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Review

Biochemical markers of alcohol use in pregnant women

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Abstract

Objectives: To describe the serious health consequences of alcohol (ethanol) use, especially as they relate to pregnancy and the development of fetal alcohol syndrome (FAS) and fetal alcohol effects (FAE). The classic markers of alcohol exposure, including blood/breath alcohol, γ-glutamyl transferase (γGT), mean corpuscular volume (MCV), hemoglobin-associated acetaldehyde (HAA) and carbohydrate deficient transferrin (CDT), are valuable and their methods of analysis are reviewed.
Conclusions: Since both FAS and FAE represent two of the leading *preventable* causes of mental retardation and birth defects, identification of alcohol use early in pregnancy is important to avoid adverse fetal outcomes. Unfortunately, the diagnosis of FAS and FAE is usually made after birth, when alcohol damage has become irreversible and permanent. The clinical laboratory can help prevent this damage and make a valuable contribution in assessing prenatal alcohol use. The clinical utility of blood/breath alcohol, γGT, MCV, HAA and CDT in alcohol use identification, especially in pregnancy, is substantial. Although none of the markers singularly has adequate sensitivity and specificity for screening, their diagnostic utility increases when measured as a panel. This is especially true in detecting alcohol use in pregnancy where the presence of several positive markers was correlated with the presence of alcohol-related fetal effects.

Keywords: Alcohol; Alcoholism; AUDIT questionnaire; Birth defects; Carbohydrate deficient transferrin; Fetal alcohol syndrome; Fetal alcohol effects; Hemoglobin-associated acetaldehyde

1. Introduction

Alcohol (ethanol) use in the United States is a widespread practice - an estimated 113 million Americans drink alcohol, which is one of the most frequently reported addictions [1,2]. A mixed message is sent to the public concerning alcohol use. Medical studies have reported that the moderate consumption of alcohol may reduce mortality from vascular diseases through its beneficial effects on lipoprotein metabolism, including increasing HDL cholesterol and decreasing triglycerides levels [3,4].

Alcohol use is a serious issue in women's health; in 1999, an estimated 53% of women consumed alcohol [5]. Nonpregnant women also engage in high risk drinking, with a binge drinking (four or more drinks on one occasion, where a standard drink is 12 oz of beer or wine cooler, 5 oz of wine or 1.5 oz of 80-proof distilled spirits) frequency of 12% [5]. Alcohol affects women differently than men because they have less body water and metabolize alcohol differently than their male counterparts, causing intoxication more readily. A woman's higher blood alcohol concentration places her at increased risk for many disorders, including alcoholic liver disease, heart disease, ulcers, reproductive problems, osteoporosis, pancreatitis, brain damage, and breast and gastrointestinal cancers [6]. Because women have a decreased tolerance for alcohol, they are also at greater risk for alcohol addiction [6].

Between 14 to 20% of pregnant women report alcohol consumption sometime during pregnancy [5,7,8]. In one study, one out of every two women admitted to alcohol use in the three months before conception and one out of every thirteen women reported alcohol use in the last trimester [9]. About one in thirty pregnant women report risk drinking, meaning that they consume seven or more drinks per week or five or more drinks on one occasion [10]. The 1999 statistics for binge and frequent drinking in pregnant women are 2.7% and 3.3%, respectively [5]. For those pregnant women who do drink, 0.2 to 1.0% are classified as heavy drinkers [7]. Because alcohol readily crosses the placenta, alcohol ingested by the pregnant woman is delivered directly to her fetus. Unfortunately, the developing fetus has very little tolerance for alcohol. As a teratogen, alcohol interferes with proper brain and other organ development in

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the fetus [11]. Adverse fetal outcomes associated with prenatal alcohol ingestion include spontaneous abortion, fetal death, preterm delivery, low birth weight, growth abnormalities, mental retardation, smaller head circumferences, lower Apgar scores at 1 and 5 min, fetal alcohol syndrome (FAS) and fetal alcohol effects (FAE –also known as alcohol-related neurodevelopmental disabilities, ARND, or fetal alcohol spectrum disorders (FASD) [12].

