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Estimated Prevalence of Harmful Alcohol Consumption in Pregnant and Non-pregnant Women in Saxony-Anhalt (North-East Germany) Using Biomarkers

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Abbreviations

Abstract

Background: Alcohol consumption is commonly accepted in western societies and is also a known risk factor in pregnancy, which could lead to fetal alcohol spectrum disorders (FASD). Prevalence of alcohol consumption during pregnancy is mostly unknown. The prevalence estimation in past publications based on questionnaires shows difficulties based on possible under-reporting due to social stigmatization. The aim of this study was to estimate the prevalence of harmful alcohol consumption in a large cohort of pregnant women in comparison to non-pregnant females using different biomarkers related to alcohol consumption.

Methods: Routine parameters known to be influenced by alcohol consumption (γ-glutamyltransferase, GGT; carbohydrate-deficient transferrin, CDT/%CDT; mean corpuscular/cell volume, MCV; combined parameter of GGT and %CDT, GGT-CDT) were analyzed in serum samples of 2,182 pregnant women and 743 non-pregnant age-matched females. Data were statistically compared (I) for changes between pregnant and non-pregnant women, and (II) for changes of the three trimesters of pregnancy.

Results: Prevalence rates differ greatly according to the parameter and cut-off reflecting the problem of assessing alcohol consumption by biomarkers. Prevalence of harmful alcohol consumption on the basis of one singular or several elevated parameters was 13.8% (95%-CI: 12.4 – 15.2) in pregnant women compared to 18.6% (95%-CI:15.8 – 21.4) in non-pregnant women; however, 85.0% of these elevated measurements were caused by isolated increase of %CDT only. Using GGT-CDT as the parameter with the highest specificity according to the literature, the estimated prevalence of harmful alcohol consumption in pregnancy is 0.5% (95%-CI: 0.2 – 0.7).

Conclusion: Estimated prevalence rates differ greatly with respect to the biomarkers and cut-offs used. The isolated measurement of CDT/%CDT might result in an overestimation of harmful alcohol consumption during pregnancy.

Key words: alcohol, pregnancy, prevalence, %CDT, GGT, GGT-CDT ratio

Introduction

Alcohol consumption is commonly accepted in western societies. 66.5% of the female population in Germany reported consumption of alcohol in the last 30 days (Atzendorf et al., 2019), 24.6% reported binge-drinking (5 or more drinks (> 70g ethanol) in one day, in the last 30 days) and 19.7% consumed 12g ethanol or more every day. Consumption of alcohol is not just known to be a risk factor for diseases like liver cirrhosis, hepatocellular carcinoma, or pancreatitis (GBD 2016 Risk Factors Collaborators, 2017), it is also known to cause fetal alcohol spectrum disorders (FASD) if consumed during pregnancy (Kraus et al., 2019). FASD summarizes all teratogenic effects of intrauterine alcohol exposure, where fetal alcohol syndrome (FAS) is the most severe form, comprising typical facial dysmorphias, neurodevelopmental and growth deficits, and variable congenital malformations (Kraus et al., 2019). The underlying pathophysiological mechanisms of FASD are still incompletely understood. It seems, that ethanol enhances the formation of reactive oxygen species (ROS), which leads to “macromolecular oxidative damage”, causing DNA-, RNA- and histone modifications as well as dysfunctional proteins. All these alterations could lead to teratogenesis resulting in FASD and FAS (Bathia et al., 2019). The prevalence of alcohol consumption in pregnancy, FASD and FAS is widely unknown or was estimated in the past using questionnaires, which are showing difficulties based on possible under-reporting due to social stigmatization (Göransson et al., 2003). Popova et al. estimated a global prevalence of alcohol consumption in pregnancy of 9.8% (European Estimate: 25.2%, 95%-CI: 21.6 – 29.6) and a prevalence of FAS of 15 per 10,000 (European Estimate: 37.4 per 10,000, 95%-CI: 24.7 – 54.2) (Popova et al., 2017). Another systematic review estimated a global prevalence of FASD of 77 per 10,000 (Europe Estimate: 198 per 10,000) (Lange et al., 2017). Several laboratory markers are associated with alcohol consumption, and therefore, are used routinely to monitor drinking behaviour. The most used biomarkers are carbohydrate-deficient transferrin (as absolute concentration (CDT) and in relation to total transferrin (%CDT)), gamma-glutamyl-transferase (GGT) and mean corpuscular/cell volume (MCV). Elevated measurements of %CDT were found in people with a daily alcohol consumption of 50 – 80g for at least one week (Stibler, 1991), and may also be able to detect binge drinking (Howlett et al., 2017). For GGT, Hietala et al. found significant higher concentrations in case of 40g or more alcohol ingestion per day (Hietala et al, 2005). Notably, these biomarkers differ greatly with respect to sensitivity and

