A pilot study to determine the feasibility of measuring the baseline level of excessive alcohol intake during pregnancy in the west of Scotland

FINAL REPORT

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Final report: This document completes the reporting of the pilot study, superseding the interim-report written before results from the Ethyl Glucuronide were concluded.

KEY FINDINGS

Based on the levels of Carbohydrate-deficient transferrin (CDT) – a biomarker of chronic alcohol use – approximately 5% of the study sample of pregnant women had results *suggestive* of recent hazardous alcohol use. This is in-line with the estimated prevalence figures. Ethyl Glucuronide – a biomarker of acute alcohol use – could not be detected, possibly related to the age of the samples (4 to 5 years old).

These results suggest that CDT, but not EtG could be used in a larger, representative study to determine baseline hazardous alcohol consumption in pregnant women at a population level.

INTRODUCTION

Reports show that there has been a significant increase in the number of women of child bearing age who drink heavily (1-2). Studies from America and Sweden identified heavy drinking in approximately 1-2% of pregnant women (3-6).

Fetal Alcohol Syndrome (FAS) is recognized in the developed world as the leading preventable cause of developmental and cognitive disability. According to the literature, the overall combined rate of FAS and Fetal Alcohol Spectrum Disorders (FASD) is estimated to be about 7-18 /1,000 births across various populations (7,8). Both the diagnosis of FAS/FASD soon after birth and the direct measurement of alcohol in the mother are difficult, posing challenges for the evaluation of interventions aimed at reducing dangerous antenatal alcohol consumption.

Ethyl Glucuronide (EtG), a minor metabolite of alcohol, is present in the urine for up to 5 days after consumption (9-15) but probably only for 24 hours in blood, possibly having utility as a marker of acute alcohol consumption. Carbohydrate-deficient transferrin (CDT) (16,17) is a marker of chronic alcohol consumption (normalising 2-4 weeks from the start of abstinence). In a pregnant population with relatively low prevalence of heavy drinking, there are challenges to using these biomarkers in isolation to accurately identify hazardous alcohol use in pregnancy. A two-stage biomarker system – which measures both CDT and EtG, would increase the positive predictive value – allowing the identification of heavy drinking in a pregnant population, albeit with a reduced sensitivity.

AIMS AND PURPOSE

The aim of this pilot study was to test the utility of a two assay system (CDT and EtG) in determining the baseline prevalence of heavy alcohol consumption in a pregnant population in the West of Scotland.

APPROACH AND METHODS

Study population: approximately 18,000 women per year from the west of Scotland undergo second trimester maternal serum screening at the West of Scotland Regional Genetics Service (WSRGS), representing about 2/3 of all registered pregnancies. For each woman undergoing screening a residual serum sample taken at 15-16 weeks gestation is available for testing. A sample of 150 pregnant women (2006 and 2007) from the most deprived postcodes of Glasgow, who were sampled on a Monday, were selected for analysis. Women from deprived regions were expected to have the highest prevalence of heavy drinking, based on the strong relationship between deprivation and health-related behaviours. Selecting samples taken on a Monday maximises the chance of detecting heavy drinking; on the assumption that pregnant women who drink heavily are more likely to do so at the weekend.

Laboratory analysis: Frozen residual serum samples were dispatched to the laboratory of Dr Roy Sherwood at the Clinical Biochemistry Department, King's College Hospital (London) with only a laboratory number for identification. All samples were assayed for both CDT (The Capillarys (Sebia, UK), a capillary electrophoresis assay) and EtG (LC-MS/MS, using a Jasco high performance liquid chromatography coupled to a triple quadruple mass spectrometer (Applied Biosystems, Cheshire, UK)). With a view to a future larger, representative study in several years time it was considered important to use a laboratory which regularly assays for both EtG and CDT (reducing effects of operator variation), and has the capacity to assay thousands of samples. This laboratory fulfils both of these criteria and is used by the DVLA to assess abstinence from alcohol for drivers banned from driving who apply to have their licence re-instated.

Sample size: Heavy alcohol consumption in the general pregnant population is estimated to be around 2% (4-7), and 3-4% in the most deprived regions of Glasgow [see Box 1, Appendix]. In the sample of 150 women we expected 4-5 samples with high CDT and/or EtG, the remainder either having lower or undetectable levels. Comparison with reference ranges from known non-heavy drinkers served as negative controls.

