OBSTETRICS

Reference ranges for liver function tests in pregnancy controlling for maternal characteristics

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BACKGROUND: Liver dysfunction complicates 3% of pregnancies, and prompt diagnosis reduces severe maternal and perinatal morbidity and mortality. Recognition of liver dysfunction relies on the creation of reference ranges. Outside pregnancy, factors such as age and sex have been shown to affect liver biomarkers. However, despite recommendations for age- and sex-adjusted reference ranges for liver function tests, these have not been widely adopted clinically. In pregnancy, only a few studies have examined changes of liver function tests with gestation, and none of them have controlled for maternal demographic characteristics.

OBJECTIVE: This study aimed to (1) provide reference ranges for liver function tests in a large population of uncomplicated pregnancies after adjusting for the effect of gestational age and maternal demographic characteristics on the median and measures of dispersion and shape (skewness and kurtosis) of the distribution of these variables, and (2) create an online calculator of z-scores of maternal liver function tests using the above-mentioned methodology.

STUDY DESIGN: This was a cross-sectional study of healthy women undergoing routine antenatal ultrasound scans at 11^{+0} to 13^{+6} weeks' gestation (visit 1), 19^{+0} to 24^{+6} weeks (visit 2), 30^{+0} to 34^{+6} weeks (visit 3), and 35^{+0} to 37^{+6} weeks (visit 4). Women with a history of liver dysfunction or adverse pregnancy outcomes were excluded from the analysis. We measured the following variables: ALP (alkaline phosphatase), ALT (alanine aminotransferase), AST (aspartate aminotransferase), GGT (gamma-glutamyl transferase), total bilirubin, and ALB (albumin). The assessment of the distribution of liver function tests across gestational age (controlling for maternal characteristics) was performed using the generalized additive model for location, scale, and shape, with the gamlss R package, which allows the implementation of distributions other than the normal distribution.

RESULTS: There were 3451 women who agreed to participate in the study and had uncomplicated pregnancies. Women participated only once in the study, with 805, 860, 886, and 900 women attending visits 1, 2, 3, and 4, respectively. The location parameter of the distribution of the liver function test variables is independently predicted by gestational age and ethnicity for all variables, by maternal body mass index for all variables except ALB, by maternal age for all variables except ALT-AST ratio and GGT, by maternal parity for all variables except total bilirubin and ALP, by maternal smoking for total bilirubin and ALB, and by maternal weight for ALP. The scale parameter of the distribution is also independently predicted by gestational age for all variables except ALP, maternal body mass index for ALT, AST, GGT, and ALP, maternal age and ethnicity for GGT, and maternal parity for ALT and AST. In contrast, the skewness and kurtosis of liver function tests are influenced nonuniformly by gestational age, maternal ethnicity, body mass index, age, and parity. An online calculator of z-scores for the above-mentioned variables is given at https://fetalmedicinefoundation.shinyapps.io/life/.

CONCLUSION: Assessing whether a pregnant woman's liver function tests deviate from the expected normal values necessitates adjusting for gestational age and maternal demographic characteristics, both for the median and measures of dispersion and shape of the distribution.

Key words: alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, dispersion and shape of the distribution, gamma-glutamyl transferase, generalized additive model for location, scale, and shape, liver function tests, maternal characteristics, pregnancy, reference ranges, total bilirubin

Introduction

Liver dysfunction complicates 3% of pregnancies.[1] Prompt diagnosis is essential because unrecognized liver disease can lead to severe morbidity and mortality of the mother and fetus.[2] Liver disease in pregnancy can develop as a consequence of conditions exclusive to pregnancy, such as hypertensive disorders or preexisting chronic conditions, such as viral hepatitis.[3–5]

Recognition of liver dysfunction relies on reference ranges derived from healthy individuals, usually defined as values above the 95th or 97.5th percentile of the reference group. Outside pregnancy, age,[6–9] sex,[7,8] ethnicity,[7,10] and body mass index (BMI)[7,11–13] have been shown to affect liver biomarkers, and the National Academy of Clinical Biochemistry recommends age- and sex-adjusted reference ranges for liver function tests (LFTs).[14] However, these recommendations have not been widely adopted. In pregnancy, the physiological changes of liver function impact biochemical markers.[15] Therefore, it is important that pregnancy-specific reference ranges are used to minimize false-negatives and false-positives in diagnosis of liver dysfunction.[16] The studies that examined changes in LFTs with gestation are limited by small numbers of patients,[16–21] incomplete examination of LFTs,[18,19] or the use of statistical methods that analyzed continuous variables as dichotomous.[16,17] Furthermore, none of these studies controlled for maternal demographic characteristics such as age, BMI, and ethnicity. The limitations of the above methodologies prevent the translation of liver function biomarker values into z-score deviations

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AJOG at a Glance

Why was this study conducted?