The frequency, intensity and timing of drinking are factors influencing fetal outcome [13]. Even moderate consumption of alcohol during pregnancy is associated with adverse fetal outcomes, and drinking is especially detrimental if ingestion occurs during critical gestational stages of organ formation, especially the first 1 to 6 weeks of gestation [14–16]. Although no relationship has been documented between alcohol ingestion during pregnancy at a level of 0.5 oz absolute ethanol/day and infant developmental outcome, with each additional ounce of absolute alcohol consumed per day, the risk of spontaneous abortion increases by about 25% [12]. With higher alcohol exposures, alcohol-related infant effects are noticeable. In those mothers who average ≥ 5 drinks/occasion at least once per week, functionally significant deficits were evident in their prenatally alcohol-exposed infants [17]. The greatest incidence of infant cognitive deficiency occurs in pregnant women who engaged in binge drinking [7,16,18–20]. Findings of microcephaly at birth, heavy (four or more drinks on one occasion or seven or more drinks per week) episodic drinking during pregnancy, and a cumulative risk index identified those neonates at risk for FAS and FAE [21]. In those women who drink during pregnancy, 10% will have a child with FAS while a greater number will have a child with FAE [22]. Alcohol ingestion during pregnancy exceeding 3 to 5 drinks/day is associated with a 30 to 50% increased risk of delivering an infant with FAE [23]. Neonatal adverse effects were also related to maternal age; no significantly affected infants were born to women < 30 yr who were moderate to heavy drinkers [17]. In older women, the risk of infant impairment with alcohol ingestion increased 2 to 5 times [17].

Both FAS and FAE result from alcohol's effects on the developing fetal nervous system, causing structural, behavioral and cognitive abnormalities. FAS causes serious birth defects, including craniofacial abnormalities, microcephaly, growth deficiency, and difficulties in cognition, mental health and social interactions [24–25]. Signs and symptoms related to FAS that are identifiable at birth and during infancy are listed in Table 1 [24]. Children with FAS exhibit very characteristic facial features, including short eye slits, a thin upper lip, flattened cheekbones and an indistinct groove between the upper lip and nose [24]. Problems with intellectual functioning in FAS include difficulties in learning, memory, problem solving, speech, hearing and attention [24]. In a study of children born with FAS, correlated variables in addition to maternal alcohol consumption levels include older maternal age, lower maternal educational level, fewer months of prenatal care, fewer prenatal visits, prematurity, low birth weight, congenital malformations and lower Apgar scores [26]. FAE is much more difficult to diagnose because the affected child exhibits only mental impairment [25].

Table 1
Fetal alcohol syndrome (FAS) indices [24]

Birth
- Growth deficiencies (small body size and birth weight).
- Skeletal deformities (deformed ribs and sternum: curved spine; hip dislocations; bent, fused, webbed or missing fingers and toes; limited movement of the joints and small head).
- Facial abnormalities (small eye openings; skin webbing between the eyes and the base of the nose; drooping eyelids; short upturned nose; sunken nasal bridge; flat or absent groove between the nodes and the upper lip; thin upper lip; opening in the roof of the mouth; small jaw; and low-set or poorly formed ears).
- Organ deformities (heart defects: heart murmurs, genital malformations, and kidney and urinary defects).
- Central nervous system handicaps (small brain and faulty arrangement of the brain cells and connective tissue).

Infant
- Growth deficiencies (small body size and decreased weight, slower than normal development and failure in catch up).
- Facial abnormalities (nearsightedness and failure of the eyes to move in the same direction).
- Central nervous system handicaps (mental retardation — usually mild to moderate but occasionally severe; learning disabilities; short attention span; irritability in infancy; hyperactivity in childhood and poor body, hand and finger coordination).

Although the incidences of FAS and FAE are thought to be significantly underreported, the prevalence of FAS in the United States ranges from 0.3 to 5.6 per 1000 live-born infants while the estimates of FAE being even higher, on the order of about 1 in every 100 live births [5,27–29]. FAS and FAE represent two of the leading *preventable* causes of mental retardation and birth defects. The prevention strategies are clear: if the mother abstains from drinking alcohol immediately before conception and throughout pregnancy, these disorders are completely avoidable. In Healthy People 2000, thwarting alcohol use in pregnancy was addressed as an objective to decrease the incidence of FAS [30]. However, during the 1990s, both the use of alcohol and the amount ingested during pregnancy increased [30]. A more recent analysis of the alcohol statistics had found that the rate of alcohol use has declined but the rates of binge drinking and frequent drinking had increased [5]. Because no progress was made on the Healthy People 2000 initiative to decrease the incidence of FAS, the objective was incorporated into Healthy People 2010, along with other alcohol-related initiatives, including to decrease infant developmental disabilities, to decrease the incidence of low birth weight infants, and to increase in alcohol abstinence during pregnancy [30].