specificity. GGT is reported to have sensitivity of up to 95% for detection of ingestion of 60g or more per day for several months (Andresen-Streichert et al., 2017), which is higher than the sensitivity of %CDT (46 - 90%). Under the same circumstances %CDT was reported to be more specific for detection of alcohol consumption than GGT (70 – 100% vs. 18 – 93%) due to GGT elevation in cases of non-alcoholic liver diseases or toxic effects of different drugs (Andresen-Streichert et al., 2017). To elevate sensitivity without losing specificity, the combined parameter GGT-CDT was established (Hietala et al., 2006). For assessing the capability of these parameters for estimating the prevalence of alcohol consumption in pregnancy, Shipton et al. conducted a pilot study and showed that CDT is able to detect and monitor "hazardous" alcohol consumption in pregnancy (Shipton et al., 2013). Howlett et al. measured %CDT and GGT in 600 random blood samples of women in early pregnancy in north-east England and estimated a prevalence of elevated measurements of 1.7% (95%-CI: 0.7 – 2.9) based on %CDT and of 4.2% (95%-CI: 2.6 – 5.9) based on GGT (Howlett et al., 2020). In order to evaluate the prevalence of harmful alcohol consumption during pregnancy in Saxony-Anhalt (North Germany), five laboratory biomarkers (CDT, %CDT, GGT, MCV, GGT-CDT) were analysed in 2,182 pregnant and 743 non-pregnant age-matched females and statistically compared between (I) pregnant and non-pregnant women and (II) for changes of the three trimesters of pregnancy.

Materials and Methods

Study Design and Study Population

Study design was composed of retrospective and prospective parts. Frozen back-up serum samples from pregnant women (undergoing toxoplasma gondii serology testing), which were stored for 12 - 15 months at -20°C, were sorted out for enough specimen volume, visible icteric, lipemic or hemolytic staining and for measurement of MCV from the same venipuncture (Figure 1). Specimens that met these requirements were used for analysing GGT, CDT, %CDT. Each sample also received a Hemolysis-Icterus-Lipaemia-Check (HIL-Check) to exclude preanalytical interferences by these three conditions. Values for MCV were taken from the measurement of the corresponding blood sample at day of sample entry (12 – 15 months in the past; 05/2016 – 09/2017). Elevated MCV values were further assessed by measurement of holo-transcobalamin (HTC) to rule out vitamin-B12-deficiency. Borderline or reduced HTC concentrations, indicating vitamin-B12-deficiency, resulted in exclusion of the case from study cohort. Samples whose

measurements were not possible or incomplete were also excluded from study cohort. All samples were barcoded at 2 levels to ensure anonymous setting of the analysis as recommended by the local Ethic Committee of the Land Saxony-Anhalt in its approval (No. 56/17). According to this approval no declaration of consent by participants was necessary. In total, 2,182 samples from pregnant women could be measured and further analyzed statistically. The control samples were selected from age-matched women from whom MCV was analysed and pregnancy was excluded by laboratory parameters. The number of control samples (n = 743) was chosen to match the average sample numbers of pregnant women in the different trimesters. Control samples were frozen to -20°C to get the same preanalytical conditions as the samples of pregnant women. According to the approval of the local Ethic Committee of the Land Saxony-Anhalt, no declaration of consent was obtained from this cohort either. All together serum samples of 2,182 pregnant women and 743 non-pregnant age-matched females were analysed (details presented in Table 1). Information about drinking habits or pre-existing chronic diseases was not available.

