Assessment of alcohol consumption in mexican pregnant women by hair testing of ethyl glucuronide

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ABSTRACT

There are no studies that have utilized both biomarkers and self-reported data to evaluate maternal alcohol use during pregnancy in Mexico. Therefore, we aimed to describe the prevalence of alcohol consumption in a cohort of 300 Mexican pregnant women. We used a validated ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method to measure hair ethyl glucuronide (EtG) in hair segments that corresponded to the first and second half of pregnancy. We compared the hair EtG values to a self-reported questionnaire on maternal drinking habits and evaluated whether the gestational alcohol use was associated with psychotropic drug use. Based on the EtG measurements, 263 women (87.7%) were alcohol-abstinent during the entire pregnancy, while 37 (12.3%) had used alcohol at least once during the pregnancy. Of these, only two women were found to have problematic alcoholic behavior during the entire pregnancy. No significant differences in sociodemographic characteristics were observed between alcohol-abstinent women and women with drinking habits. The self-reporting data and hair EtG gave heterogeneous results: although 37 women had selfreported alcohol use during pregnancy, only 54.1% of these women tested positive for hair EtG. Of the women who tested positive for hair EtG, 54.1% tested positive for psychoactive substances. In our cohort, the use of drugs of abuse was independent of gestational drinking. This study provided the first objective evidence of prenatal ethanol consumption in a cohort of Mexican pregnant women.

INTRODUCTION

Ethyl alcohol (simply defined as alcohol) is one of the most prevalent psychoactive substances used worldwide and has a significant impact on public health. Alcohol use can result in harm not only to the drinker, but also to other related individuals such as family members, friends, co-workers, and unfamiliar persons (WHO, 2018). A common case of this harm to others is the damage caused by consuming alcohol during pregnancy. Prenatal exposure to ethanol can lead to irreversible damage of the fetus such as Fetal Alcohol Syndrome (FAS), which causes permanent, severe, and irreversible brain damage, dysmorphologic features, and serious growth problems. FAS is the most severe condition within an umbrella of Fetal Alcohol Spectrum Disorders (FASD) (de Sanctis, Memo, Pichini, Tarani, & Vagnarelli, 2011).

No amount of alcohol could be considered safe to drink during pregnancy. Indeed, although most pregnancies are unplanned and many women consume alcohol without knowing they are pregnant, abstinence is important not only throughout all the gestation period but even when planning a pregnancy (Bakhireva, Leeman, Roberts, Rodriguez, & Jacobson, 2021; Bennett & Bowden, 2022; Gomez et al., 2020; Pichini, Busardò, & Garcia-Algar, 2020). Despite public health warnings, a non-negligible number of women keep on drinking alcohol during pregnancy with variable prevalence across countries (Busardò et al., 2022; Gimenez et al., 2022; Mårdby, Lupattelli, Hensing, & Nordeng, 2017; Popova, Lange, Probst, Gmel, & Rehm, 2017). According to the meta-analysis of Popova and co-workers, a global estimate is that 9.8% of pregnant women use alcohol during pregnancy (Popova et al., 2017). However, due to the sensitivity of the issue and recall bias, self-reported alcohol use data during pregnancy only provides underestimations or inaccurate estimations of the true alcohol consumption (MartínEstal & Castorena, 2020).

The prevalence of worldwide alcohol consumption during pregnancy significantly varies across different countries. South American countries show a higher prevalence: 75% of pregnant women in Argentina reported consuming alcohol during pregnancy (López, Filippetti, & Cremonte, 2015), while 63% reported doing so in Uruguay, and 57% in Chile (Gimenez et al., 2022).

Mexico is among the countries in which information regarding women's use of alcohol during pregnancy is dated (Borges et al., 1997; Casco & Natera, 1993; Romero, Mondragón, Cherpitel, Medina-Mora, & Borges, 2001) and based on self-reported data. Data from the National Addiction Survey carried out in Mexico in 1988 indicated that 17% of urban women between the ages of 12 and 65 who reported at least one pregnancy, drank some alcohol during the pregnancy (Villatoro-Velázquez et al., 2017). In a more recent study (Gorn, Romero Mendoza, Marcela Tiburcio Sainz, Medina-Mora Icaza, & Rojas Guiot, 2007), 57% of 134 women having been pregnant at least once admitted to having consumed alcoholic beverages during pregnancy. However, around 66% of drinkers reduced alcohol intake after the confirmation of pregnancy, but 26% continued drinking as usual and 6.5% began drinking during this period. In addition, a retrospective study carried out at the Hospital Civil de Guadalajara Dr. Juan I. Menchaca to assess the percentage of women consuming alcohol during pregnancy between 1991 and 1998 reported a prevalence of 2.42% in the analyzed years (Pe ña & Matute, 2010). Yet, there are no studies that would have used biomarkers to verify maternal alcohol use during pregnancy that would have compared these to the self-reported information.