This study aimed to provide reference ranges for liver function tests in a large population of uncomplicated pregnancies after adjusting for the effect of gestational age and maternal demographic characteristics on the median and measures of dispersion and shape (skewness and kurtosis) of the distribution of these variables.

Key findings

The median and measures of dispersion and shape of the distribution of liver function tests in pregnancy are influenced significantly by gestational age, maternal age, ethnicity, body mass index, parity, and smoking.

What does this add to what is known?

Calculating deviations from the median of a distribution for liver function tests in pregnancy, such as z-scores, necessitates adjustment for gestational age and maternal demographic characteristics, both for the expected median and for measures of dispersion and shape of the distribution.

from a central (median) value, adjusted for confounding characteristics. This is particularly important because it is established that correcting placental hormone or angiogenic values for maternal characteristics improves the performance of screening for Down syndrome[22,23] or preeclampsia,[24] respectively.

The aim of this study was to (1) determine reference ranges for LFTs from a large sample of healthy pregnant women (by analyzing data as continuous variables and correcting for maternal characteristics), and (2) provide a free online application to obtain z-scores for liver variables in pregnancy.

Patients and methods

Target population

Data for this study were obtained from women undergoing routine ultrasound scans during their pregnancy at King's College Hospital, London, United Kingdom, between November 2009 and November 2017. During pregnancy, women are offered 4 scans at distinct gestational periods, which cover different screening aims. The first visit, at 11^{+0} to 13^{+6} weeks' gestation, is focused on screening for aneuploidies and pregnancy complications. The second visit at 19^{+0} to 24^{+6} weeks' gestation, the third visit at 30^{+0} to 34^{+6} weeks, and the fourth visit at 35^{+0} to 37^{+6} weeks include ultrasound examination of the fetal anatomy and estimation of fetal size. Gestational age (GA) is determined by the measurement of fetal crown–rump length at 11 to 13 weeks or fetal head circumference at 19 to 24 weeks.[25,26]

During these visits, women were asked to participate in a study on screening for adverse pregnancy outcomes, which was approved by the National Ethics Committee. Women agreeing to participate in the study provided informed consent, and maternal blood was collected, immediately centrifuged, and serum frozen at −80°C. Data on pregnancy outcomes were collected from the hospital maternity records or the general medical practitioners.

Inclusion criteria for the current study encompassed healthy women, with singleton uncomplicated pregnancies, delivering phenotypically normal infants at ≥37 weeks' gestation and without any risk factors or history of liver disease. In detail, women with preexisting chronic hypertension, diabetes mellitus, systemic lupus erythematosus or antiphospholipid syndrome, and liver (such as gallbladder disease or cholestasis of pregnancy), renal, autoimmune, and infectious diseases (such as HIV) were excluded from the study. For the above-mentioned conditions, we reviewed patients' hospital records for the years predating pregnancy and up to 5 years after pregnancy, and excluded patients with any relevant diagnosis. In addition, pregnancies complicated by preterm delivery, gestational diabetes,[27] hypertensive disorders of pregnancy,[28] infections, aneuploidy, or major fetal abnormalities, and those ending in termination, miscarriage, or fetal death were excluded from the study.

Patient characteristics

Patient characteristics included maternal age, self-declared ethnicity (White, Black, South Asian, mixed), method of conception (spontaneous or assisted with ovulation drugs or in vitro fertilization), cigarette smoking (nonsmoker or smoker), and parity (nulliparous or parous). Maternal height was measured at the first visit, and maternal weight and BMI at all visits. This was a prospective, cross-sectional study, and each patient's variables were recorded at 1 visit during their pregnancy.

Measurement of liver function biomarker variables

For the analysis, reagents were distributed by Siemens Healthcare Diagnostics Ltd (Frimley, United Kingdom). Samples were processed using ADVIA 2400 analyzers (Siemens Healthcare, Frimley, United Kingdom).

We measured ALP (alkaline phosphatase), ALT (alanine aminotransferase), AST (aspartate aminotransferase), GGT (gamma-glutamyl transferase), total bilirubin (TBIL), and ALB (albumin). The ALT-AST ratio was calculated. The details of measurement of each variable are appended in Supplemental Material A.

