



Review

Detection of alcohol consumption during pregnancy—Current and future biomarkers

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Abstract

Alcohol, one of the most frequently reported addictions, is a significant public health problem in the USA. Early identification is important and would aid in intervention for the pregnant woman who continues to drink and for the affected infant. To date, there isn't a definitive test which identifies either alcohol abuse during pregnancy or newborns exposed to alcohol prenatally. The existing biomarkers can detect varying degrees of alcohol exposure but further research is needed to improve sensitivity/specificity and to validate these markers.

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1. Introduction

Alcohol, one of the most frequently reported addictions, is a significant public health problem. In the USA, an estimated 120 million people, aged 12 or older, are current drinkers of alcohol (Bearer et al., 2005). Due to its

teratogenic effects, efforts to educate the public, especially women of childbearing age, are continuously being done. Despite these efforts, the prevalence of alcohol use among pregnant women is similar to those reported in past years (12.4% in 1991; 11.4% in 1997; 12.8% in 1999 and 10.1% in 2002) (CDC, 2002). Most women who drink before pregnancy substantially reduce their consumption or completely stop drinking once they become pregnant. One study found that only 4.6% of women reported

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drinking an average of one drink per day by the end of the third trimester of pregnancy, compared with 44% before pregnancy (Day et al., 1989). Unfortunately, some women choose to drink heavily, even when pregnant, as reflected in substantially unchanged rates of reported binge and frequent drinking during 1991–99 (CDC, 2002).

Since alcohol crosses the placenta, it can interfere with proper brain and organ development (Goodlett, 2001). Adverse fetal outcomes include spontaneous abortion, fetal death, preterm delivery, low birth weight, growth abnormalities, mental retardation, smaller head circumferences and lower Apgar scores (Russell and Skinner, 1998). The frequency, intensity and timing of drinking are factors influencing fetal outcome (Eckardt et al., 1998). Heavy drinking during pregnancy can cause fetal alcohol syndrome (FAS), the most common preventable cause of mental retardation. Even moderate alcohol consumption during pregnancy can result in a spectrum of effects known as fetal alcohol spectrum disorder (FASD). Streissguth et al. (1990) found learning problems in school age children whose mothers recalled one binge episode of consuming more than five drinks during pregnancy. Another study by Jacobson et al. (1994), showed deficits on the Fagan Test of Infant Intelligence in 6.5-month-old infants whose mothers consumed on average one drink per day. FASD includes a wide range of disorders such as: alcohol-related birth defects, alcohol-related neurodevelopmental defects and subtle deficits on a variety of behavioral, educational and psychological tests (Stratton et al., 1996). FASD is more prevalent than FAS and estimated to affect 1% of all newborns (Sampson et al., 1997).

The effects of both FAS and FASD are irreversible. The cumulative costs for society are enormous and health expenditures related to FAS alone are estimated between \$75 million to \$9.7 billion annually (Stratton et al., 1996). Since educational efforts alone have not proved to be effective, it is important to identify women who continue to drink during pregnancy and newborns that have been exposed to alcohol in utero. Early identification will facilitate interventions that can stop alcohol use during this and future pregnancies, provide close monitoring for infants at risk for FASD, and if needed provide a more stable living environment and special services (Bearer et al., 1999; Stoler and Holmes, 2004; Stoler et al., 1998). It is particularly important to identify at-risk children before age 6 since early intervention reduces the likelihood of secondary problems associated with FAS and FASD, such as mental health problems, school failure, delinquency, inappropriate sexual behavior, and alcohol or other drug problems (Grant et al., 2004; Streissguth et al., 1996). In addition, developing effective methods to detect prenatal alcohol consumption may promote better scientific understanding of alcohol research on dose–response relationships between alcohol exposure and FASD.

To date, there isn't a definitive laboratory test available. Identifying women who drink during pregnancy or newborns that were exposed to alcohol prenatally is difficult as

demonstrated by Little et al. who found that the diagnosis of FAS was missed in 100% of newborns which were identified later in childhood (Little et al., 1990).