Direct alcohol biomarkers, such as ethyl glucuronide (EtG), fatty acid ethyl esters (FAEEs), and phosphatidylethanol (PEth), measured from maternal and neonatal biological matrices provide an objective tool to assess alcohol consumption during pregnancy and consequent prenatal

exposure to this teratogen (Biondi, Freni, Carelli, Moretti, & Morini, 2019; Kulaga, Pragst, Fulga, & Koren, 2009; Montag, 2016; Wurst et al., 2008). Whereas PEth can be detected in maternal blood at a maximum of 4e6 weeks after alcohol consumption, FAAEs and EtG accumulate in the hair (Montag, 2016). In fact, hair EtG has been used to assess both abstinence and heavy drinking (Biondi et al., 2019; SoHT, 2019). Thus, measuring EtG in the maternal hair shaft corresponding to the pregnancy period (9e10 cm or 3.5e3.9 inches of maternal hair cut after delivery) or in subsequent segments representing different gestation periods can be used to assess gestational alcohol consumption (Gomez-Roig et al., 2018; Joya, Mazarico, et al., 2016). Metabolites in the hair can also be used to screen for drug use (Gómez-Ruiz et al., 2022; Marchei et al., 2022). According to a Mexican report on the consumption of psychoactive drugs (CONADIC, 2021), amphetamine-type stimulants (30.2%), alcohol (30.0%), and marijuana (15.1%) are the greatest causes of the demand for medical treatment. In our recent study, we objectively assessed the prevalence of gestational consumption of classical drugs of abuse, new psychoactive substances, and prescription drugs by screening hair samples from a cohort of 300 Mexican pregnant women (Gómez-Ruiz et al., 2022). Out of the 300 examined hair samples, 127 (42.3%) tested positive for psychoactive substances (with methamphetamine and cocaine among the most used drugs), 82.6% tested positive for a prescription drug (paracetamol being the most used), and 27.4% tested positive for environmental tobacco smoke (Gómez-Ruiz et al., 2022). Because 27.5% of drug consumers were poly-users and 13.3% self-declared alcohol consumption, it is important to assess how alcohol consumption is associated with drug use in pregnant women.

Therefore, our aims were to describe the prevalence of gestational alcohol use by measuring maternal hair EtG levels, to compare EtG prevalence to that of women's self-reported information in a questionnaire on drinking habits, and to evaluate whether the gestational alcohol use was associated with psychotropic drug use, in the cohort of 300 pregnant women in México.

MATHERIALS AND METHODS

Cohort

The study was a retrospective observational study carried out between 1 November 2019 and 31 January, 2020 in the neonatology unit of Nuevo Hospital Civil de Guadalajara "Dr. Juan I. Menchaca. The women were recruited at the time of delivery and those who agreed to participate in the study signed the informed consent document. During a follow-up visit, 3 or 4 weeks after delivery, hair samples were collected together with a medical questionnaire, which included data about maternal sociodemographic characteristics (e.g., age, nationality, education level, occupation, civil status) and consumption of psychotropic substances (drugs of abuse, alcohol, and tobacco) before and during pregnancy. In particular, regarding alcohol consumption, delivering women were asked whether they had consumed alcohol during the first and/or second half of pregnancy, the type of consumed drink (beer, wine, whiskey, and other distilled alcoholic beverages), how frequently it was consumed (e.g., every day, some days a week, once a week, once a month) and how many drinks were consumed during the drinking occasion. The survey data and collected hair were anonymized by code. The local Human Research Ethics Committee (CONBIOETICA14-CEI-008-20 161 212) approved the study protocol. Estimated gestational age (EGA) at delivery (weeks), birth weight (grams), head circumference (centimeters), length (centimeters), and pathologies of the newborns were obtained from medical records.

Hair EtG measurement

The collected hair shafts were divided into two segments. The proximal segment, from 0.0 (root) to around 4.5 cm (or 1.7 inch) corresponded to the last half of pregnancy and the distal segment, from 4.5 to 9.0 cm (or 1.7e3.5 inches) corresponded to the first half of the pregnancy. EtG was measured in each hair segment by applying a previously published method, which was revalidated inhouse with some analytical improvements (Gomez-Roig et al., 2018; Joya, Marchei, et al., 2016). Briefly, aliquots of 25 mg of finely cut hair were extracted employing 1 h incubation at 100 ◦C in 0.5 mL of an acidic-buffered M3 reagent containing 1.5 ng/mL of EtG-d5. Then, the extracted hair was dried under nitrogen flow and resuspended in 0.1 mL of mobile phase A. After vortex mix and ultracentrifugation at 10 000 g for 10 min, 10 mL of the clear supernatant was injected into UHPLC-MS/MS. Chromatography was carried out using a Luna Omega Polar C18 (100 2.1 mm, 1.6 mm) using a linear gradient elution with two solvents: 0.1% formic acid in water (mobile phase A) and methanol (solvent B). Solvent B was maintained at 2.0% for the first 0.5 min. It was increased to 95.0% from 0.5 to 2.0 min and held to 95.0% from 2.0 to 2.5 min. Then it was decreased back to 2.0% from 2.5 to 2.6 min and held to 2.0% from 2.6 to 5 min to re-equilibration. The flow rate was kept constant at 0.3 mL/min during the analysis. EtG and the internal standard (EtG-d5) were detected in negative electrospray ionization mode with the triple quadrupole mass spectrometer operated in multiple reaction monitoring (MRM). Mass spectrometry conditions were the following: capillary voltage 2.5 kV, desolvation temperature 650 ◦C, source temperature 150 ◦C, cone gas flow rate 40 L/h, desolvation gas flow rate 900 L/h, and collision gas flow rate 0.07 mL/min. Cone energy voltages were 20V and collision energy voltages were 15 and 18eV for both EtG and EtG-d5. MRM transitions were m/z 221.1 / 75.0, 221.1 / 85.0 for EtG and m/z 226.0 / 75.0, 226.0 / 85.0 for EtG-d5. Underlined transitions were selected for quantification. The limit of quantification was 5 pg/mg.

EtG and the internal standard, ethyl glucuronide-d5 (EtG-d5) used in this study, were purchased from Cerilliant (Austin, Texas, United States). M3 (acidic aqueous buffer) reagent was provided

by Comedical s.a.s. (Mattarello, Trento, Italy). HPLC-MS grade solvents (methanol, acetonitrile, and water) were purchased from Carlo Erba (Milan, Italy). All other chemicals used for experiments were of analytical reagent or HPLC grade from commercial resources.