The effects of both FAS and FAE are permanent and irreversible. Since no cure or effective therapy exists, both the affected child and his/her family must endure a lifetime

of consequences resulting from the handicaps generated by *in utero* alcohol exposure. The associated costs of alcohol use during pregnancy are astronomical; health expenditures related to FAS alone are estimated between $75 million to $9.7 billion annually [31].

Since a safe blood alcohol level for the fetus has yet to be determined, the American Academy of Pediatrics and the American College of Obstetricians and Gynecologists recommend alcohol abstinence for all women who are pregnant. Since the worst alcohol-related damage occurs to the embryo even before the pregnancy is clinically recognized, alcohol abstinence is also recommended before conception.

Women who abuse alcohol are less likely than male drinkers to be recognized and treated for alcohol problems by their physicians. When abuse is recognized, women typically have more severe alcohol-related health issues than men, indicating delayed intervention [32]. When women are identified as having alcohol problems, a greater number of them accept counseling and thus have a better chance of recovery. Educating women on the risks of alcohol to her unborn child may be more successful during pregnancy when women are particularly receptive to intervention [7]. Prenatal visits provide an educational avenue to initiate intervention for those women exhibiting high-risk behaviors. Sadly, only one in four women reported that their health care provider informed them of the harmful effects of alcohol on their developing fetuses [9]. When pregnant women were alerted to alcohol's adverse fetal effects during a one-hour motivational interview, 81% significantly reduced their alcohol consumption and their peak intoxication level [33]. One interventional alcohol treatment program found a significant decrease in both chronic and binge-drinking episodes in women aged 18 to 40 yr over the 4-yr follow-up period [34]. In those study participants who became pregnant during the trial, the greatest decreases in alcohol consumption were seen [34]. Significant effects were attainable through brief intervention - two 15 min, physician-delivered counseling visits [34]. An interventional program initiated at 16 weeks gestation found that those women who selected abstinence as their alcohol reduction goal and who identified FAS as their reason for alcohol avoidance showed the greatest reduction in alcohol consumption [8]. Healthcare providers must emphatically motivate and support pregnant women in their efforts to change their high-risk alcohol lifestyle behaviors [33,35]. Intervention services should address those barriers to recovery that are typically gender-specific, such as child rearing [36]. Additionally, referral to women-only alcohol treatment sessions has been shown to improve dropout rates and outcomes [26].

2. Alcohol Use Questionnaires

Identification of alcohol use early in pregnancy is important to reduce the risk of adverse alcohol-related fetal outcomes, and the reduction of alcohol ingestion both before and during pregnancy has the potential to reduce the incidence of FAS and FAE. One alcohol screening method involves the administration of a questionnaire that identifies high-risk behaviors indicative of alcohol abuse. Most of these questionnaires have been validated only in men and the queries into high-risk behavior consequences may not apply to women, especially those who do not work outside the home. A literature review of standard alcohol screening questions administered to women found that the AUDIT (Alcohol Use Disorders Identification Test) questionnaire gave one of the best performances [37]. The AUDIT questionnaire was developed through a 1988 World Health Organization-sponsored project. This test is designed to indicate the severity of alcohol abuse in three categories - the harmfulness of the drinking pattern, how much harm is currently being experienced and whether the indicated drinking pattern implies some degree of physical or psychological dependence on alcohol [38]. The AUDIT questionnaire has the advantage over others because it provides additional insights into women's alcohol consumption and symptoms of dependence. Because of women's decreased tolerances for alcohol, a lower positive screening scoring cut-off is recommended, such as a cut-off of ≥ 4 points (0–40 total points possible) for the AUDIT questionnaire instead of the currently recommended cut-off of ≥ 8 points [37].