In this review, we will briefly describe the current methods and biomarkers for identifying women who consume alcohol during pregnancy or newborns that have been exposed to alcohol in utero.

2. Maternal self report

Currently no laboratory test can identify and quantify prenatal alcohol use that takes place over a protracted period. Since alcohol itself and the main product of its metabolism, acetaldehyde, break down rapidly in the blood, they cannot be used to distinguish between a single drinking episode and chronic, intermittent alcohol use. Testing blood, breath, or urine is useful only for assessing very recent alcohol exposure. Unfortunately, the biological markers currently in use may not be effective in screening for alcohol use occurring over the longer term, forcing clinicians to rely on maternal self-reports for assessing drinking patterns (Chang, 2001; Russell et al., 1994, 1996; Savage et al., 2002).

Major disadvantages of self-reports are that it is often difficult for people to recall the amount and frequency of their alcohol intake, and the stigma and fear of punishment (e.g., incarceration or involuntary commitment associated with drinking alcohol during pregnancy) makes women reluctant to reveal prenatal alcohol use, especially if they drink heavily (Bearer et al., 2003; Chan et al., 2003).

Some screening instruments attempt to circumvent pregnant women's reluctance to disclose prenatal alcohol use by including questions that assess prenatal alcohol use indirectly—for example, by asking women to report the number of drinks they can consume before passing out or falling asleep (Russell et al., 1994, 1996). Although some research indicates that such screening instruments can effectively flag heavy drinking, they do not provide a long-term, objective measure of the full range of prenatal alcohol use. Supplementing maternal reports with a biological marker for prenatal alcohol use would allow earlier identification and intervention for exposed infants and would make it easier to recognize women who are at risk for drinking during their next pregnancy (Bearer et al., 2001, 2003).

3. Biomarkers

Biomarkers are a cellular or molecular indicator of exposure, disease, or susceptibility to disease. Biomarkers are detectable and measurable by a variety of methods including physical examination, laboratory assays and medical imaging.

Biomarkers can be categorized to three large groups: (1) biomarkers of exposure, (2) biomarkers of effect and (3) biomarkers of susceptibility. While biomarkers of exposure are designed to detect exposure (usually will be tested

during or close in time after the exposure), biomarkers of effect are more proximal to clinical disease (i.e., they are designed to detect the early effects of exposure, or the development of disease). Obvious biomarkers of susceptibility are genetic polymorphisms.

Developing a biomarker is a long and difficult process. Initially, it is necessary to identify a biological marker that would indicate exposure or effect, remain present over time and is easy to detect. In addition, the biological sample containing this marker should be easy to obtain and involve minimally invasive techniques. Finally, obtaining and processing the sample should be inexpensive and the sample size should be relatively large to increase sensitivity.

Once a potential biomarker and a potential biological sample have been selected, it is important to develop a fast, reliable and cheap method for analytical quantification of the biomarker in the specific sample. Issues to consider include determination of recovery and the stability of the biomarker in the chosen sample.

The most challenging step in developing a biomarker is validating that it correctly identifies exposure without false positives or false negatives. Markers of exposure must be validated according to their ability to assess the true exposure (i.e., sensitivity) and lack of exposure (i.e., specificity). Estimates of sensitivity must consider the background level of the biomarker in a population without exposure as well as the marker's ability to detect levels of exposure leading to a biological effect. Estimates of specificity also must consider variations in the population, including age and gender, time of day of the measurement, and the effect of other diseases or developmental processes. Ideally, the marker should be specific for the given exposure (i.e., it should not find false positives). When comparing biomarkers, it is necessary to compare their sensitivity and specificity for identifying a similar exposure, or, in this case, a similar level of drinking. Also, since gender and pregnancy also affect biomarkers, biomarkers should be compared within populations of the same sex or pregnancy state.

Since maternal self-report is not reliable in most cases, researchers are looking for an animal model. Animal models are useful in the validation process because they can be used to study the mechanisms behind the expression of markers and the relationships between markers and exposure. Currently, two animal models for the study of alcohol teratology are being evaluated, the pregnant ewe model (Cudd, 2005) and the guinea pig model (Caprara et al., 2005). Further studies for validating a biomarker of maternal alcohol exposure are in progress.

