Toxicology Letters 275 (2017) 49–56

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Ethylglucuronide in the urine as a marker of alcohol consumption during pregnancy: Comparison with four alcohol screening questionnaires

Giampiero Ferraguti[a], Paola Ciolli[b], Valentina Carito[c], Gemma Battagliese[d], Rosanna Mancinelli[e], Stefania Ciafrè[f], Paola Tirassa[c], Rosaria Ciccarelli[d], Alessia Cipriani[b], Marisa Patrizia Messina[d], Marco Fiore[c,*], Mauro Ceccanti[d]

[a] Department of Cellular Biotechnologies and Hematology, Sapienza University of Rome, Italy
[b] Dipartimento di Scienze Ginecologico-Ostetriche e Scienze Urologiche, Sapienza University of Rome, Italy
[c] Institute of Cell Biology and Neurobiology (IBCN-CNR), Rome, Italy
[d] Centro Riferimento Alcologico Regione Lazio, Sapienza University of Rome, Italy
[e] Centro Nazionale Sostanze Chimiche, ISS, Rome, Italy
[f] Institute of Translational Pharmacology (IFT-CNR), Italy

ARTICLE INFO

Keywords:
Ethylglucuronide
Alcohol-screening questionnaires
AUDIT-C
T-ACE
TWEAK
Food diary

ABSTRACT

Ethyl glucuronide (EtG) is an ethanol metabolite and EtG is used as a biomarker of alcohol drinking. EtG can be detected in the blood and in several biological matrices including urine, hair and nails. Alcohol consumption during pregnancy is a strong risk factor for fetus health so in the recent years different strategies to reveal alcohol use have been planning including the use of screening questionnaires as the AUDIT-C, T-ACE and TWEAK. The present study aims to investigate in pregnant women the specificity and predictive value of the AUDIT-C, T-ACE and TWEAK plus a food diary in use in Sapienza University Hospital compared with the results of urine EtG measurement. Seventy pregnant women were enrolled and examined. Urine samples were provided by pregnant women immediately after the interviews. EtG determinations were performed by Enzyme Immunoassay with a cut-off established at 100 ng/mL.

Data show that 34.28% of the enrolled pregnant women overcame the EtG cut off. No direct correlation was found between EtG data and the alcohol screening interviews showing lower levels of alcohol consumption, although T-ACE revealed the same at risk percentage. However, a significant concordance was observed with food diary data and T-ACE only in patients with higher EtG urinary concentration. This study provides clinical evidence that the diagnosis of maternal alcohol consumption during pregnancy only based on indirect methods, such as questionnaires and food diary, may significantly underestimate alcohol use.

1. Introduction

The negative and highly harmful effects of drinking before and during pregnancy are well documented and known for some time both in animal models (Ceccanti et al., 2016; Fiore et al., 2009) and humans (Abel, 2009; Murawski et al., 2015; Ornoy and Ergaz, 2010). Recently, the impact of genetic factors in modulating behavioural and physical effects of prenatal alcohol consumption has been taken into consideration. Neurochemical experiments have shown that genetic factors could be responsible for selective susceptibility to behavioral alterations induced by developmental alcohol consumption (Cagiano et al., 2002). It has been also shown that maternal CYP17 genotype modulates the association between prenatal alcohol exposure and intrauterine fetal growth (Delpisheh et al., 2008). Moreover, the role of genetic variants involved in the neurobiology of addiction as well as those involved in alcohol metabolic pathways and in response to medication in alcoholism therapy still represent arguments to further investigate (Ferraguti et al., 2015). Other studies have also yielded some insights into epigenetic mechanisms affecting brain development and how these processes can be related to prenatal alcohol exposure (Ungerer et al., 2013).