Using self-report to diagnose alcohol use in pregnant women may be unsuccessful; pregnant women are influenced by societal pressures to misrepresent or deny their alcohol intake because of the stigma associated with drinking during pregnancy. The use of biochemical markers is useful as a corrective tool to self-reports of alcohol consumption, especially, in those groups exhibiting a high degree of deception [39–40]. In abstinence-orientated treatment programs, self-reports were validated through comparison with the biologic markers, γ-glutamyl transferase (γGT) and carbohydrate deficient transferrin (CDT) [41]. AUDIT and CDT were found to be complementary instruments for alcohol screening because each uses independent criteria for identifying those engaging in abusive drinking behaviors [42].

3. Prenatal Indicators of Fetal Alcohol Syndrome (FAS)

To date, there are no standardized criteria for the prenatal diagnosis of FAS or FAE. *In utero* ultrasound findings exist that are suggestive of FAS, but none definitive, thus permitting only the exclusion of FAS [43]. The classic ultrasound results that are indicative of FAS are listed in Table 2. Ultrasound studies in the second trimester undercover fetal structural abnormalities while an ultrasound examination in the third trimester assesses fetal intrauterine growth retardation, both alcohol-associated findings.

Table 2
Prenatal ultrasound results indicative of fetal alcohol syndrome

- Symmetrical intrauterine growth retardation
- Organ defects (hypertelorism, cardiac anomalies, renal anomalies)
- Skeletal and rib abnormalities (micrognathia, footlength)
- Vascular abnormalities (presence of a single umbilical artery, hydraminos).

4. Diagnosis of Fetal Alcohol Syndrome and Effects

A craniofacial anomalies tally taken at birth is a sensitive indicator of fetal alcohol exposure [44]. The Fetal Alcohol Survey Group of the Research Society on Alcoholism proposed criteria for the diagnosis of FAS [45]. The finding of one abnormality from each of three categories, for a total of three criteria, is required to make the diagnosis of FAS. The criteria in each category are listed in Table 3 [45].

To date, the diagnosis of FAE cannot be made reliably until the child is school-aged.

5. Alcohol Biochemistry

Genetic factors and gender influence alcohol metabolism and its subsequent effects [15]. In the body, ethanol is first metabolized to acetaldehyde, primarily by the enzyme alcohol dehydrogenase. Besides the major isoenzyme present in the liver, there is also a minor stomach isoenzyme exhibiting lower activity. After the consumption of one alcoholic drink, blood alcohol concentrations reach peak levels within 30 to 45 min. With increased alcohol ingestion, blood alcohol concentrations peak later and reach much higher levels because the rate of alcohol absorption through the intestine exceeds its hepatic liver metabolism. Acetaldehyde is metabolized to acetate and eventually to carbon dioxide and water for elimination from the body. The half-life of blood alcohol is 4 h, and generally by 8 to 10 h after ingestion, ethanol has been metabolized and excreted [46]. Before its complete metabolism, a small quantity of alcohol is detectable in breath and body fluids.

Because of acetaldehyde's extreme reactivity, not all of it is metabolized to acetate. Acetaldehyde also forms both covalent and noncovalent adducts with proteins, with the reversible adducts converted to irreversible complexes within 3 days. The reversible adducts are responsible for the adverse dose and time dependent cytotoxic and neurotoxic effects of alcohol while the irreversible adducts cause enzyme inactivation [14–15]. Acetaldehyde is transported in the bloodstream by erythrocytes and plasma proteins. This reversibly bound, transported acetaldehyde can be transferred to other cells, explaining the increased cancer risk associated with alcohol abuse [47]. Additionally, antibodies to these acetaldehyde-modified proteins may contribute to the tissue damage seen in alcoholics [48].

Table 3
The Fetal Alcohol Survey Group of the Research Society on Alcoholism criteria for the diagnosis of fetal alcohol syndrome (FAS) [45]

1. Prenatal and/or postnatal growth retardation; failure to thrive, defined as weight, length and/or head circumference $<10^{th}$ percentile).
2. Central nervous system involvement including signs of neurologic abnormalities (irritability in infancy and hyperactivity during childhood), developmental delay, hypotonia or intellectual impairment (mild to moderate mental retardation).
3. Characteristic facial dysmophology (at least two out of three).
   a. Microcephaly (head circumference $<3^{rd}$ percentile).
   b. Microphthalmia and/or short palpebral fissures.
   c. Poorly developed philtrum, thin upper lip (vermillion border) and flattening or absence of the maxilla.