3.1. Biomarkers of maternal drinking during pregnancy

(1) *Alcohol*—Alcohol can be detected on the breath, skin, urine, blood, or cord blood. These rapid, sensitive and inexpensive tests are used as a biomarker for acute alcohol effects but do not play a rule as a biomarker of

chronic exposure due to the rapid elimination of alcohol from the body.

(2) *Alcohol metabolites and products*—In the body, after alcohol reaches the liver, placenta, and other metabolic organs of both the mother and the fetus, it is broken down or conjugated by several enzymes. Break down is by alcohol dehydrogenase (ADH) or cytochrome P450 2E1, which oxidizes alcohol to form acetaldehyde. The conjugation process is done by fatty acid ethyl ester (FAEE) synthase, which produces FAEEs, and glucuronyl transferase, which produces ethyl glucuronide.

Acetaldehyde can be detected in the blood. Because it is difficult to measure accurately and is quickly eliminated, it is an insensitive marker for chronic intermittent alcohol exposures.

FAEEs are products of the nonoxidative ethanol metabolism and considered a stable metabolite. FAEEs have been studied in different species (mouse, rat, sheep and human) and can be detected in blood, hair, placenta, cord blood and meconium of newborn exposed in utero to ethanol (Bearer et al., 1996; Mac et al., 1994; Soderberg et al., 1999). In addition, it was shown that maternal FAEEs are not transferred to the newborn since they are degraded extensively by the placenta (Chan et al., 2004). In the blood, FAEEs's short half life (in serum about 24 h after alcohol intake) (Soderberg et al., 1999) make it ineffective as a biomarker for chronic alcohol abuse. However, evidences from several studies showed that FAEEs could serve as a reliable biomarker for prenatal alcohol use when measured in other materials (Bearer et al., 2003, 1999; Chan et al., 2003; Klein et al., 1999). Nevertheless, FAEEs's half-life in the human placenta, hair, cord blood and meconium is unknown. Further research is needed to determine it.

In 1992, Bearer suggested FAEEs in cord blood as biomarkers for in utero ethanol exposure (Bearer et al., 1992). Mac et al. (1994) suggested FAEEs extracted from meconium of newborns as a biomarker for in utero ethanol exposure. In 1999, Bearer et al. showed that the presence of one FAEE, ethyl linoleate, in meconium was associated with higher weekly levels of maternal alcohol use (based on self-report) during the month prior to pregnancy, in the first trimester, and overall. Sensitivity and specificity of the test were 72% and 51%, respectively, to distinguish women who reported 1 or more drinks/week in the third trimester from women who denied use, and 68% and 48%, respectively, to distinguish women who used ≥ 1 drink/week from women who used < 1 drink/week in the month before pregnancy (Bearer et al., 1999). Analysis of meconium from two abstaining populations, one from Amman, Jordan and the other, women from Cleveland who reported no alcohol, tobacco, cocaine or marijuana use, showed a significant difference in 6 of 7 FAEEs (all but palmitoleic) from the group who reported any use of alcohol, tobacco, marijuana and or cocaine. A ROC analysis showed a sensitivity of 92% and specificity of

61% for ethyl oleate to detect women reporting 7 or more drinks/day in the month prior to pregnancy. Chan et al. (2003), confirmed that basal FAEE levels in a normal population without ethanol exposure are low. Recently, Bearer et al. (2003) found a strong association between ethyl oleate with self-reported drinking during the second and third trimester of pregnancy. The reported sensitivity and specificity in this study were 84% and 83% respectively to detect women who reported 3 or more drinks per occasion. In addition, the study found that FAEE concentration in meconium was more strongly related to the mother's self-reported alcohol consumption per occasion than to the overall average she consumed per week. This finding may prove especially useful, as recent research has suggested that the number of drinks per occasion may be the best indicator of risk for alcohol-related impairment (Bearer et al., 2003; CDC, 2002).