According to the latest international consensus on the use of alcohol markers in hair for the assessment of abstinence and chronic alcohol consumption (Biondi et al., 2019; SoHT, 2019), a concentration lower than or equal to 5 pg/mg EtG in the proximal head hair segment (3e6 cm, 1.18e2.36 inch) does not contradict self-reported abstinence. Conversely, a concentration greater than 5 pg/mg EtG in the proximal head hair segment strongly suggests repeated alcohol consumption, which we defined as “social drinking”. A concentration greater than or equal to 30 pg/mg EtG in the proximal head hair segment with a length of 3 cm up to 6 cm (1.18 inch up to 2.36 inch) strongly suggests chronic excessive alcohol consumption.

Data analysis

The statistical analyses were carried out with the SPSS software version 28.0 (SPSS Inc.; Chicago, Illinois, United States). Descriptive analyses were used to characterize the samples. The estimates of the rates for each categorical variable were described in frequencies and percentages, and for the continuous variables in mean and standard deviations. Associations between sociodemographic and lifestyle characteristics of pregnant women with “negative to hair EtG testing” and “positive to hair EtG testing at any time during pregnancy” groups were analyzed by an independent t test for quantitative variables and a chi-square test for qualitative variables. We considered p values < 0.05 statistically significant.

Only a trend was reported regarding the group of “positive to hair EtG testing at any time during pregnancy”, given the limited number of samples.

Results

Hair EtG concentration

Based on the results obtained in the 600 hair segments coming from 300 participants, 263 women (87.7%) were abstinent during the whole pregnancy, 35 (11.7%) had been drinking at any time during their pregnancy, and 2 (0.6%) were chronic excessive drinkers.

Of the 37 women with hair EtG 5 pg/mg, 18 (48.6%) maintained a social drinking behavior during the first part of pregnancy and then quit, 5 (13.5%) drank to the end of gestation, and 2 (5.4%) showed an excessive drinking behavior in the whole pregnancy. Surprisingly, 12 women (32.4%) abstained from alcohol in the first part of pregnancy, but in the second started a social drinking habit. No measurable EtG level in hair was found in the 17 women enrolled in the study who reported alcohol drinking during pregnancy. Most of them (58.8%) declared to have drunk only during the first half of pregnancy, 29.4% only in the second half, and 11.7% reported drinking during all 9 months. The majority of these women (88.2%) reported drinking beer sporadically and only on some weekends during pregnancy.

Self-reported ethanol consumption

98.7% of the 300 women included in the study had a Mexican origin with a mean maternal age at recruitment of 24.1 ± 6.3 years (range: 12e44 years). The estimated gestational age at hospital admission was 38.5 weeks, with 32.7% of the participants experiencing their first pregnancy. With regard to educational level, only 5.0% of participants had a university degree and 47.7% had a secondary education.

Out of 300 enrolled pregnant women, 263 (87.7%) women selfreported no ethanol consumption during pregnancy, whereas the remaining 37 (12.3%) declared some ethanol consumption at any gestational time. Specifically, 21 women declared drinking only during the first half of pregnancy (mean 1.7 alcohol unit [AU]/week,

0.19 standard drinks/day) while 10 women drank during the second half (mean: 1.1 AU/week; 0.14 standard drinks/day) of pregnancy, respectively. Finally, 6 participants reported consumption during all the pregnancy (mean 1.0 AU/week; 0.14 standard drinks/day). Out of the 37 gestational drinkers, 33 (89.2%) drank only beer, 2 (5.4%) drank only wine, and another 2 (5.4%) declared having consumed more than one alcoholic drink (e.g., whiskey and tequila in one case and beer, wine, and tequila in the other case).

Comparison of hair EtG and self-reported data

No significant differences in sociodemographic characteristics were observed between women in the “negative for hair EtG” group and women in the “positive for hair EtG” group. In both groups, women had similar age, a low-medium educational level, and most of them were cohabitating. The only exception was the case of married women who drank during pregnancy in a lesser percentage than unmarried women (2.7% married women drinking vs. 20.2% unmarried women drinking, chi-square $p < 0.05$). We observed lower self-reported alcohol consumption in women who tested negative for hair EtG compared to women who tested positive for hair EtG (6.5% vs. 54.1%, chi-square $p < 0.05$). Finally, no statistically significant differences of neonatal characteristics and pathologies between the two women's groups were observed. Table 1 shows the socioeconomic and demographic characteristics in relation to the analytical results obtained by hair biomarker analysis.

Association between gestational alcohol and psychotropic drug use

Of the 37 women who tested positive for hair EtG, 20 (54.1%) tested positive for psychoactive substances, measured in the same hair shaft for a previous study (Marchei et al., 2022). Specifically, 5 (25%) tested positive for cannabis only, 4 (20%) tested positive for methamphetamine only, 1 (5.0%) tested positive for cocaine only, 1 (5.0%) tested positive for NeN-dimethyltryptamine only, and 9 (45.0%) tested positive for more than one psychoactive substance. In this latter case, 5 cases (55.6%) of polydrug use were represented by cannabis and methamphetamine, followed by 3 cases (33.3%) of polydrug use of cannabis, methamphetamine, and cocaine (33.3%), and 1 case (11.1%) of polydrug use of methamphetamine and cocaine. Out of 263 abstinent women, 107 (40.7%) tested positive for the consumption of one of more drugs of abuse. No statistically significant difference was observed in the gestational consumption of drugs of abuse between drinking and abstinent pregnant women (54.1% of drinking pregnant women vs. 40.7% of abstinent pregnant women, $p > 0.05$).