6. Biochemical Markers

Accurate and reliable biochemical markers for alcohol use would be very valuable for identifying women who drink alcohol and for determining the risk of FAS and FAE in pregnant women. Although all women should be counseled to avoid alcohol during pregnancy, it is clear that some women will continue to drink throughout gestation, putting their fetuses at high risk for adverse alcohol-related effects. If accurate biochemical markers of alcohol use were available, they could be used to identify those at-risk women needing more aggressive intervention. Validated biomarkers are also important for identifying those women who abuse alcohol before pregnancy so that intervention can be initiated before conception. Markers of alcohol ingestion are given in Table 4 [49].

Because the frequency, intensity and duration of alcohol consumption can vary from individual to individual, a panel of biochemical markers for identifying the alcohol abuse - acute and chronic - is needed. Alcohol, determined in either blood, urine or exhaled air, serves as a marker of acute alcohol ingestion. Determination of breath alcohol provides an almost immediate indication of the subject's alcohol status. Since alcohol and its metabolites are detected in blood and urine for only a few hours after consumption, identification of nonacute alcohol abuse must be determined through the objective analysis of biochemical markers reflective of the changes or damage caused by alcohol or its metabolites.

Markers showing historical promise for detecting chronic alcohol abuse are HAA, γGT, MCV and CDT. All four markers are indirect assessments of alcohol consumption and the elevations of γGT and MCV are related to alcohol-induced organ lesions [50].

6.1. Gamma-glutamyltransferase (γGT)

γGT is an enzyme produced predominately in the liver. It is involved in glutathione metabolism and in the renal reabsorption of amino acids. Increased serum levels of γGT serve as an indicator of hepatobiliary disease. It is a sensi-

Table 4
Markers of alcohol ingestion [49]

Acetaldehyde
Acetaldehyde-modified albumin
Acetaldehyde-protein adducts
Acetate, blood
Acetone
Adenylyl cyclase
Alanine aminotransferase
Aldehyde dehydrogenase, RBC
Alkaline phosphatase, intestinal
Alpha-amino-n-butyric acid
Alpha-amino-n-butyric acid / leucine ratio
Apolipoproteins
Apolipoprotein E, sialic acid deficient
Aspartate aminotransferase (AST)
Carbohydrate deficient transferrin
Dolichol, urinary
Early detection of alcohol consumption score (EDAC)
Ethanol
Fatty acid ethyl esters
Gamma-glutamyl transferase
HDL cholesterol
Hemoglobin-associated acetaldehyde
β-Hexosaminidase
β-Hexosaminidase isoenzyme B activity
5-Hydroxytryptophol
5-Hydroxytryptophol/5-hydroxyindole-3-acetic acid ratio, urinary
IgA reactivity with acetaldehyde-modified proteins
Mean corpuscular volume
Mitochondrial AST
Phosphatidylethanol
Platelet adenylyl cyclase activity
Sialic acid
Sialidase

tive marker of alcohol ingestion, especially when chronic alcoholic liver disease results. Elevations are not specific for alcohol damage since increases also occur in liver disease and with certain drugs [48,51].

6.2. Mean corpuscular volume (MCV)

Alcohol and its metabolites have toxic effects on the production of hematologic precursor cells and on red cell morphology. Macrocytosis, enlarged erythrocytes, is a common finding in chronic alcoholics. MCV is an average estimate of the volume of erythrocytes and serves as an indicator of macrocytosis [52].

6.3. Hemoglobin-associated acetaldehyde (HAA)

HAA formation peaks 30 min post alcohol ingestion as the reactive acetaldehyde, generated by the action of oxygenated hemoglobin on alcohol, initially forms a reversible adduct with erythrocyte hemoglobin [14,47,53–54]. These HAA reversible complexes, detectable in the bloodstream for up to 48 h after the last drink, are converted to irreversible HAA [14,47]. The irreversible HAA accumulates with time and is still detectable in the bloodstream for at least 28 days [53–54].