The correlation between FAEEs in meconium and prenatal alcohol use is not perfect (Bearer et al., 2003; Chan et al., 2003). Possible explanations are: (1) FAEEs may accumulate unevenly in meconium over time, so that samples do not appear to reflect reported drinking; (2) genetically determined variations in alcohol metabolism may influence the synthesis of FAEEs; and (3) illness or the use of some medications and food additives may affect FAEE concentrations.

In addition to meconium, one of the places that FAEEs are incorporated is hair. Studies have shown that FAEEs levels in the hair of adults correlated with amount of ethanol abuse and could be detected 2 months after abstinence. Also there was no difference in FAEEs concentration between pubic, chest or scalp hair (Pragst et al., 2001). Wurst et al. (2004) used the sum of four FAEEs in hair to distinguish between 18 alcohol-dependent patients in detoxification and a group of 10 social drinkers and 10 teetotalers. By using a cut-off of 0.29 ng/mg, sensitivity and specificity were 100% and 90%, respectively. There was no correlation between FAEEs and the total grams of ethanol consumed in last month, blood alcohol concentration (BAC) on admission to the hospital or traditional markers of heavy drinking (CDT, MCV, or GGT). FAEEs are incorporated into hair mainly from sebum. For this reason, their concentration in hair is influenced by interindividual differences of the activity of the sebum glands and hair care or hair cosmetics. Hence, interpretation of the results may be difficult.

Ethyl glucuronide—is a direct metabolite of ethanol that can be measured in body fluids, blood and urine. It can detect ethanol intake up to 80 h after the complete elimination of alcohol from the body (Wurst et al., 1999) and it is stable in urine samples up to 5 weeks (Schloegl et al., 2005), making it a potential marker in numerous settings such as relapse, in settings where alcohol use may be risky (e.g. such as driving, work-place, pregnancy or monitoring physicians or other professionals who are in recovery and working) or for resolving forensic questions

of relapse. To date, no studies involving pregnant women or newborns have been done.

Cocaethylene—is a metabolite of alcohol formed in the presence of cocaine, perhaps by the same enzymes that catalyze the formation of FAEE. It can be detected in the blood, urine, hair and meconium. It would only be useful as a biomarker in populations with concurrent cocaine use.

3.2. Markers of early biological effect

3.2.1. Enzymes involved in alcohol metabolism

Ethanol ingestion may affect some of the enzymes required for its metabolism. Usually, ethanol will cause an induction of enzymes and their level will increase. Those enzymes may be useful as biomarkers of alcohol exposure.

Cytochrome P450 2E1 (CYP450 2E1)—is the cytochrome P450 enzyme that metabolizes alcohol. An elevated CYP450 2E1 activity may occur as a result of chronic drinking but it is not specific. The highest level of CYP450 2E1 is in the liver. However, some P450s are also expressed at lower levels in extrahepatic tissues, most notably gut, lung, kidney, brain and hematopoietic tissue. In alcoholics, the elevated levels of CYP450 2E1 in blood leukocytes decreased rapidly during withdrawal of 8 days, with an estimated half-life of 2.5 days (Lucas et al., 1995). In a rabbit model, P450 2E1 levels in lymphocytes could be induced 6–10 fold after exposure to 15% alcohol in their drinking water for 12 days. The researchers found that the extent of the induction correlated well with BAC and could be demonstrated at BACs as low as 50 mg/dl (Raucy et al., 1995). In contrast, induction of P450 2E1 levels in human is less substantial when compared to animals and was found to be only a 2.3 fold increase between alcohol abusers and controls (Raucy et al., 1997). No studies of pregnant women or newborns have been reported.