Table 1. Sociodemographic characteristics associated with alcohol consumption during pregnancy measured by hair EtG in a cohort of Mexican pregnant women.

Variables (%)	All Cases		Social drinkers			Excessive drinkers
	Negative for hair EtG (n ¼ 263)	Positive for hair EtG (n ¼ 37)	1st half of pregnancy[a] (n ¼ 18)	2nd half of pregnancy[b] (n ¼ 12)	The whole pregnancy[c] (n ¼ 5)	The whole pregnancy[d] (n ¼ 2)
Nationality						
Mexican	98.5 (259)	100.0 (37)	100.0 (18)	100.0 (12)	100.0 (5)	100.0 (2)
Others	1.1 (3)	e	e	e	e	e
NA	0.4 (1)	e	e	e	e	e
Age (mean ± SD)	24.4 ± 6.3	22.3 ± 5.9	22.3 ± 6.9	24.5 ± 4.5	18.6 ± 2.1	17.5 ± 0.7
Academic level						
No study	1.5 (4)	e	e	e	e	e
Primary school	24.0 (63)	29.7 (11)	27.7 (5)	25.0 (3)	20.0 (1)	100.0 (2)
Secondary school	49.0 (129)	37.8 (14)	38.9 (7)	25.0 (3)	80.0 (4)	e
High school	20.9 (55)	24.3 (9)	16.7 (3)	50.0(6)	e	e
College	4.6 (12)	8.1 (3)	16.7 (3)	e	e	e
Social role						
Housewife	83.3 (219)	70.3 (26)	66.7 (12)	75.0 (9)	60.0 (3)	100.0 (2)
Student	2.3 (6)	e	e	e	e	e
Employed	7.2 (19)	10.8 (4)	16.7 (3)	e	20.0 (1)	e
Day worker	0.4 (1)	2.7 (1)	e	8.3 (1)	e	e
Dealer	3.8 (10)	10.8 (4)	11.1 (2)	16.7 (2)	e	e
Unemployed	3.0 (8)	5.4 (2)	5.5 (1)	e	20.0 (1)	e
Civil status						
Single	14.4 (38)	27.0 (10)	33.3 (6)	16.7 (2)	20.0 (1)	50.0 (1)
Married	20.2 (53)	2.7 (1)*	5.6 (1)	e	e	e
Cohabitant	64.3 (169)	70.3 (26)	61.1 (11)	83.3 (10)	80.0 (4)	50.0 (1)
Widow	1.1 (3)	e	e	e	e	e
Self—reported use						
Drugs of abuse	1.1 (3)	13.5 (5)*	11.1 (2)	e	40.0 (2)	50.0 (1)
Tobacco	3.4 (9)	21.6 (8)*	5.6 (1)	25.0 (3)	40.0 (2)	100.0 (2)
Alcohol	6.5 (17)	54.1 (20)*	44.4 (8)	75.0 (9)	60.0 (3)	e
Inhalant	e	8.1 (3)	16.7 (3)	e	e	e
Newborn characteristics						
Gestational age (week, mean ± SD)	38.5 ± 2.2	38.4 ± 3.3	38.3 ± 1.3	39.8 ± 2.1	35.9 ± 7.7	36.7 ± 2.4
Weight (g, mean ± SD)	3010.8 ± 550.6	2963.5 ± 600.0	2916.4 ± 358.3	3236.7 ± 678.0	2623.0 ± 1158.3	2600.0 ± 311.1
Height (cm, mean ± SD)	49.2 ± 3.4	48.9 ± 4.3	49.1 ± 1.6	50.7 ± 3.0	44.8 ± 9.5	46.5 ± 3.5
Head circumference (cm, mean ± SD)	34.0 ± 3.2	33.9 ± 2.1	34.1 ± 1.2	34.5 ± 1.4	32.1 ± 4.7	32.8 ± 0.4
Neonatal pathologies						
YES	16.0 (42)	16.2 (6)	11.1 (2)	16.7 (2)	20.0 (1)	50.0 (1)

NA: no answer; *p < 0.05: statistically significant differences between hair EtG in negative and positive group.

[a] Distal segment: 4.5e9.0 cm (1.7e3.5 inches) hair with EtG ≥5 and EtG ≤30.0 pg/mg.

[b] Proximal segment: 0.0e4.5 cm (0e1.7 inches) hair with EtG ≥5 and EtG ≤30.0 pg/mg.

[c] Both distal and proximal segment hair with EtG ≥5 and EtG ≤30.0 pg/mg.

[d] Both distal and proximal segment hair with EtG ≥30 pg/mg.

Discussion

The value of screening hair analysis for identifying use and/or exposure to licit and illicit substances in any population group, such as pregnant women, is gaining recognition (Biondi et al., 2019; Cortes et al., 2018; Friguls et al., 2012; Gómez-Ruiz et al., 2022; Lendoiro et al., 2013; Marchei et al., 2022). Although some authors have questioned the clinical usefulness (Bennett & Bowden, 2022; Hayes et al., 2019) of hair ETG for detecting maternal alcohol consumption during pregnancy and despite the fact that analytical data should be interpreted carefully, especially in the presence of specific conditions and pathologies (Pragst & Yegles, 2008; Triolo et al., 2022), analysis of maternal hair for EtG as a biomarker of alcohol use offers the opportunity to investigate not only suspected gestational alcohol use but also to identify newborns at risk for possible disabilities associated with prenatal exposure to ethanol, such as FAS or FASD (Jarque et al., 2018; Joya, Marchei, et al., 2016; Messina et al., 2020; Ordenewitz et al., 2021; Pichini et al., 2020).