Research outcomes: This pilot study aimed to identify if the two assay system, involving CDT and EtG, could be used at the population level to determine the baseline prevalence of heavy drinking in pregnant women, in preparation for a larger, representative epidemiological study.

FINDINGS

Samples from the most deprived 23% of postcodes in Glasgow City (as determined by the Scottish Index of Multiple Deprivation (SIMD)) were selected for analysis. Characteristics of the 150 selected samples are shown in Table 1.

Table 1: Baseline characteristics of the 150 study samples at "booking" appointment

Characteristic	N (%)	Mean	Median	Minimum	Maximum
Gestation (wks)		17	16	15	21
Maternal weight (kg)		69.9	65.0	41.0	139.0
Maternal smokers*	48 (32)				
Maternal age (at EDD, yrs)		26	25	16	40
SIMD Score (maternal residence)		71	71	57	88

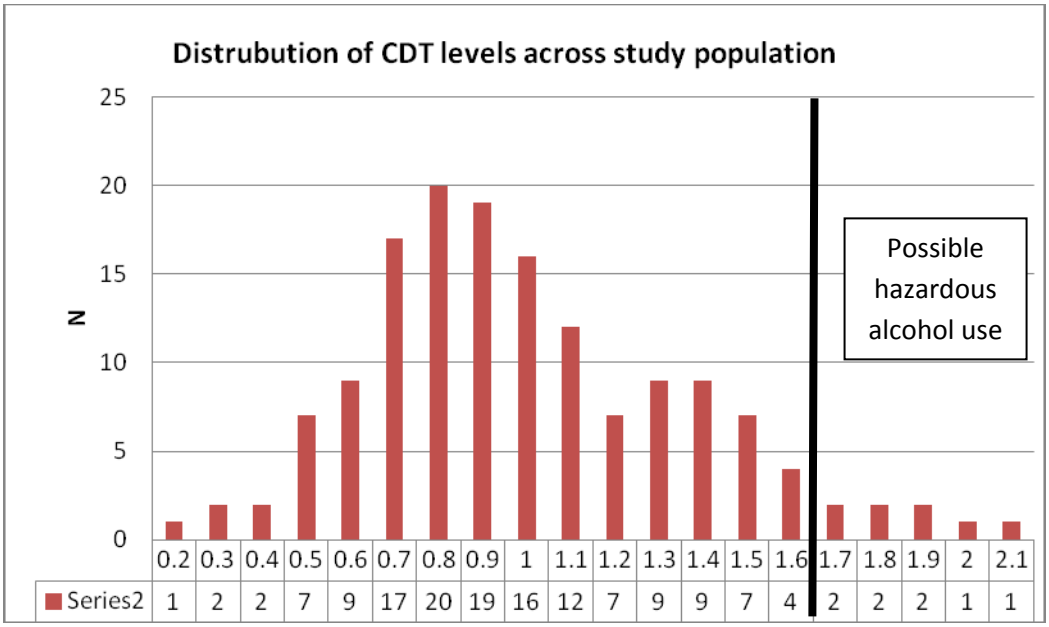
* n=6 smoking status unknown

A considerably larger proportion of mothers from the pilot sample reporting smoking compared to the Scottish average of 18% (ISD Scotland) and all were residing in the most deprived decile in Scotland (score range of 45-89).

Chronic hazardous alcohol use

Carbohydrate-deficient transferrin levels were obtained from 149 samples. The distribution of CDT levels across the pilot samples is shown in Figure 1. CDT is measured as a percentage of total transferrin.

Figure 1:



Levels above 1.6% were taken to indicate *possible* chronic hazardous drinking and levels above 1.9% were considered indicative of chronic hazardous drinking.

Five percent of the pilot sample had CDT levels indicative of *possible* chronic hazardous drinking (Table 2), of these n=2 (1%) had levels indicative of *probable* chronic hazardous alcohol consumption (Figure 1).