FAEE synthase—an enzyme that conjugates ethanol to free fatty acids and produces FAEE, has been found to be active in most organs of the body and in peripheral blood, cord blood, placenta, and meconium. Several reports have shown that tissue specific FAEE synthase activity can be altered following chronic alcohol exposure. Gorski et al. (1996) demonstrated an induction of FAEE activity in white blood cells of healthy non-alcoholic volunteers to nearly 2-fold upon ingestion of 2 ounces of Scotch whiskey per day for 6 days. A decline of the activity to control levels occurred despite continued ingestion of 2 ounces of Scotch whiskey for an additional 3 days. Furthermore, FAEE synthase activity in white blood cells taken from control subjects was twice that observed in alcoholics admitted to a detoxification unit (Gorski et al., 1996). Similar results were found in rats, when treatment with ethanol for 4 weeks increased pancreatic and liver gene expression of FAEE synthase enzyme (Pfutzer et al., 2002).

These results suggest that FAEE synthase activity may be a useful marker of alcohol use for binge drinkers, although the dynamics of enzyme expression appear to be complex and the changes described occur at high alcohol

doses. FAEE synthase activity has not been studied in pregnant women or newborns.

3.3. Products of the interaction of alcohol metabolites and cellular components

The following potential biomarkers are formed when products of alcohol metabolism interact with other cellular components in the body to create compounds that can be detected in the blood or urine.

Acetaldehyde-protein adducts—due to a short half-life, free acetaldehyde has limited potential as a biomarker. However, the bound form of acetaldehyde (to various proteins), tends to increase during chronic alcohol exposure and also seems to be more stable. It is measured by using high-performance liquid chromatography and fluorescence detection—known as the whole blood-associated acetaldehyde assay (WBAA) and can be used to test for heavy alcohol consumption (Halvorson et al., 1993). Proteins with detectable acetaldehyde adducts include hemoglobin, serum proteins, albumin, CYP450 2E1 and red blood cell membrane proteins. The prolonged half-life of these proteins, allows this marker to provide a picture of alcohol consumption over time. In a study by Halvorson et al. (1993), levels of protein-bound acetaldehyde measured in adults, remain high for approximately a month after alcohol consumption. Sillanaukee et al. (1992) found that the concentration of hemoglobin acetaldehyde (Hb-Ac) was significantly higher in 18 heavy drinkers and 20 alcoholics compared with 22 control subjects. In addition, the sensitivity to determine heavy drinking (50%), was higher than the sensitivity for two of the traditional biomarkers of alcohol abuse, gamma glutamyltransferase and mean corpuscular volume (Sillanaukee et al., 1992). In another study on 182 adults admitted to the drug and alcohol treatment unit, Hb-Ac levels were used to detect drinking of more than six drinks per day versus less than six drinks per day, with a sensitivity and specificity of 67% and 77%, respectively (Hazelett et al., 1998). However, the finding that one dose of alcohol significantly increased the concentration of Hb-Ac in control subjects indicates that this test cannot distinguish between chronic versus acute exposure. There is only one study which evaluated acetaldehyde adducts in pregnant women. In this study, Hb-Ac adducts were measured in 19 pregnant problem drinkers (Niemela et al., 1991). Four women became abstinent during the pregnancy and 15 continued to drink 10–35 drinks per week. Among the 15 drinkers 7 had Hb-Ac levels higher than the abstainers for a sensitivity of 47% and specificity of 100%.

Oxidation products—Alcohol metabolism involves a number of processes, one of which is oxidation. Through oxidation, alcohol is detoxified and removed from the blood, preventing the alcohol from accumulating and destroying cells and organs. Oxygen radicals produced during oxidation can then react with lipids to form lipid peroxidation products, compounds such as dienes and

malondialdehyde, which may act as biomarkers detectable in the blood. Baldi et al. (1993) used serum malondialdehyde levels to distinguish between 15 healthy control subjects and 3 groups of alcoholics: those with normal liver function, those with non-cirrhotic alcoholic liver disease, and those with cirrhotic alcoholic liver disease. The researchers found that, irrespective of the presence of liver disease, using malondialdehyde as a biomarker for alcohol use had a sensitivity of 70% and a specificity of 100%. In another study, the serum concentration of dienes was higher in an alcohol-using population compared with normal controls (Butcher et al., 1993). Nevertheless, other conditions such as preeclampsia can cause an elevation in malondialdehyde (Takacs et al., 2001) and dienes (Uotila et al., 1993) levels, limiting the usefulness of this marker in complicated pregnancies.