Measurement Methods

CDT and Transferrin were measured using the immunonephelometric N Latex CDT and the N Antisera to Human Transferrin assay on BN ProSpec analyzer platform by Siemens (Siemens Healthcare Diagnostics Products, Marburg, Germany); %CDT was calculated automatically. For quantification of GGT the standardized (IFCC/Szasz) GGT-2 kinetic assay on Roche cobas c analyzer was applied (Roche Diagnostics, Mannheim, Germany). Calculation of MCV (from measured haematocrit and erythrocyte count) was done using the fully automated routine hematology analyzers by Sysmex (Sysmex Germany, Norderstedt, Germany). Equation of GGT-CDT was done using the formula published by Hietala et al (Hietala et al., 2006) as follows: GGT-CDT = 0.8 * ln(GGT) + 1.3 * ln(%CDT).

Cut-Offs

Cut-offs used to estimate the prevalence rate of harmful alcohol consumption on the basis of biomarkers were selected from two publications by Niemelä et al, that used the same test kit to measure %CDT as used in this study (Niemelä et al., 2016a, Niemelä et al., 2016b). The cut-off for %CDT (≥ 1.79) reflects the mean +2 standard deviations from women not consuming alcohol. Due to the high degree of international standardization of GGT assays, using cut-offs derived by

studies based on the same test kit was refrained. For GGT and GGT-CDT the cut-offs with the highest specificity were selected.

Statistical Analysis

Statistical analysis was done using R (version 4.0.3, Vienna, Austria). Testing for normal distribution was performed by using Shapiro-Wilk-test. Since none of the parameters analysed was normally distributed, non-parametrical tests were used. Prevalence rates were compared by using Chi-squared test. Calculation of confidence intervals was done by using the normal approximation. For comparisons of means between two independent groups Mann-Whitney-U-test (U-test) was applied. For comparison of means between more than 2 independent groups, Mann-Whitney-U-test with correction using the Bonferroni adjustment method was used. Level of significance was set to 0.05 ($p < 0.05$ was considered statistically significant). Due to the exploratory character of the study, no further adjustment for considering several endpoints in parallel has been applied.

Results

Analysis of five alcohol consumption-associated biomarkers in both groups revealed highly significant differences for four parameters, whereas MCV was found to be similar with a tendency of higher values for pregnant compared to non-pregnant women (Table 2). MCV values were therefore not further evaluated.

Prevalence rates of harmful alcohol consumption based on biomarkers were calculated for both, pregnant and non-pregnant women on the basis of previously published cut-offs as shown in Table 3. Estimates show prevalence rates between 0.5% and 11.9% for pregnant women and between 3.8% and 11.4% for non-pregnant women (details presented in Table 3). 301 pregnant (13.8%) and 138 (18.6%) non-pregnant women demonstrated at least one elevated biomarker (Table 3 and 4). Various constellations of one or more elevated biomarker for the prevalence estimates are shown in Table 4.

Trimester-specific analysis of biomarkers

Further analysis estimated prevalence rates based on biomarkers for each trimester of pregnancy. GGT- and GGT-CDT-values showed a decreasing trend, whereas %CDT-values were found to be increased (Table 5). The cut-off for %CDT showed a higher prevalence of elevated measurements in the second and third trimester in comparison with the first trimester.

