT, at least in part as a reflection of alcohol related skeletal damage. This is the reverse of the normal pattern in acute hepatocellular disease (for example acute viral hepatitis) where the ALT exceeds the AST.

False positive results are found in non alcoholic liver disease, muscle damage and myocardial damage. Despite these, the specificity is reasonably high at >90% (Table 2).

Table 2 Sensitivity and specificity of markers for detection of hazardous and dependent alcohol use

		Sensitivity(%)	Specificity(%)
MCV	Hazardous consumption	20-30	64-100
	Dependence/alcoholism	40-50	64-100
AST	Hazardous consumption	10-30	>90
	Dependence/alcoholism	35-50	>90
ALT	Hazardous consumption	10-20	>80
	Dependence/alcoholism	20-50	>80
GGT	Hazardous consumption	20-50	55-100
	Dependence/alcoholism	60-90	55-100

Adapted from Conigrave *et al*[23]

AST itself has a mitochondrial (mAST) and cytosolic component. It appears that alcohol selectively affects the mitochondrial component following damage to this organelle so that the serum increase in alcohol misusers is mAST. This has been proposed as a more sensitive marker of alcohol misuse. There is also a small increase in non alcoholic liver disease and it has therefore been suggested it should be used as a ratio of mAST to total AST[26].

Serum gamma glutamyl transferase (GGT) increases in alcohol misuse in a dose-dependent manner, and is often the first marker to be elevated[23]. It is less sensitive in women than men[27,28]. The exact mechanism of elevation of GGT in alcohol misuse is unclear. The enzyme may be released by hepatic cell injury or by induction following exposure to alcohol. In alcoholic liver disease a component of the increase is also from hepatocyte cholestasis and hepatocyte damage. It increases after five weeks of drinking more than 50 g per day. It usually increases to three times the upper reference limit, but will normalise within five weeks of abstinence, with a half-life of 26 days, although this is lengthened in chronic liver disease[29].

Some individuals misusing alcohol never have an elevated GGT; in some chronic alcohol misusers initially high levels fall despite continued drinking. False positives are seen in non-alcoholic liver disease, including fatty liver, biliary tract disease, obesity, diabetes, pancreatitis, hyperlipidaemia, trauma and heart failure, and with microsomal inducing drugs such as anti-epileptics[25].

The varying sensitivity and specificity makes it an unsuitable marker to be used alone for screening, but it is useful to confirm a clinical suspicion of alcohol misuse. Several isoforms of GGT exist and can be separated by electrophoresis. The pattern in alcohol abuse is distinctly different from not only that of healthy volunteers, but also from non alcoholic liver disease. It is, however, the same as that in those taking anti-epileptic drugs since both result in enzyme induction. It has been suggested that the analysis of GGT isoforms may improve the specificity of GGT for alcohol misuse[30].

Combinations of markers

As can be seen from Table 2 none of the routinely available markers has sufficient sensitivity or specificity to be used alone, and in practice a combination is usually used. AST and GGT both have higher sensitivity in men than women while MCV is higher in women. Each of MCV, AST and GGT are raised by a different mechanism and so used in combination will pick up varying parts of the alcohol misusing population. If two or more markers are positive then the number of false positives fall and the specificity is seen to increase[29,31].

The clinical case mix affects the test performance of any given marker. The sensitivity of the test is highest where there are a high number of

severe alcohol misusers, in for example the alcohol treatment centre. The sensitivity is lowest in the general community[23]. Chick *et al* found a sensitivity of 40% for in patient alcohol misusers and 23% for those in the setting of employment screening[31]. Sillanukee *et al* found a sensitivity of 4. 7% for MCV for detecting alcoholics in the context of a detoxification centre, but only 22% for detecting heavy drinkers voluntarily attending for health screening. Similarly using GGT there was a sensitivity of 65% in the detoxifica tion centre, but only 35% within the community[32].

Other markers

Serum urate is routinely available and may be elevated in 40% of male and 25% of female alcohol misusers. False positives results are seen in gout, renal disease and with some drugs.

Serum triglycerides are often measured for other reasons but increase after one - week of drinking in 40% of alcohol misusers, and normalise within one week of abstinence. False positives are seen in hyperlipidaemia, diabetes, obesity and with some drugs.

It can be seen that the currently available routine markers do not have sufficient sensitivity or specificity to be used alone to detect alcohol misuse. Considerable research has been undertaken to try to find any other potentially more useful markers. One such marker is carbohydrate deficient transferrin (CDT).

Transferrin is a large glycosylated protein which binds and transports iron in the plasma. It has terminal carbohydrate units containing sialic acid. Alcohol intake of greater than 60 g per day for two weeks results in loss of some or all of the sialic acid component of transferrin and hence the term - carbohyd rate deficient transferrin. Initial research gave promising results with sensitivities of 100% and specificities of 97%[33], confirmed by other workers[34-37] and prompted the development of commercial assays: CDTect (Pharmacia and Upjohn, Sweden) and AXIS %CDT (AXIS Biochemicals, Norway). There has been a considerable amount of research using these commercial assays and variation in the reported results[38,39]. It seems that in development of the assays there has been some loss of sensitivity and specificity, particularly in women and those with liver disease. This marker is increa singly being used in Europe, and often in combination with other markers.

SUMMARY AND CONCLUSIONS

Alcohol has been used in society over centuries and all the evidence we have ind icates that, to society as a whole, the risks are heavily outweighed by the benefits and it is particularly expensive in health terms. A means to identify those at risk is required so that these individuals can be targeted for help. This in turn requires a means for monitoring. Ideally detection should screen for alcohol misuse at a level at which damage occurs.

Histories and questionnaires are still the commonest initial means of detection of alcohol misuse. They are cheap, easily administered but are subjective. They still provide the 'gold-standard'. If the history remains uncertain and there is a suspicion of alcohol misuse biological markers provide objectivity, and a combination of markers remains essential in detection. The three commonest markers in current practice were GGT, AST and MCV. However these show problems with detection, particularly in the context of liver disease. Serum carbohydrate deficient transferrin initially showed promise as having a high sensitivity and specificity and could be ideally suited for both screening and monitoring. However following development of commercial assays, the sensitivity and specificity is not as promising as early work had suggested. Research continues in both investigating and refining markers of misuse.

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Edited by Ma JY