5-Hydroxytryptophol (5-HTOL)—is produced when the neurotransmitter serotonin is broken down. Under normal circumstances and without the intake of alcohol, the ratio of 5-HTOL/5-hydroxyindole-3-acetic acid (5-HIAA) is small. During alcohol metabolism, the level of 5-HTOL increases. 5-HTOL is secreted in the urine, where it can be detected for approximately 5–15 h longer than standard alcohol measurements (Beck and Helander, 2003). Thus, 5-HTOL could be used as a reliable 24-h 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 (Beck and Helander, 2003). Another indicator of alcohol consumption is the ratio of 5-HTOL to another serotonin metabolite, 5-HIAA. Under normal conditions, this ratio is small, however, alcohol intake will cause an increase in this ratio (Johnson et al., 2004). A disadvantage of this marker is its short half-life. In a study that measured the mean ratio of urinary 5-HTOL/5-HIAA in 16 heavy drinkers, the ratio was significantly elevated both at 5 and 9 h after ethanol administration, but returned to baseline 13 h after ethanol administration (Bisaga et al., 2005). No studies have been conducted in pregnant women or newborns.

3.4. Altered structure/function

Markers indicating ethanol-induced alterations of normal body structures or functions include altered target proteins and early indications of target organ damage.

Carbohydrate-deficient transferrin (CDT)—is the most sensitive and specific biologic marker of chronic alcohol abuse. CDT is a modified form of transferrin (a molecule responsible for carrying iron within the bloodstream) and its levels are increased after alcohol consumption. The half-life of CDT is 17 ± 4 days, resulting in prolonged presence in the bloodstream after its formation (Reynaud et al., 1998).

CDT has been widely used by clinicians 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. Researchers also found that, in general, women tend to have higher CDT levels than men, regardless of their drinking history (Arndt, 2001). Also, pregnancy was found to be correlated with increased CDT levels (Sillanaukee et al., 2000). In cord blood, CDT concentrations are significantly higher than in maternal blood, but are not correlated with reported maternal alcohol intake (Whitty et al., 1997).

In recent years, several improvements in techniques (i.e., immunological reagents) (Bean et al., 2001), and measuring CDT levels as a percentage of total transferrin rather than an absolute value (Anttila et al., 2004), resulted in higher sensitivity of this marker.

Studies have indicated that heavy drinkers have higher amounts of CDT than nondrinkers. One review of 16 studies that examined CDT as a biomarker in women with alcohol problems (Allen et al., 2000) reported that the median sensitivity was 51% for all studies distinguishing different degrees of severity of drinking over a range of drinking behaviors from women drinking less than 2 drinks per day, with a median specificity of 92%. However, the sensitivity falls dramatically when comparing heavy drinkers with moderate drinkers. The promise of this biomarker increases when used in combination with other tests.

Proteomics—A number of proteins are known to be affected, either directly or indirectly, by alcohol. The rapidly advancing field of proteomics offers promise for developing sophisticated biomarkers that can detect subtle biological changes associated specifically with alcohol use (Anni and Israel, 2002), or can distinguish between currently drinking women who will continue to drink during pregnancy and those who will stop (Neuhold et al., 2004). Recently, investigators used a powerful technique, surface-enhanced laser desorption/ionization–time of flight–mass spectrometry (SELDI–TOF–MS), to study serum samples from alcoholics who had consumed more than 10 drinks a day for at least 10 years (Nomura et al., 2004). 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 [alpha]E 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 1 week into the treatment program. Nomura et al. (2004) concluded that the two protein fragments have potential as markers of excessive alcohol consumption in heavy drinkers seeking treatment.