It is well known that women are physiologically more vulnerable to the effects of alcohol and drinking alcohol during pregnancy exposes the unborn child to a toxic substance to which the fetus has no tolerance, that may be the cause of adverse fetal events known as Fetal Alcohol Spectrum Disorders (FASD) (Mancinelli et al., 2013; Mattson et al., 2011). FASD is a collection of diverse disorders, including craniofacial dismorphisms, physical birth defects, growth

* Corresponding author at: Institute of Cell Biology and Neurobiology, IBCN – CNR Via del Fosso di Fiorano 64, 00143 Roma, Italy.
E-mail address: marco.fiore@cnr.it (M. Fiore).

http://dx.doi.org/10.1016/j.toxlet.2017.04.016
Received 9 March 2017; Received in revised form 19 April 2017; Accepted 24 April 2017
Available online 25 April 2017
0378-4274/

retardation, and alteration of brain development as well as cognitive and behavioral defects (Del Campo and Jones, 2017; Mattson et al., 2011; Olson et al., 2009). At the present time, it is not possible to establish a safe threshold of consumption, the only feasible recommendation for all pregnant women is the total abstention from alcohol use (Coriale et al., 2013; et al., 2011; Kodituwakku and Kodituwakku, 2011). Hence, the importance of identifying women who drink during pregnancy becomes ever greater (Lange et al., 2014; Wurst et al., 2008). Actually, the first step in the identification of a possible FASD child is investigating the alcohol consumption of the mother during pregnancy. In this regard, several simple alcohol-screening instruments have been set up (Chang, 2001). Unfortunately the screening tests developed to identify at risk consumption in the general population often fail to identify pregnant women at risk (Chang, 2001). Indeed, assessing the mother's alcohol consumption is rather difficult as the admission of alcohol drinking during pregnancy may be accompanied by heavy guilt feelings. To overcome these difficulties and to obtain a reliable picture of the alcohol behaviour in pregnant women, new specific methodologies and new strategies based for example on the identification of dietary habits during pregnancy have been proposed (Flegal, 1990; Witte and Haile, 1996). In this context, specific screening instruments for pregnant women have been developed, and three of these, the TWEAK (Chan et al., 1993), the AUDIT-C (Bush et al., 1998; Saunders et al., 1993) and the T-ACE (Sokol et al., 1989), are commonly used by FASD experts.

It is possible that an assessment made only on the basis of questionnaires (even those based on the investigation of the eating habits of pregnant women) and not measuring objective values indicating the real presence of alcohol or drugs in the body (as for example the levels of alcohol and drugs in the urine), could be not sufficient. Therefore, because of the difficulty of objectively assessing the actual gestational alcohol consumption, in the last decades a number of biological markers, considered much more reliable to document the use of ethyl alcohol, have been proposed.