The prevalence of alcohol consumption during pregnancy in North American countries is estimated between 10% and 15%. Among these countries, it is estimated that between 1% and 5% of Mexican pregnant women drink (Popova et al., 2017). Moreover, the estimated prevalence of alcohol consumption (any amount) during pregnancy among the general population of Mexico based on a meta-analysis of the literature (Lange et al., 2017) was 1.2%, ranging from 0.4% to 2.4%. As previously stated, the estimate of maternal alcohol consumption during pregnancy in Mexico relied on self-reported data, making it difficult to establish measures to prevent harmful and irreversible effects on the fetus. For this reason, in this study, for the first time, the hair biomarker EtG in two subsequent maternal hair segments corresponding to the first and the second half of pregnancy was measured to objectively assess gestational alcohol consumption in a cohort of 300 pregnant Mexican women. In agreement with a case control study conducted in Naucalpan, Mexico, which found that 11% of the women interviewed admitted drinking alcoholic beverages during pregnancy (Borges, Garrido, C´ardenas, Ibarra, & Bobadilla, 1993), our study showed a 12.3% prevalence of alcohol consumption at any time during pregnancy with only two (0.6%) women found to have problematic alcoholic behavior during the whole pregnancy.

Self-reported questionnaires disclosed the same 12.3% gestational consumption of alcohol, but the declarations did not match (were not coincident) with the measurement of hair EtG. As already reported in our previous studies (Pichini et al., 2012) on substances use during gestation, the lack of concordance between questionnaire and hair EtG data in moderate and low gestational drinking can lead to heterogeneity of results, as in this study, due to both a misreporting of drinking behavior during a 9-month period, and also that small amounts of consumed alcohol most likely did not result in measurable hair EtG. Sporadic consumption does not always lead to a detectable amount of EtG in the hair, and this is true for both alcohol biomarkers and the use of legal and/or illicit substances. In particular, the lack of detection of EtG in the hair of low and sporadic drinkers may be due to the fact that only a small amount of alcohol is non-oxidatively conjugated to form nonmeasurable amounts of metabolite. Furthermore, in one of our previous studies on alcohol maternal-neonatal biomarkers (Joya, Marchei, et al., 2016), we demonstrated that prenatal ethanol exposure could be predicted when meconium EtG >30 ng/g and when maternal hair ETG is above 11 pg/mg. This means that sporadic alcohol consumption resulting in non-measurable hair EtG also consequently results in non-measurable meconium EtG, not objectively proving maternal intake and consequent fetal exposure. In agreement with other authors (Bakhireva et al., 2021; Muggli, Cook, O'Leary,

Forster, & Halliday, 2015), more specific questions about “special occasions” can help women more accurately report occasional events of alcohol intake, even high, that may not be captured by questions about “typical" drinking behavior of alcohol. Moreover, any element that could result in false negatives has to be carefully evaluated when interpreting hair EtG data (Biondi et al., 2019). Hair hygiene, regular use of hair products, and chemical treatments should be taken into account as they may lead to false negative results (Kerekes & Yegles, 2013; Luginbühl, Nussbaumer, & Weinmann, 2018; Petzel-Witt et al., 2018). Unfortunately, in our study, no specific question on alcohol habits and no cosmetic treatment (all the hair samples were of brown-black colors) was reported or observed. Furthermore, in the absence of a sufficient hair sample, it was not possible to perform the analysis of ethyl palmitate as suggested by the Society of Hair Testing in doubtful cases (SoHT, 2019). Finally, in agreement with Wurst et al. (2008), for pregnant women with low or sporadic gestational alcohol intake, the combined use of the AUDIT questionnaire and direct ethanol metabolite testing in biological matrices was more predictive than utilizing only the biomarker measurement. On the contrary, our results confirmed that the questionnaires tend to underestimate the problem and are unreliable and sometime useless, especially in the presence of heavy drinkers (the two excessive drinkers did not declare alcohol consumption). In these cases, the use of biomarkers may prove useful in highlighting high-level alcohol exposure, where denial/underreporting is common and where consequences such as FASD are more likely.

The unreliability of the questionnaire is revealed not only in relation to alcohol consumption but also in relation to the use of licit and illicit substances. In fact, we found that only 1.1% of alcohol-abstinent and 13.5% of drinking women declared the use of substances of abuse during pregnancy. Actually, our previous study (Gómez-Ruiz et al., 2022), which analyzed classic drugs and new psychoactive drugs in the hair of the same cohort of women, disclosed that 40.7% of alcohol-abstinent and 54.1% of drinking women consumed one or more drugs of abuse during pregnancy. The same was highlighted with respect to self-reported tobacco smoking (3.4% alcohol-abstinent and 21.6% drinking women) and real use. Analyzing the hair of the same cohort of Mexican women, we disclosed that 25.8% of alcohol-abstinent and 40.5% of drinking women were positive for nicotine, used as a biomarker of tobacco consumption. With respect to the concomitant consumption of alcohol and drugs of abuse, our results show that in this cohort, pregnant women consumed drugs of abuse independently from their gestational drinking and vice versa. It can be hypothesized that in Latin cultures such as in Mediterranean countries drinking is also considered a food habit, which is not completely prohibited by some health professionals, despite the international recommendations (Pan American Health Organization and World Health Organization, 2020; Pan American Health Organization and World Health Organization, 2023).