Furthermore, a significant increase of %CDT values for second compared to first trimester (mean: 1.43% vs. 1.57%, $p < 0.001$, U-test) was demonstrated, whereas the second and the third trimester as well as the non-pregnant women showed comparable levels of %CDT (medians: 1.56% vs. 1.55% vs. 1.52%) as shown in Figure 2. To further evaluate the observed increase of %CDT from first to second trimester, analysis of absolute CDT revealed significant higher values in pregnant women compared to controls (mean: 47.0 vs. 40.3 mg/l; $p < 0.001$, U-test, Table 2). Subanalysis in context to the 3 trimesters confirmed the known increase of absolute CDT during pregnancy (means: 40.7 vs. 54.9, $p < 0.001$, 54.9 vs. 62.2, $p < 0.001$, U-test) in dependence of gestational age (Figure 3) as already published by Bakhireva et al (Bakhireva et al., 2012). GGT values were significantly lower in pregnant vs. non-pregnant women (mean: 13.8 vs. 22.5 U/l, $p < 0.001$, U-Test, Table 2). Subanalysis concerning trimesters revealed a significant decrease from first to second trimester (mean: 15.9 vs. 10.5, $p < 0.001$, U-test) with comparable values between second and third trimester (mean: 10.5 vs. 10.5, $p = 0.87$, U-test, Figure 4). Similar pattern was observed for GGT-CDT between pregnant and non-pregnant women (mean: 2.47 vs. 2.84, $p < 0.001$, U-test, Table 2). GGT-CDT among the three trimesters demonstrated an almost identical pattern with significant reduction between 1st and 2nd trimester (mean: 2.55 vs. 2.35, $p < 0.001$, U-test, Figure 5).

Discussion

The aim of this study was to estimate the prevalence of harmful alcohol consumption in pregnancy using biomarkers. As shown in Table 3, estimated prevalence rates of harmful alcohol consumption differs significantly between the different biomarkers and the cut-offs used, resulting in a range of 0.5% and 11.9% for pregnant women and 3.8% and 11.4% for non-pregnant women. These findings are based on the different levels of sensitivity and specificity of each biomarker. Comparing different studies using different protocols, testing methods and cut-offs, broad variations exist among prevalence rates of harmful alcohol consumption based on biomarkers reported in pregnant women. Using increased GGT values alone (> 45 U/l) Howlett et al. 2020 identified 4.2% of pregnant women in early pregnancy with elevated measurements, whereas the analogous GGT-based rate of women in the first trimester in our cohort was lower (2.9% (95%-CI: 2.0 – 3.8) even a slightly lower cut off (> 40 U/l) was applied. Notably, using elevated %CDT as only biomarker, corresponding rates of 1.7 % and 7.2% for Howlett et al. and our study, respectively, were identified. This striking discrepancy between both rates in the same cohorts by using two different biomarkers linked to alcohol consumption reflects the problem of assessing alcohol consumption by laboratory biomarkers. The estimated prevalence rate of harmful alcohol consumption in pregnant women, defined by one or more elevated biomarkers, in our cohort was 13.8% compared to 25.2% reported by Popova et al. who assessed alcohol consumption of any kind in pregnancy in Europe using questionnaires (Popova et al., 2017). This higher estimation of alcohol consumption by Popova et al. compared to our data is plausible since analysed biomarkers (GGT, CDT, %CDT) are only increased in case of harmful alcohol consumption as outlined in introduction. These biomarkers are not able to identify women who drink only little amounts of alcohol during pregnancy (Hietala et al., 2006). Comparing the estimated prevalence rate with recently published data about riskful drinking patterns in the general population in Germany, our result seems to fit well (13.8% vs. 19.7% (pregnant women vs. women in general, indicating a lower rate of harmful alcohol consumption in pregnancy) and 18.6% vs. 19.7% (non-pregnant women vs. women in general, showing a comparable level of harmful alcohol consumption in non-pregnant women), Atzendorf et al., 2019). Using the most specific biomarker to detect harmful alcohol consumption in pregnancy according to the literature (GGT-CDT, cut-off: ≥ 3.8, Niemelä et al., 2016a) one would expect 83

cases of new-borns in 2019 in Saxony-Anhalt (0.5% of 16,619 new-borns in 2019), respectively 3,890 cases in Germany in 2019 (0.5% of 778,100 new-borns in 2019) who have been exposed to harmful alcohol consumption during pregnancy. Interestingly these estimates are in the range of reported numbers of 100 "FAS-like" malformations per year in average, published by the Malformation-Monitoring Centre of Saxony-Anhalt (Rissmann et al., 2014) and 2.930 cases calculated for whole Germany (95%-CI: 1,720 – 4,500, Kraus et al. 2019). However, it should be noted that these corresponding numbers represent primarily an association only, and do not provide any evidence of a causal linkage between our estimated prevalence rates and the numbers of "FAS-like" malformations. Firstly, FASD- and FAS-affected new-borns, children and adolescents show more symptoms than just "FAS-like" malformations. Secondly, the biomarkers used in our study identify only women with a certain amount of alcohol consumption that we termed "harmful". Other types of alcohol consumption, which might also lead to FASD- and FAS-affected new-borns, remain undetected.