HAA levels in males are greater than those found in females but the gender difference is explained by the sex-dependent hemoglobin concentrations [55–56]. HAA levels in alcohol abusers have been found to be significantly higher than levels found in teetotalers [55]. HAA increases significantly with alcohol ingestion, even after a single high dose of ethanol (2 g/kg), when the conventional markers, γGT and MCV, show no change [57]. HAA was found to have better sensitivity and specificity than γGT, ALT, AST and MCV in one alcohol treatment program [56]. HAA has high specificity and serves as a good marker of alcohol abuse in women [57]. In pregnant women, the highest concentrations of HAA were found in those women who subsequently delivered an infant with FAE [23].

6.4. Carbohydrate-deficient transferrin (CDT)

Variations from a protein's "normal" glycosylated pattern are potentially indicative of pathologic states. The most sensitive and specific biologic marker of chronic alcohol abuse is carbohydrate-deficient transferrin (CDT), a modified form of transferrin [58]. Transferrin (Tf) is the body's major iron transfer protein, ferrying iron from sites of absorption and storage to sites of utilization. This 80,000 Dalton glycoprotein, consisting of a single polypeptide chain, is synthesized and metabolized in the liver. Posttranslational modification of this protein results in the addition of two asparagine-linked carbohydrate moieties containing up to three sialic acid residues each to the native transferrin molecule [59]. The major form of modified transferrin present in the bloodstream is the tetrasialoTf, with small amounts of tri- and disialoTf also being present [60].

Clinical studies have shown that transferrin isoforms that are deficient in sialo residues and/or carbohydrate moieties are indicative of chronic alcohol ingestion [61]. CDT, technically defined as transferrin isoforms having pIs $\geq$ 5.7, consists of the a-, mono- and disialoTF isoforms; tetrasialoTf has a pI of 5.4 [59,62].

The mechanism of CDT formation in alcohol abuse is proposed to involve the acetaldehyde-mediated inhibition of protein glycosylation processes, a change in protein transport, the inhibition of protein synthesis, and/or the increased trimming of completely glycosylated transferrin [59]. Alcohol metabolites modify and subsequently inactivate the enzyme hepatic glycosyl transferase, which is responsible for forming glycosylated transferrin; loss of this protein's activity leads to the formation of CDT [62]. Another study has proposed that chronic alcohol ingestion down regulates the expression of sialyltransferase and destabilizes its mRNA, thus resulting in decreased hepatic synthesis of sialyltransferase, the enzyme responsible for sialylation of transferrin's carbohydrate moieties [62–63]. Additionally, alcohol ingestion results in increased sialidase activity, causing increased removal of sialic acid residues from modified

transferrin [62]. Besides the loss of transferrin terminal sialic acid residues, alcoholics with liver disease may also be deficient in transferrin asparagine-N-linked oligosaccardies [61]. The half-life of CDT is 17 ± 4 days, resulting its prolonged presence in the bloodstream after its formation [64].

The appearance of CDT in the bloodstream is induced by the ingestion of 60 g (one bottle of wine or five beers) of alcohol daily for at least seven days [14]. Even moderate consumption of alcohol, such as 375 mL of wine/day for 4 weeks, causes significant elevations in CDT in healthy individuals [65]. Because of their lower tolerance for alcohol when compared to men, several researchers have recommended the use of different cutoffs for alcohol ingestion to indicate excessive consumption in women, including 30 and 40 g/day, respectively [66–67].

A serum CDT level < 60 mg/L is normal, between 60 to 100 mg/L is indicative of probable alcoholism while > 100 mg/L implies a very high probability of alcohol abuse [64]. Because CDT was found to increase during gestation, a higher cut-off, 33 U/L for teetotaler pregnant women instead of the 26 U/L recommended for abstinent nonpregnant females, is suggested [68]. Note that different methods report CDT in different units, such as mg/L or U/L. No established correlation between the units exists for CDT so interconversion is difficult.