Table 2: CDT levels in the study population

Range	Description	N	Valid %
<1.7	"Normal"	141	95
1.7+	*Possible* chronic hazardous drinking	6	4
3.0+	*Probable* chronic hazardous drinking	2	1
Missing		1	-
Total		**150**	100%

Acute hazardous alcohol use

Ethyl Glucuronide – a marker of acute alcohol use – could not be detected in any of the samples. Although the lack of EtG may be a result of true negative results, there was evidence of protein participation suggestive of sample degradation. Sample degradation is likely to result in the loss of assay sensitivity and may be related to the age of the samples (4 to 5 years old).

If possible, the samples will be run on another machine (Thermo TSQ Vantage LC/MS/MS) with much greater sensitivity - to determine if there are samples positive for EtG in the pilot population. It would not be possible to use this machine routinely.

CONCLUSION

Based on the CDT results alone – 5% of the sample have results suggestive of *possible* hazardous alcohol consumption. This is in-line with the estimates from the epidemiological data (see appendix). Our laboratory work suggests that the EtG assay is not a sufficiently robust assay to be used on older samples.

This pilot aimed to identify an assay system which could be used in an epidemiological study using anonymised samples to measure and monitor prevalence of hazardous alcohol consumption (at the second trimester) in pregnant women – at a population level. As such, the assay will need to produce reliable, repeatable estimates on samples several years old. This pilot suggests that CDT but not EtG could be used to achieve this. The use of one assay is likely to be less sensitive than the initially conceived two-assay system – i.e. a smaller proportion of the samples from women drinking hazardous levels of alcohol will be identified. This may have implications for the size of the study needed to provide an accurate baseline estimate of hazardous alcohol consumption.

Implications for future studies:

We aim to run a larger, representative study of maternal serum samples to provide a baseline estimate of the prevalence of hazardous alcohol consumption in pregnancy in the west of Scotland using the CDT assay, with similar studies repeated periodically allowing a pattern of trends in hazardous alcohol consumption to be built.

Practicalities: To obtain reproducible results, minimally affected by operator variation, it is important to use a laboratory that routinely runs the CDT assay and has the capacity to run several thousand samples. The laboratory run by Dr Sherwood is one of the few facilities in the UK which fulfils these criteria evidenced by its use by the DVLA to assess abstinence from alcohol for drivers banned from driving who apply to have their licence re-instated.

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APPENDIX

Box 1: Deriving a "Best Guess" estimate of the prevalence of heavy drinking in the most deprived regions of Glasgow, using two approaches:

(1) Based on smoking figures alone: in the three most deprived regions of Glasgow smoking during pregnancy is approximately 60-80% higher than the Scottish average (source: GCPH profiles). If we assume that heavy drinking during pregnancy has a similar excess then we might expect the prevalence of heavy drinking in these areas to be around **3.2-3.6%.**

(2) Based on drinking and smoking figures:

(a) *Based on smoking data, the prevalence of hazardous behaviour in pregnancy is estimated to be approximately **5-20%** less than the prevalence of the same behaviour in the general (non-pregnant) population.* Generally the prevalence of smoking in the pregnant population is less than that for smoking in the general population (e.g. in Castlemilk 45% of the general population smoke (males & females) compared to 40% in the pregnant population). Across the three most deprived neighbourhoods listed above, smoking in the pregnant population is approximately **5-20%** lower than in the non-pregnant population (e.g. for Castlemilk the difference is 13%, [(45-40)/40]).

(b) Using alcohol related deaths to examine the difference in alcohol use by area deprivation, the excess (compared to national figures) in alcohol related deaths in these three areas is approximately 130-166%. Excesses in women are likely to be half that of men (based on the sex patterning in alcohol related GP consultations [ongoing work, DeS]), giving an estimated excess of alcohol related deaths in these neighbourhoods of around 60-80% compared to the Scottish estimates. The excess of heavy drinking during pregnancy is likely to be lower still, based on that seen with smoking figures (2a, above). Reflecting this, the estimated excess in heavy drinking of 60-80% can be reduced by a further 5-20% giving an estimated excess of heavy drinking during pregnancy of 48-76% in the most deprived neighbourhoods compared to Scottish figures. Applying this to the prevalence of hazardous alcohol use in pregnancy, derived from the literature (i.e. 2%), an estimated **3-3.5%** of the pregnant population in these most deprived neighbourhoods will consume hazardous levels of alcohol.