Although alcohol damage is permanent, the disabilities caused by FAS and FASD can be mitigated with early diagnosis and subsequent follow-up and disorder management (Jarque et al., 2018). It can be hypothesized that in this cohort of women, there was a belief that small amounts of alcohol consumption during pregnancy did not harm their health and the health of their babies (54.1% of women who tested positive for EtG in hair reported alcohol consumption during pregnancy). This hypothesis is sustained by what was reported in a recent study conducted in northwest England. Although most of the women interviewed believed that avoiding alcohol during pregnancy was the safest option, some others declared that there was not enough evidence to show the harms of low-level alcohol consumption. All participants reported that lowlevel alcohol consumption during pregnancy could be acceptable (Ujhelyi Gomez, Goodwin, Chisholm, & Rose, 2022).

Screening and brief intervention (BI) is an effective individual-level approach that is of critical importance when developing multi-level policy solutions to combat alcohol-related harms. Despite the evidence of its effectiveness, few countries implement screening and brief intervention widely as a component of routine medical care and there is no evidence of this practice in South America (Gimenez et al., 2022; Popova et al., 2023; PAHO & WHO, 2020). It is known that after learning they were pregnant, a certain percentage of women spontaneously stopped ethanol consumption (Joya, Mazarico, et al., 2016). In agreement with that observation, our study reported a decrease in the number of pregnant women who drink in the 2nd half of pregnancy. Among the social gestational drinkers, 5 (14.3%) decreased their consumption during pregnancy while 18 (51.4%) totally quit the habit. Unfortunately, 34.3% started drinking in the second half of pregnancy. Of these, 75% declared their consumption, but the reason for starting to drink in the second half of pregnancy was not reported in the questionnaire. Our hypothesis, supported by the above-reported studies (Ujhelyi Gomez et al., 2022), is that at the end of the pregnancy, when the fetus is almost completely formed, low-level alcohol consumption is perceived as acceptable and less harmful.

Nevertheless, brief interventions (BI) at antenatal visits are also important for those women in our cohort who did not drink in the first part of the pregnancy because they believe that it was harmful for the fetus, but who began drinking in the second half of pregnancy, believing that at this point alcohol drinking was no longer harmful. Providing clear and concise information to pregnant women or expectant mothers could motivate them not to drink or to stop drinking for a future pregnancy.

Conclusions

Hair testing is a unique tool in pharmacotoxicology that allows objective assessment of the history of consumption or passive exposure to xenobiotics including tobacco, classical and new psychoactive substances, and alcohol, and consequent possible prenatal exposure to these substances. Our results confirmed the usefulness of maternal hair analysis to ascertain alcohol use during pregnancy, and how the measurement of exposure biomarkers in this matrix is essential to demonstrate real consumption. The present study has been the first in evaluating the real prevalence of alcohol use in a cohort of Mexican pregnant women through an objective assessment of these conditions by measuring EtG in the hair. This information can serve as a basis for directing political and public health interventions aimed at preventing alcohol consumption in women during pregnancy.

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Author contributions

E. Marchei wrote the first draft of the manuscript and revised subsequent versions with S. Pichini and O. García-Algar. E. Marchei and C. Lombroni conducted the lab analysis. L.M. Gómez-Ruiz, A. Acosta-López, R.Y. Ramos-Gutiérrez, M.B. Varela-Busaka, V. Andreu-Fernandez, S. Pichini, and O. García-Algar conceptualized and designed the analysis. L.M. Gómez-Ruiz, A. Acosta-López, R.Y.

Ramos-Gutiérrez, and M.B. Varela-Busaka collected samples and questionnaires. All authors contributed to writing, critically reviewing, and revising the manuscript.

Declaration of competing interest

None to disclose.

References

Bakhireva, L. N., Leeman, L., Roberts, M., Rodriguez, D. E., & Jacobson, S. W. (2021). You didn't drink during pregnancy, did you? Alcoholism: Clinical and Experimental Research, 45(3), 543e547.

Bennett, R., & Bowden, C. (2022). Can routine screening for alcohol consumption in pregnancy be ethically and legally justified? Journal of Medical Ethics, 48(8), 512e516.

Biondi, A., Freni, F., Carelli, C., Moretti, M., & Morini, L. (2019). Ethyl glucuronide hair testing: A review. Forensic Science International, 300, 106e119.

Borges, G., Garrido, F., Cárdenas, V., Ibarra, J., & Bobadilla, J. L. (1993). Parental alcohol consumption and postneonatal mortality. Journal of Community & Applied Social Psychology, 3(1), 17e27.

Borges, G., Tapia-Conyer, R., López-Cervantes, M., Medina-Mora, M. E., Pelcastre, B., & Marina, F. F. (1997). Alcohol consumption and pregnancy in the Mexican national addiction survey. Cadernos de Saúde Pública, 13(2), 205e211.

Busardò, F. P., La Maida, N., Pellegrini, M., Di Giorgi, A., Montana, A., & Pichini, S. (2022). Assessment of prenatal exposure to alcohol and gestational consumption by EtG testing in neonatal meconium and maternal hair and neonatal: First Italian national survey. Toxicologie Analytique et Clinique, 34(3). S33eS34.

Casco, M., & Natera, G. (1993). El alcoholismo en la mujer: La explicación que ellas mismas dan. [Alcoholism in women: The explanation that they themselves give]. Salud Mental, 16(1), 24e29.
Comisión Nacional contra las Adicciones (CONADIC). (2021). Informe sobre la Situación de la Salud Mental y el Consumo de Sustancias Psicoactivas en M´exico 2021. Available on: https://www.gob.mx/cms/uploads/attachment/file/648021. (Accessed 26 December 2022).

Cortes, L., Almeida, L., Sabra, S., Muniesa, M., Busardò, F. P., Garcia-Algar, O., et al. (2018). Maternal hair and neonatal meconium to assess gestational consumption and prenatal exposure to drugs of abuse and psychoactive drugs. Current Pharmaceutical Biotechnology, 19(2), 136e143.