In men, CDT has adequate sensitivity and specificity to detect chronic alcohol abuse because its response to alcohol dosing is continuous, even within the range of alcohol considered nonhazardous [65]. In fact, a 10% change in CDT is diagnostically sensitive and specific enough to detect a change of at least two standard drinks per day in men [69]. CDT was found to respond to moderate changes in alcohol consumption in alcohol-dependent patients [39]. It those alcoholics who relapsed following a period of abstinence, CDT very quickly attained elevated levels again [66].

In women, the diagnostic utility of CDT receives mixed reviews [70–71]. Generally, CDT exhibits lower diagnostic sensitivities and specificities for the detection of alcohol abuse when compared to men [72]. CDT is theorized to have decreased clinical utility in women because CDT formation is related to hormonal and iron status [62,72–74]. CDT is decreased in postmenopausal women and in women on oral contraceptives [72–73]. Increased levels of CDT are associated with pregnancy and the premenopausal state; in fact, CDT concentrations vary with the phase of the menstrual cycle with higher levels occurring during menstruation [73]. Blood loss during menstruation may explain the higher CDT values associated with premenopausal women [72–73]. In women, CDT levels appear to be influenced by drinking pattern, with drinking intensity (drinks/day) being a stronger influence than frequency (number of days drinking) [75–76]. In males, both the intensity and frequency of alcohol ingestion strongly affect CDT concentrations [76–77].

In an interestingly aside, in patients admitted to ICU following trauma, those who had an elevated CDT (> 20 U/L on ER admission) experienced prolonged ICU stays and more complications when compared to those whose CDT levels were < 20 U/L [78]. These findings have been generalized to pregnant women and their offspring in predicting alcohol-associated complications; however, there are no outcome studies validating this generalization [78].

Possibly the identification of a specific isoform of CDT, by capillary electrophoresis (CE), may serve as the best predictor of chronic alcohol abuse in women, thus increasing its diagnostic utility. Transferrin isoforms are generated differentially in response to alcohol and that results are gender specific [79]. Women who consumed low levels are alcohol had increased levels of a- and monosialoTf when compared to male low-level alcohol consumers [80]. Women who were high or chronic alcohol consumers, when compared to women who were low alcohol consumers, showed a 68% and 249% increase in asialoTf, a 36% and 58% increase in monosialoTf, a 54% and a 225% increase in disialoTF and a 52% and 192% increase in total CDT, respectively [81]. Tri- through hexasialoTfs were not increased in women drinkers [81]. Studies evaluating the efficacy of CDT for detecting alcohol abuse in pregnant women are limited. But the determination of an individual CDT isoform may serve to increase the diagnostic sensitivity and specificity of this test in pregnant women who abuse alcohol.

6.5. CDT by capillary electrophoresis (CE)

CDT analysis by CE allows for the separation, identification and quantitation of the individual CDT isoforms. In a typical method for the determination of CDT isoforms, serum samples are first pretreated with Fe^{+3} to eliminate charge differences between transferrin molecules due to percent iron saturation [82–83]. Interference caused by complement C3 co-migrating with the CDT isoforms is eliminated by one of two ways. Use of inulin to activate the alternate complement pathway converts complement C3 into its degradation products [84]. Alternatively, the use of a mobile phase containing anti-C3c antibodies eliminates the coincident electrophoretic mobilities [85]. The pretreated sample is injected under positive pressure into a fused-silica capillary [82–83,85]. Capillary zone electrophoresis separation utilizes a mobile phase containing sodium tetraborate buffer at pH 8.3 to produce the endoosmotic flow and diaminobutane to minimize protein-wall interactions [82–83,86]. In-line ultraviolet detection of the separated CDT isoforms is done at 214 nm [82–83,85]. This analysis, performed under nondenaturing conditions, produces baseline resolution of the a-, mono-, di- tri- and tetrasialoTf isoforms as well as detection of genetic Tf mutants and carbohydrate-deficient glycoprotein syndromes (CDGS) [82–89]. CDGS are a group of genetic, multisystem diseases characterized by the deficiency of terminal

N-acetylneuraminic or sialic acid residues in posttranslationally modified proteins [90–92].