The clinical parameters routinely used for the diagnosis of chronic use/abuse of alcohol are actually indirect indicators of alcohol-induced diseases, for example the hepatic enzymes such as the Aspartate Aminotransferase (AST), the Gamma-Glutamyl Transferase (GGT), the Alanine Aminotransferase (ALT), but also the Mean Corpuscular Volume (MCV) of red blood cells and the Carbohydrate Deficient Transferrine (CDT). Even though CDT has a sensitivity/specificity generally higher than AST, ALT, GGT, or MCV, CDT is less sensitive/specific in women than men and needs an alcohol consumption above 50–80 g/day for 2–3 weeks to increase serum levels. Further, changes in transferrin glycosylation occurring during pregnancy can alter CDT values (Kenan et al., 2011) with a progressive elevation from the first to the last pregnancy trimester (Bianchi et al., 2011). These biomarkers are indirect markers and for the most of these, the single increase is not indicative of alcohol abuse, also their meaning is very limited by several variables. For example, the alteration of the enzymes AST and ALT may be linked not only to alcohol abuse but also to pathologies occurring during pregnancy, such as hyperemesis in the first trimester or cholestasis in the third trimester, or biliary calculosis. As well as triglyceride levels are elevated in the abuse of alcohol, as well as in pregnancy, and this may be confounding (Nanau and Neuman, 2015; Stoler et al., 1998; Topic and Djukic, 2013). However, to overcome these problems, a quantification of direct biomarkers has been advocated (Cabarcos et al., 2015; Kummer et al., 2016). These direct biomarkers include, firstly, ethanol itself, and then some of its metabolites, such as Ethylglucuronide (EtG), Fatty Acid Ethyl Esters (FAEE), Ethylsulfate (EtS) and Phosphatidylethanol (PEth) (Morini et al., 2010, 2011). In particular, the measurement of EtG in several biological matrices, including neonatal and maternal hair, neonatal meconium and maternal urine, is receiving increasing interest. EtG was firstly described, in 1953, by Kamil and collegues (Kamil et al., 1953) as a minor metabolite of ethanol and since then it has been studied as an important marker of alcohol consumption. EtG is a phase II metabolite of ethanol formed by the enzymatic conjugation of ethyl alcohol with glucuronic acid, catalyzed by UDP-glucuronosyltransferase (Cabarcos et al., 2015). Indeed, detection of EtG in urine indicates recent alcohol consumption (Cabarcos et al., 2015) and is considered to be a specific, highly sensitive, and reliable marker of recent (up to 3 days) alcohol intake (Schmitt et al., 1995; Skipper et al., 2004; Weinmann et al., 2004) or even up to 5 days (Wurst et al., 2005). The first methods for EtG determination in blood and urine date back to the middle of the 90 s of the previous century and were based on analytical techniques in gas chromatography-mass spectrometry (Borucki et al., 2005). To date, several other analytical methods for the determination of this biomarker have been developed and used (Janda and Alt, 2001; Shah and Lacourse, 2006; Zimmer et al., 2002), including the EtG immunoassay that we used in this study (Helander and Zheng, 2009). Indeed, EtG commercial immunoassays have been shown to properly correlate with the liquid chromatography–mass spectrometry (LC–MS) reference method (Helander and Zheng, 2009; Leickly et al., 2015; Turfus et al., 2013). Thus, the main aim of the present study was to investigate in pregnant women the specificity and predictive value of three alcohol screening questionnaires (T-ACE, AUDIT-C and TWEAK) associated with a food diary when directly compared with the detection of urinary EtG, in order to verify the reliability of the questionaries.

2. Materials and methods

2.1. Participants selection and study design

The study was conducted on 96 pregnant women in follow up at the ambulatory of Gynaecology and Obstetrics of the Sapienza University Hospital “Policlinico Umberto I” of Rome, Italy, visited between the months of April and June 2016. Main exclusion criteria for the enrolment in the investigation for all pregnant women included history of head injury, loss of consciousness, history of organic mental disorder, present assumption of psychoactive legal and illegal drugs (as cocaine, opioids, amphetamine, other recreational drugs, anxiolytics, euphoriants, antipsychotics, barbiturates, benzodiazepines, antidepressants, hallucinogens), seizure disorders or central nervous system diseases (all informations based on interviews), hypertension at the time of recruitment to avoid any kind of interference with the EtG measurement. Another exclusion criterium was the use of mouthwash containing alcohol since it has been known that it may alter EtG urine levels (see Methods, EtG Assay). Pregnant women that could not communicate in Italian were also excluded from the study. Following the previously described exclusion criteria 80 pregnant woman were included. All the tests were always carried out on the Thursday day of the week.

A schedule was used for collecting information about socio-demographic data (age, educational level, marital status, professional condition and what kind of job) and information about pregnancy (the trimester of pregnancy, intended or unintended pregnancy, after how many weeks she pregnancy was discovered) were provided.

As recommended by the University Hospital guidelines, firstly, the gynaecologists, with motivational interviewing and supportive dialogue, talk to pregnant women about alcohol use by administering also the food diary interview to investigate alcoholic habits. Afterwards, pregnant women were asked to fill out the three structured questionnaires (AUDIT-C, T-ACE and TWEAK) and a food diary. Immediately after the interviews, they were asked to provide a sample of their urine to assess the concentration of EtG. In the final sample, 70 pregnant women were included since 10 of them refused to provide urine samples (Figs. 1 and 2).

All the interviews were conducted in a quiet room, without any relatives or parents, within the eyesight of the investigators. The questionnaires were anonymous, to guarantee the privacy of the women that can feel free to say the true, and were paired to the biological markers with a progressive number. It took approximately