Friguls, B., Joya, X., Garcia-Serra, J., Gómez-Culebras, M., Pichini, S., Martinez, S., et al. (2012). Assessment of exposure to drugs of abuse during pregnancy by hair analysis in a Mediterranean island. Addiction, 107(8), 1471e1479.

Gimenez, P. V., Lichtenberger, A., Cremonte, M., Cherpitel, C. J., Peltzer, R. I., & Conde, K. (2022). Efficacy of brief intervention for alcohol consumption during pregnancy in argentinean women: A randomized controlled trial. Substance Use & Misuse, 57(5), 674e683.

Gomez-Roig, M. D., Marchei, E., Sabra, S., Busardò, F. P., Mastrobattista, L., Pichini, S., et al. (2018). Maternal hair testing to disclose self-misreporting in drinking and smoking behavior during pregnancy. Alcohol, 67, 1e6.

Gómez-Ruiz, L. M., Marchei, E., Rotolo, M. C., Brunetti, P., Mannocchi, G., Acosta-López, A., et al. (2022). Prevalence of licit and illicit drugs use during pregnancy in Mexican women. Pharmaceuticals, 15(3), 382.

Gomez, K. U., Goodwin, L., Jackson, L., Jones, A., Chisholm, A., & Rose, A. K. (2020). Are psychosocial interventions effective in reducing alcohol consumption during pregnancy and motherhood? A systematic review and meta-analysis. Addiction, 116(7), 1638e1663.

Gorn, S. B., Romero Mendoza, M., Marcela Tiburcio Sainz, M., Medina-Mora Icaza, M. E., & Rojas Guiot, E. (2007). Riesgos asociados al consumo de alcohol durante el embarazo en mujeres alcohólicas De la ciudad de México. Salud Mental, 30, 31e38.

Hayes, N. A., Baldwin, A., Magri, R., Ostrander, E., Smith, M., & Fleming, M. (2019). Limitation of nail and hair ethyl glucuronide (Etg) levels to assess maternal alcohol use. Drug Dependence and Addiction, 1, 19e25.

Jarque, P., Marchei, E., Roca, A., Gomila, I., Pichini, S., Busardò, F. P., et al. (2018). The importance of biomarkers of fetal exposure to alcohol and psychotropic drugs in early diagnosis: A case report. Drug Testing and Analysis, 10(5), 895e898.

Joya, X., Marchei, E., Salat-Batlle, J., García-Algar, O., Calvaresi, V., Pacifici, R., et al. (2016). Fetal exposure to ethanol: Relationship between ethyl glucuronide in maternal hair during pregnancy and ethyl glucuronide in neonatal meconium. Clinical Chemistry and Laboratory Medicine, 54(3), 427e435.

Joya, X., Mazarico, E., Ramis, J., Pacifici, R., Salat-Batlle, J., Mortali, C., et al. (2016). Segmental hair analysis to assess effectiveness of single-session motivational intervention to stop ethanol use during pregnancy. Drug and Alcohol Dependence, 158, 45e51.

Kerekes, I., & Yegles, M. (2013). Coloring, bleaching, and perming influence on EtG content in hair. Therapeutic Drug Monitoring, 35(4), 527e529.

Kulaga, V., Pragst, F., Fulga, N., & Koren, G. (2009). Hair analysis of fatty acid ethyl esters in the detection of excessive drinking in the context of fetal alcohol spectrum disorders. Therapeutic Drug Monitoring, 31(2), 261e266.

Lange, S., Probst, C., Heer, N., Roerecke, M., Rehm, J., Monteiro, M. G., et al. (2017). Actual and predicted prevalence of alcohol consumption during pregnancy in Latin America and the caribbean: Systematic literature review and meta-analysis. Revista PanAmericana de Salad Publica, 41, e89.

Lendoiro, E., González-Colmenero, E., Concheiro-Guis´an, A., de Castro, A., Cruz, A., López-Rivadulla, M., et al. (2013). Maternal hair analysis for the detection of illicit drugs, medicines, and alcohol exposure during pregnancy. Therapeutic Drug Monitoring, 35(3), 296e304.

López, M. B., Filippetti, V. A., & Cremonte, M. (2015). [Alcohol consumption before and during pregnancy in Argentina: Prevalence and risk factors]. Revista PanAmericana de Salud Publica, 37(4e5), 211e217.

Luginbühl, M., Nussbaumer, S., & Weinmann, W. (2018). Decrease of ethyl glucuronide concentrations in hair after exposure to chlorinated swimming pool water. Drug Testing and Analysis, 10(4), 689e693.

Marchei, E., Rotolo, M. C., Mannocchi, G., Capomassi, A., Gómez-Ruiz, L. M., Acosta-López, A., et al. (2022). Assessment of licit and illicit drugs consumption during pregnancy by Ultra-High Performance Liquid Chromatography-High Resolution Mass Spectrometry (UHPLC-HRMS) target screening in Mexican women hair. Journal of Pharmaceutical and Biomedical Analysis, 211, Article 114607.

Mårdby, A. C., Lupattelli, A., Hensing, G., & Nordeng, H. (2017). Consumption of alcohol during pregnancy e a multinational European study. Women and Birth, 30(4), e207ee213.

Martín-Estal, I., & Castorena, F. (2020). El consumo de alcohol durante el embarazo y su impacto en la salud. El sitio de divulgación científica del Tec de Monterrey.

https://transferencia.tec.mx/2020/09/03/el-consumo-de-alcohol-durante-elembarazo-y-su-impacto-en-la-salud. (Accessed 26 December 2022).