It is remarkable that 85.0% of all pregnant women with elevated biomarkers (as illustrated in Table 4) showed an isolated elevation of %CDT. This effect seems to be caused by the physiological increase of CDT in pregnancy (Bakhireva et al., 2012). Therefore, it has been recommended to report the relative amount of CDT in correspondence to total transferrin (%CDT) to avoid false high results of CDT (Kenan et al., 2011). However, our data suggest that there is a slightly increase of %CDT between the first and second trimester and comparable concentrations of %CDT in the second and third trimester. In addition to higher %CDT values in context to gestational age, the higher numbers of elevated %CDT measurements in second and third trimester in comparison with non-pregnant females strongly implies that not only absolute levels of CDT, but values of %CDT increase with gestational age as well. This phenomenon would cause falsely high prevalence rates of harmful alcohol consumption in pregnant women using a single cut-off for evaluation of all three trimesters. This finding implies the need for new evaluation of %CDT cut-offs in pregnancy in general since there are no trimester-specific reference intervals either. In case of GGT, trimester-specific reference intervals were published in 2008, showing slightly increased upper reference limits from 7th to 17th gestational week (34.8 U/l) than at higher gestational age (2nd /3rd trimester: 24.0 U/L and 25.8 U/L, respectively, Larsson et al., 2008) but all estimated upper reference limits were below the cut-off (40 U/l) used

in this study. Taking a closer look at GGT and GGT-CDT our data showed a significant decrease in means between the first and the second trimester, while the difference between the second and third trimester was significantly lower and even medically negligible. This finding is supported by a recent analysis of alcohol consumption in pregnant women from the US (England et al., 2020). England et al. found a prevalence of current drinking (at least one drink in the last 30 days) in pregnant women of 19.6% in the first trimester with a significant decrease to 4.7% in second and third trimester. Main strengths of this study are the high number of pregnant women and age-matched controls from the same area, sample processing in the same laboratory, prevalence estimation using cut-offs established with the same test kit as used in this study, and the usage of various alcohol-related biomarkers for monitoring harmful alcohol consumption. Furthermore the fact that no consent of participants was needed reduces the rate of possible under-reporting due to social stigmatization. Although, the used biomarkers are widely available in routine laboratories, other biomarkers with better specificity and sensitivity (e.g. ethyl glucuronide) exist and might be more suitable for separating harmful from medically neglectable alcohol consumption.

Conclusion

This study revealed two main findings. First, estimated prevalence rates differ greatly with respect to the biomarkers and cut-offs used. Secondly, the isolated measurement of CDT/%CDT might result in an overestimation of harmful alcohol consumption due to the physiological increase in CDT during pregnancy that seems to be insufficiently corrected using %CDT. Therefore, new studies focusing on trimester-specific reference limits and cut-offs for CDT and %CDT are needed.

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Figure legends

Figure 1: Selection of specimens. Numbers of back-up samples sorted out for enough specimen volume, visible icteric, lipemic or hemolytic staining, measurement of MCV from the same venipuncture, exclusion of multiple measurements from one woman, not or incompletely measurable samples and holo-transcobalamin concentrations indicating vitamin-B12-deficiency.

Figure 2: %CDT in pregnant (grey boxes) and non-pregnant (white box) women. Data shows a significant increase of %CDT from the first to the second trimester. Second and third trimester as well as non-pregnant women showed comparable levels of %CDT values. Significant and non-significant differences are marked as **** ($p < 0.001$), ** ($p = 0.01$) and n.s., respectively (Mann-Whitney-U-test). Data are shown as boxplots (25^{th}- 75^{th} range + median), whiskers showing the last data point in ± 1.5 * inter-quartile-range. Outliers are shown by dots.

Figure 3: Absolute CDT concentration (mg/l) in pregnant (grey boxes) and non-pregnant women (white box) in context to gestational age. Data shows a significant increase of absolute CDT by trimester; data are shown as box plots as outlined in legend of Figure 2.