In a preliminary study, Robinson et al. (1995), searched for serum protein variations associated with FAS. These researchers studied 12 children diagnosed with FAS (indicating that prenatal alcohol exposure had to have occurred) and 8 control subjects. Their analysis found eight proteins whose concentrations differed significantly be-

tween the two groups of children. No single protein distinguished children with FAS from children in the control group, but analyses revealed clusters of proteins that collectively distinguished between the two groups. This study demonstrates the power of looking at patterns of response to alcohol, and it can be used not only to identify children with prenatal alcohol damage, but also women, who because of their biological response to drinking, may be at risk to drink during their pregnancies.

Dolichols—are chemicals found in high levels in urine and serum of alcoholics. There are controversial results in the literature about urinary dolichols as a marker of alcoholism. While some studies did not find any effect of moderate alcohol consumption (60 g of alcohol daily) on the levels of urinary dolichols (Roine, 1988) or a low sensitivity (9–19%) (Stetter et al., 1991), others showed significantly higher urinary dolichol concentrations in alcoholics compared to social drinkers (Roine et al., 1992). An interesting work by Wisniewski et al. (1983) studied urinary dolichols among 16 infants who were small for gestational age. Six infants, who were born to mothers that were heavy drinkers had 4–5 times higher urinary dolichol levels compared with infants without prenatal exposure to alcohol. In addition there is a significant increase in urinary dolichol levels in patients with bacterial infections and malignancies as well as pregnancy (Roine et al., 1989). Additional research is needed on this potential biomarker.

Sialic acid (SA)—is an acetylated derivative of neuraminic acid, which is attached to carbohydrate chains of glycoproteins and glycolipids. It can be detected in serum, saliva, and urine and probably has the same half-life of the glycoproteins (about 10 days), to which it is linked. Increased levels of total SA and/or lipid associated SA have been observed in alcoholics but also in pregnancy (Crook et al., 1997) and various diseases including cancer, diabetes and renal disease. The first reports evaluated the diagnostic specificity in alcoholism up to 95–100% and the sensitivity of 51–57% (Romppanen et al., 2002; Sillanaukee et al., 1999). Another study that measured SA serum levels in alcoholics reported an overall sensitivity and specificity of 63% and 64% respectively. When a subgroup consuming >400 g alcohol per day was tested, the sensitivity increased to 100% but specificity remained the same (Idiz et al., 2004). The low specificity of total SA may limit its clinical value as a marker of alcohol abuse, but its use as part of diagnostic test for identifying alcohol abuse should be further investigated.

Gamma glutamyltransferase (γ GT)—is an enzyme produced predominately in the liver and involved in glutathione metabolism. Increased serum levels of γ GT serve as an indicator of hepatobiliary disease. However, γ GT is not a very sensitive marker, showing up in only 30–50% of excessive drinkers in the general population (Conigrave et al., 2003). Nor is it a specific marker of chronic heavy alcohol use, because other liver diseases or certain drugs also increase serum levels of γ GT. In pregnancy, γ GT levels are usually decreased.

Aspartate/alanine aminotransferase (AST/ALT)—these liver enzymes, which can be detected in the blood, can be useful markers for alcohol abuse. However, those enzymes are more useful as an indication of liver disease and are less specific to alcohol consumption. Of the two enzymes, ALT is the more specific measure of alcohol-induced liver injury because it is found predominantly in the liver, whereas AST is found in several organs, including the liver, heart, muscle, kidney, and brain. Very high levels of these enzymes (e.g., 500 units per liter) may indicate alcoholic liver disease and often the ratio of AST to ALT is used to confirm an impression of heavy alcohol consumption. However, because these markers are not as accurate in patients who are under age 30 or over age 70, they are less useful than some of the other more comprehensive markers (Halvorson et al., 1993). One study found that AST and ALT were comparable in sensitivity and specificity to distinguish social drinkers from alcoholics (Sillanaukee et al., 1999). In pregnant women, despite the tendency for decreased levels of AST/ALT, these enzymes are not sensitive as shown by a study measuring several markers in which AST and ALT were poor indicators of abusive drinking (Halmesmaki et al., 1992).

Mean corpuscular volume (MCV)—an index of red blood cell size, is associated with heavy chronic drinking (Neumann and Spies, 2003), as the MCV in heavy drinkers tends to exceed the average range. This marker is less useful clinically and when used as a single marker showed low sensitivity for recent excessive intake and low specificity for alcohol.