Messina, M. P., D'Angelo, A., Battagliese, G., Coriale, G., Tarani, L., Pichini, S., et al. (2020). Fetal alcohol spectrum disorders awareness in health professionals: Implications for psychiatry. Rivista di Psichiatria, 55(2), 79e89.

Montag, A. C. (2016). Fetal alcohol-spectrum disorders: Identifying at-risk mothers. International Journal of Women's Health, 8, 311e323.

Muggli, E., Cook, B., O'Leary, C., Forster, D., & Halliday, J. (2015). Increasing accurate self-report in surveys of pregnancy alcohol use. Midwifery, 31(3), e23ee28.

Ordenewitz, L. K., Weinmann, T., Schlüter, J. A., Moder, J. E., Jung, J., Kerber, K., et al. (2021). Evidence-based interventions for children and adolescents with fetal alcohol spectrum disorders e a systematic review. European Journal of Paediatric Neurology, 33, 50e60.

Pan American Health Organization (PAHO) and World Health Organization (WHO). (2020). Regional status report on alcohol and health in the americas 2020. International Repository for Information Sharing.

Pan American Health Organization (PAHO) and World Health Organization (WHO). (2023). Regional office for the americas. Infographic: Health impacts of women's alcohol consumption. Available on: https://www3.paho.org/hq/index.php? option com_content&view article&id 12274:infographic-healthimpacts-of-womens-alcohol-consumption&Itemid 0&lang en#gsc. tab 0. (Accessed 9 May 2023).

Peña, J., & Matute, E. (2010). [Alcohol consumption in pregnant women treated at the Dr. Juan I. Menchaca civil hospital of Guadalajara, between 1991 and 1998]. Spiral (Guadalajara), 16(47), 211e229.

Petzel-Witt, S., Pogoda, W., Wunder, C., Paulke, A., Schubert-Zsilavecz, M., & Toennes, S. W. (2018). Influence of bleaching and coloring on ethyl glucuronide content in human hair. Drug Testing and Analysis, 10(1), 177e183.

Pichini, S., Busardò, F. P., & Garcia-Algar, O. (2020). Only total abstinence from alcohol consumption during pregnancy guarantees absolute absence of any deleterious effect in the fetus and Child. Journal of Studies on Alcohol and Drugs, 81(2), 220e221.

Pichini, S., Marchei, E., Vagnarelli, F., Tarani, L., Raimondi, F., Maffucci, R., et al. (2012). Assessment of prenatal exposure to ethanol by meconium analysis: Results of an Italian multicenter study. Alcoholism: Clinical and Experimental Research, 36(3), 417e424.

Popova, S., Dozet, D., Pandya, E., Sanches, M., Brower, K., Segura, L., et al. (2023). Effectiveness of brief alcohol interventions for pregnant women: A systematic literature review and meta-analysis. BMC Pregnancy and Childbirth, 23(1), 61.

Popova, S., Lange, S., Probst, C., Gmel, G., & Rehm, J. (2017). Estimation of national, regional, and global prevalence of alcohol use during pregnancy and fetal alcohol syndrome: A systematic review and meta-analysis. Lancet Global Health, 5(3), e290ee299.

Pragst, F., & Yegles, M. (2008). Determination of fatty acid ethyl esters (FAEE) and ethyl glucuronide (EtG) in hair: A promising way for retrospective detection of alcohol abuse during pregnancy? Therapeutic Drug Monitoring, 30(2), 255e263.

Romero, M., Mondragón, L., Cherpitel, S., Medina-Mora, M. E., & Borges, G. (2001). Characteristics of Mexican women admitted to emergency care units. Alcohol consumption and related problems. Salud Pública de M´exico, 43(6), 537e543.

de Sanctis, L., Memo, L., Pichini, S., Tarani, L., & Vagnarelli, F. (2011). Fetal alcohol syndrome: New perspectives for an ancient and underestimated problem. Journal of Maternal-Fetal and Neonatal Medicine, 24(Suppl 1), 34e37.SoHT. (2019). Consensus for the use of alcohol markers in hair for supporting the assessment of abstinence and chronic alcohol consumption. Retrieved from https://www.soht.org/images/pdf/Revision_2019_Alcoholmarkers.pdf.

Triolo, V., Spanò, M., Buscemi, R., Gioè, S., Malta, G., C˘aplinskiene, M., et al. (2022). EtG quantification in hair and different reference cut-offs in relation to various pathologies: A scoping review. Toxics, 10(11), 682.

Ujhelyi Gomez, K., Goodwin, L., Chisholm, A., & Rose, A. K. (2022). Alcohol use during pregnancy and motherhood: Attitudes and experiences of pregnant women, mothers, and healthcare professionals. PLoS One, 17(12), Article e0275609.

Villatoro-Vel´azquez, J. A., Resendiz Escobar, E., Mujica Salazar, A., Bretón-Cirett, M., Cañas-Martínez, V., Soto-Hern´andez, I., et al. (2017). Encuesta nacional de Consumo de Drogas, alcohol y tabaco. https://www.gob.mx/salud%7Cconadic/ acciones-y-programas/encuesta-nacional-de-consumo-de-drogas-alcohol-ytabaco-encodat-2016-2017-136758.

World Health Organization (Who). (2018). Global status report on alcohol and health 2018. https://www.who.int/publications/i/item/9789241565639. (Accessed 26 December 2022).

Wurst, F. M., Kelso, E., Weinmann, W., Pragst, F., Yegles, M., & Sundstro€m Poromaa, I. (2008). Measurement of direct ethanol metabolites suggests higher rate of alcohol use among pregnant women than found with the AUDIT–a pilot study in a population-based sample of Swedish women. American Journal of Obstetrics and Gynecology, 198(4), e1ee5.