Figure 4: GGT in pregnant (grey boxes) and non-pregnant (white box) women. Data show a significant decrease in GGT values from first to second trimester as well as the significant difference between all trimesters and the non-pregnant women; data are shown as boxplots as outlined in legend of Figure 2.

Figure 5: GGT-CDT in pregnant (grey boxes) and non-pregnant (white box) women.
GGT-CDT ratios show similar pattern as concentration of GGT alone (Figure 4); data are shown as boxplots as outlined in legend of Figure 2.

Table 1: Study population characteristics

Characteristics	Pregnant women	Non-pregnant women
Evaluable samples (n)	2,182	743
Age Range (years)	14 – 46	15 – 43
Age Mean (years)	29.5	29.8
Age Standard Deviation (years)	4.8	5.2
Gestational Age (n) (reported or estimated)	1st Trimester: 1,356 2nd Trimester: 551 3rd Trimester: 275	-

Trimester were defined as 1st trimester from 1st to 13th gestational week, 2nd trimester from 14th to 26th gestational week and 3rd trimester from 27th gestational week to delivery.

Table 2: Values of alcohol consumption-associated biomarkers in pregnant and non-pregnant women.

Characteristics	Pregnant women (n = 2,182)		Non-pregnant women (n = 743)		P-value
CDT Median (Range)	**44.5**	(21.5 – 99.8)	**39.7**	(21.8 – 80.2)	-
CDT Mean (SD)	**47.0**	(13.8)	**40.3**	(9.4)	< 0.001
%CDT Median (Range)	**1.47**	(0.83 – 2.47)	**1.52**	(0.96 – 3.60)	-
%CDT Mean (SD)	**1.48**	(0.25)	**1.53**	(0.24)	< 0.001
GGT U/l Median (Range)	**11.4**	(1.2 – 163.2)	**16.2**	(5.4 – 814.8)	-
GGT U/l Mean (SD)	**13.8**	(9.7)	**22.5**	(35.4)	< 0.001
GGT-CDT Median (Range)	**2.45**	(0.34 – 4.67)	**2.77**	(1.66 – 5.89)	-
GGT-CDT Mean (SD)	**2.47**	(0.44)	**2.84**	(0.49)	< 0.001
MCV fl Median (Range)	**86**	(57 – 133)	**85**	(60 – 106)	-
MCV fl Mean (SD)	**86**	(5)	**85**	(5)	0.067

Data are presented as medians and complete range of values and means including standard deviation (SD). Statistical analysis was performed by Mann-Whitney-U-test.

Table 3: Estimated prevalence rates of harmful alcohol consumption based on biomarkers in pregnant and non-pregnant women

Biomarker	Cut-Off [Ref]	Sens (%)	Spec (%)	Pregnant (all) n = 2,182 % (95%-CI)	Non-pregnant n = 743 % (95%-CI)	p
%CDT	≥ 1.79 [2]	*	96.4	11.9 (10.5 - 13.2)	11.4 (9.2 - 13.7)	0.75
GGT U/l	≥ 40 [1]	33.0	96.4	2.1 (1.5 – 2.7)	8.1 (7.0 – 9.2)	< 0.001
GGT-CDT	≥ 3.80 [1]	33.5	98.0	0.5 (0.2 - 0.7)	3.8 (2.4 - 5.1)	< 0.001
one or more elevated biomarker	%CDT: ≥ 1.79 GGT: ≥ 40 GGT-CDT: ≥ 3.80	-	-	**13.8 (12.4 – 15.2)**	**18.6 (15.8 – 21.4)**	0.002

* Sensitivity was reported in dependence of birth of a child with (39.5%) or without FAS (4.2%). Rows 2-4 present cut-offs, sensitivity, and specificity, respectively together with corresponding publication that were used for calculation of prevalence rates (%) that are shown as estimate with 95% confidence interval. Statistical analysis was performed by Chi-squared test. [1] Niemelä et al., 2016a, [2] Niemelä et al., 2016b

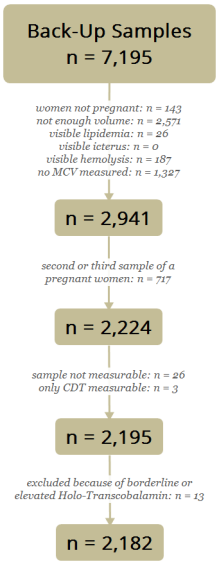
Table 4: Observed constellations of one or more elevated biomarkers for both pregnant and non-pregnant women.