Beta-hexosaminidase (beta-Hex)—is a lysosomal glycosidase that can be detected in the blood or urine, and its levels are elevated in heavy drinkers (Javors and Johnson, 2003). Karkkainen et al. (1990) have shown that beta-Hex has high sensitivity (85.7%) and specificity (97.6%) in recognizing heavy drinking (defined as over 60 g ethanol daily). Other studies showed similar result with a sensitivity of 94% and a specificity of 91% in detecting heavy drinking (Stowell et al., 1997). Although beta-Hex subsides to normal levels after only 7 to 10 days of abstinence the assay is difficult to obtain and levels may be influenced by other conditions, such as diabetes, hypertension and liver disease. In addition, pregnancy itself can cause an elevated serum levels of beta-Hex (Hultberg and Isaksson, 1981). This biomarker should be further investigated in pregnant women.

4. Test batteries

Since utilizing a single laboratory test usually lacked adequate performance as a stand-alone test, several models of multiple biomarkers have been explored under the assumption that it would increase the diagnostic efficiency (e.g. increased sensitivity and specificity). Early in the 1980s, several researchers showed that multivariate statistical analyses of a large number of blood tests were able to differentiate between heavy and light drinkers (Hawkins

et al., 1984; Hillers et al., 1986; Lichtenstein et al., 1989). However, those studies did not validate their models in the general population. Hartz et al. (1997) reviewed several models using multiple biochemical tests for identifying heavy drinkers. Only one of the models, a logistic regression equation of 40 routine laboratory tests, was able to identify 98% of heavy drinkers and 95% of light drinkers, while the performance of other studies varied widely (Hartz et al., 1997). Even though some models seemed to be promising, the high cost of computing in the 1980s made statistical analysis impractical, leading researchers to abandon this approach.

In the past 10 years, the rapidly changing technology making computing and statistical calculation accessible and cheap paved the way for further studies. Brinkmann et al. (2000), using an alcohol index of four markers including γ GT and CDT, were able to demonstrate 100% specificity and 93% sensitivity in distinguishing alcoholics from three groups of non-alcoholics. Other studies using both CDT and γ GT in alcoholics entering treatment or heavy drinkers showed a median sensitivity of 44% for each test separately and 72% when used in combination (Allen et al., 2000). Stoler et al. (1998) used a combination of CDT, γ GT, MCV, and Hb-Ac in pregnant women. It appeared that women who reported drinking at least 14 drinks per week were positive for 1 or more markers. Having two or more positive markers was more predictive of infant outcome than any measure of self-reported drinking. However, sensitivity and specificity were not reported in this study (Stoler et al., 1998). Different results were demonstrated by Sarkola et al. (2000), who did not find any association between hemoglobin-acetaldehyde adducts and CDT levels and reported level of drinking in pregnant women. Harasymiw et al. (2000) reported in a series of studies that a panel of 34 blood chemistry tests called the Early Detection of Alcohol Consumption (EDAC) score, could be practical and cost effective method for detecting excessive drinking. Based on this score, Harasymiw et al. (2000) demonstrated 100% sensitivity and 82% specificity in identifying women who drank at least three drinks per day, and 42% sensitivity and 90% specificity when identifying women who drank at least 7 drinks per week or more than 3 on any occasion. When EDAC was combined with CDT, sensitivity (92%) and specificity (94%) for diagnosis of heavy drinkers were improved (Harasymiw and Bean, 2001).

Overall, panels of routine laboratory tests for identifying alcohol abuse seem to be a cost effective and practical approach and may serve as first line screening tool. Further studies are needed for improvement of sensitivity and specificity.

5. Conclusions

Detecting alcohol use among pregnant women is an important step toward preventing alcohol-related birth defects. In addition, the early identification of exposed

infants may lead to new modalities of therapy. Currently, no laboratory test can definitively detect and quantify prenatal alcohol use. Further research is still needed to validate potentially useful biomarkers and identify further areas of possibilities.

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