6.6. CD by immunoassay

The majority of CDT analyses are performed using immunoassays. However, these methods suffer from recovery and specificity issues and the measurement of undesired isoforms. Immunoassays require specimen pretreatment before analysis that causes decreased recoveries of the a- and disialoTf residues [93]. False positive results may occur in patients with primary biliary cirrhosis, chronically active hepatitis, carbohydrate-deficient glycoprotein syndrome and genetic D-variants of transferrin [94]. The immunoassay methods for CDT detection in females have average sensitivities of 52% with 92% specificity [95]. Cross-reaction with trisialoTf, not considered "CDT" because it contains more than the 0 to 2 sialo residues required by definition, is typically a problem in these immunoassays [93,96–97].

A typical immunoassay method for total serum CDT involves saturating serum with Fe^{+3}, applying the treated sample to an ion exchange column to chromatographically separate the CDT isoforms, and quantitating the CDT isoforms and total transferrin through turbidimetry using anti-transferrin antibodies.

7. Biomarker Diagnostic Performances

The diagnostic efficacy of CDT, γGT, MCV and HAA as markers of alcohol abuse appears to vary from study to study [2,40,50,56,58,64,67–72,95,97–131,134,139,143,146–148, 150]. Confounding variables which influence these markers' diagnostic performance include gender, age, pregnancy, hormonal (estrogen) status, alcohol status (frequency, intensity, withdrawal, relapse, abstinence), disease states, smoking, certain medications, body mass index, hemoglobin concentration, iron concentration, methodology, and certain metabolic disorders [40–41,60,64,67–68,70,72–77,80,90–91,99,101–102, 105–106,132–142]. One important advantage of CDT over conventional markers such as γGT is that its relative specificity for excessive alcoholism detection remains high in the presence of other pathologies, most notably, liver disease [58,80,143].

The confusion surrounding CDT's diagnostic utility was clarified recently with the finding that the diagnostic performance of CDT is method dependent [62]. The newer, modified immunoassays for CDT are not as diagnostically accurate as the original immunoassays [144]. Insufficient data are available to assess the promising newer methods of CDT analysis, including chromatofocusing, HPLC, fast protein liquid chromatography (FPLC), isoelectric focusing (IEF) and capillary electrophoresis (CE) [144]. CE methods for CDT offer the advantage of separation and quantitation of the individual isoforms, are relatively rapid and do not require any significant specimen pretreatment. CE also detects CDT genetic variants that generate false positives in immunoassay procedures [145].

Unfortunately, the diagnostic performances of CDT, γGT and MCV are too low for general population screening [104,134]. An increase in diagnostic performance is achieved by using markers in combination, since each is an independent predictor of alcohol abuse [40,101,104, 135,139,146–150].

8. Biochemical Marker Studies in Pregnancy

Studies evaluating the efficacy of these markers for detecting alcohol abuse in women, especially pregnant women, are also limited. Similar to alcoholism studies conducted in men, the best marker for women varies by study [39,101,103,106]. The use of a panel of biomarkers composed of CDT, γGT and MCV increases the diagnostic sensitivity of each marker taken individually, especially in women [101,135,147,150].

One study to assess the biochemical markers HAA, CDT, GGT and MCV for their ability to detect alcohol use in pregnant women found that infants born of mothers having ≥ 2 positive markers had significantly smaller birth weights, lengths and head circumferences than infants born to mothers who were negative for all markers [151]. The presence of ≥ 2 positive markers was more predicative of fetal outcome than any self-report measure [151]. Another study correlated CDT, γGT, MCV and HAA with excessive alcohol consumption during pregnancy and the risk of delivering an infant with FAE. Here, γGT and MCV were found to be the better markers [68]. A third study correlated alcohol abuse in pregnancy with the biomarkers γGT, MCV, ALT and AST. γGT was found to be the best indicator of abusive drinking [103]. Note that CDT was not evaluated in this study [103].

Current biochemical markers of chronic alcohol use are not as diagnostically effective in women when compared to men. Studies evaluating the efficacy of biochemical markers for detecting alcohol use in women, especially pregnant women, are very limited. Therefore, validated biomarkers having greater diagnostic sensitivity and specificity for detecting alcohol use in pregnant women are needed. Since none of the conventional biomarkers have adequate diagnostic sensitivity and specificity alone to detect alcohol use in pregnancy, a panel of biomarkers is probably the best approach.

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