Constellation	pregnant n = 2,182 (n (% of all elevated))	non pregnant n = 743 (n (% of all elevated))
isolated %CDT (≥ 1.79%)	**256 (85.0)**	76 (55.0)
isolated GGT (≥ 40 U/l)	35 (11.6)	34 (24.6)
isolated GGT-CDT (≥ 3.80)	0 (-)	0 (-)
GGT + GGT-CDT elevated	7 (2.4)	19 (13.8)
%CDT + GGT-CDT elevated	0 (-)	2 (1.5)
%CDT + GGT + GGT-CDT elevated	3 (1.0)	7 (5.1)
sum of cases	301 (100.0)	138 (100.0)
prevalence of elevated measurements (95%-CI)	**13.8 (12.4 – 15.2)**	**18.6 (15.8 – 21.4)**

Table 5: Estimated prevalence rates of harmful alcohol consumption in trimesters of pregnancy.

Biomarker	Cut-Off	1st Trimester n = 1,356 % (95%-CI)	2nd Trimester n = 551 % (95%-CI)	3rd Trimester n = 275 % (95%-CI)	p – values for comparison of means	
%CDT	≥ 1.79	7.2 (5.8 - 8.6)	20.9 (17.5 - 24.3)	17.1 (12.6 - 21.6)	1st vs. 2nd: 1st vs. 3rd: 2nd vs. 3rd:	< 0.001 < 0.001 1
GGT U/l	≥ 40	2.9 (2.0 – 3.8)	0.5 (0.0 – 1.1)	1.1 (0.0 – 2.3)	1st vs. 2nd: 1st vs. 3rd: 2nd vs. 3rd:	< 0.001 < 0.001 1
GGT-CDT	≥ 3.80	0.7 (0.3 - 1.1)	0.2 (0.0 - 0.6)	0.0 (-)	1st vs. 2nd: 1st vs. 3rd: 2nd vs. 3rd:	< 0.001 < 0.001 1

Prevalence rates (%) are shown with 95% confidence interval. P-values for comparison of means between the three trimesters were estimated using Mann-Whitney-U-test with Bonferroni adjustment.



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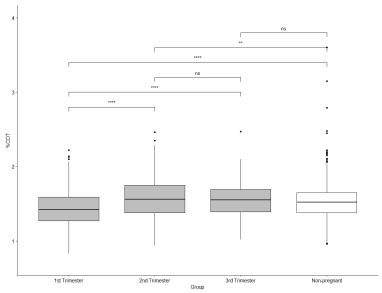

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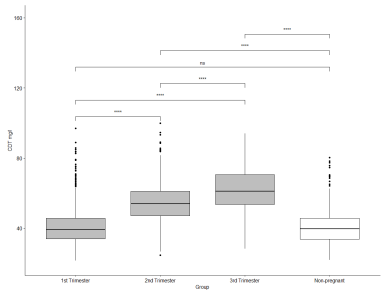

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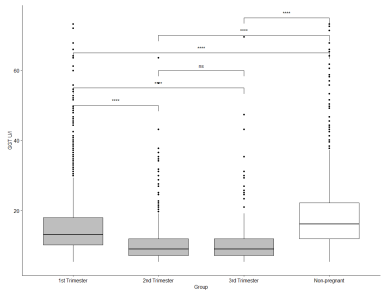

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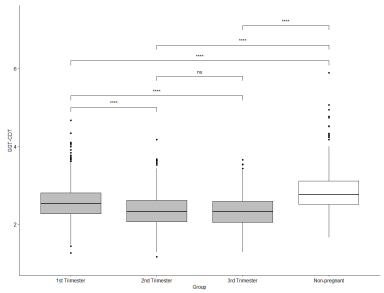

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