Statistical analysis methodology

Generalized additive model for location, scale, and shape

The assessment of the distribution of LFTs across GA (controlling for maternal characteristics) was performed using the generalized additive model for location, scale, and shape (GAMLSS), with the gamlss R package,[29–31] which allows the implementation of distributions other than the normal distribution.[32]

GAMLSS are distributional regression models in which the response variable is assumed to be generated from a flexible

TABLE 1
Demographic characteristics, pregnancy outcomes, and liver function tests at the 4 visit intervals

Characteristics	Group 1 11+0 to 13+6 wk (N=805)	Group 2 19+0 to 24+6 wk (N=860)	Group 3 30+0 to 34+6 wk (N=886)	Group 4 35+0 to 37+6 wk (N=900)	*P*-value
Age (y)	31.2 (26.9–35.0)	31.7 (27.6–35.2)	31.9 (27.6–35.3)	32.1 (28.6–35.5)	.008
Height (cm)	162.6 (158.0–167.6)	163.0 (159.0–168.0)	163.0 (159.0–168.0)	164.0 (159.6–168.0)	.004
Weight (kg)	65.0 (57.0–74.1)	68.0 (61.2–78.6)	73.6 (66.0–83.0)	76.8 (69.6–86.0)	<.0001
Body mass index (kg/m^2)	24.2 (21.8–27.8)	25.6 (23.1–29.2)	27.6 (24.9–31.0)	28.6 (25.9–32.0)	<.0001
Ethnicity					<.001
White, n (%)	285 (35.4)	296 (34.4)	307 (34.7)	306 (34.0)	
Black, n (%)	264 (32.8)	288 (33.5)	294 (33.2)	296 (32.9)	
South Asian, n (%)	256 (31.8)	276 (32.1)	285 (32.2)	276 (30.7)	
Mixed, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	22 (2.4)	
Nulliparous, n (%)	352 (43.7)	421 (49.0)	419 (47.3)	469 (52.1)	.006
Conception					.01
Spontaneous, n (%)	805 (97.8)	860 (96.7)	886 (95.8)	900 (96.3)	
Ovulation drugs, n (%)	4 (0.5)	4 (0.5)	9 (1.0)	0 (0.0)	
In vitro fertilization, n (%)	14 (1.7)	24 (2.8)	28 (3.2)	33 (3.7)	
Smoking, n (%)	45 (5.6)	29 (3.4)	25 (2.8)	25 (2.8)	.005
Gestational age at visit (wk)	12.6 (12.0–13.2)	21.8 (20.9–22.1)	32.1 (31.8–32.4)	36.0 (35.6–36.4)	<.0001
Pregnancy outcome					
Gestational age at delivery (wk)	40.1 (39.3–40.9)	40.1 (39.2–41.1)	40.1 (39.3–40.9)	40.1 (39.3–41.0)	.39
Birthweight in grams	3380 (3178–3616)	3395 (3150–3612)	3381 (3120–3639)	3440 (3211–3660)	<.0001
Liver function tests					
ALT (alanine aminotransferase) in IU/L	11.7 (9.2–14.7)	12.5 (8.9–17.4)	11.9 (9.6–15.1)	11.8 (8.8–15.1)	.004
AST (aspartate transaminase) in IU/L	21.5 (19.2–24.8)	20.9 (17.5–26.7)	21.7 (18.7–25.6)	22.6 (19.0–28.7)	<.001
AST-ALT ratio	1.8 (1.5–2.3)	1.6 (1.3–2.1)	1.8 (1.4–2.2)	1.9 (1.6–2.6)	<.0001
Total bilirubin in μmol/L	7.1 (5.6–9.0)	5.9 (4.1–7.6)	7.2 (5.9–8.9)	7.4 (6.0–9.3)	<.0001
GGT (gamma-glutamyl transferase) in IU/L	11.0 (8.4–15.9)	8.9 (6.7–13.1)	8.6 (6.4–12.8)	8.2 (5.9–12.9)	<.0001
ALB (albumin) in g/L	42.7 (41.3–44.1)	39.0 (37.5–40.3)	37 (35.6–38.7)	37.3 (36.0–38.6)	<.0001
Alkaline phosphatase in IU/L	49.9 (41.9–59.7)	58.2 (49.0–68.9)	93.4 (78.0–112.0)	120.1 (100.0–146.5)	<.0001

Numerical data are presented as median (interquartile range) and categorical data as number (percentage). *P*-values for the corresponding Kruskal–Wallis or chi-square test are shown.

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TABLE 2
Summary of the effect of maternal demographic variables on the generalized additive model for location, scale, and shape analysis of liver function tests for μ, σ, ν and τ models (L = linear, Q = quadratic, C = cubic, QN = Quintic, ie, polynomial order 5)

Mu model

Variable	Distribution	Gestational age	BMI	Weight	Age	Race	Parity	Smoking
ALP	BCTo	Q	L	L	C	South Asian		
ALT	BCTo	Q[a]	C[a]		L	Black	Multiparous	
AST	logSHASH	Q	C		L	Black[b]	Multiparous	
ALT/AST	BCTo	Q	L			Black	Multiparous[b]	
GGT	logSHASHo	C	C			Black, South Asian	Multiparous	
TBIL	logSHASHo	L	L		L	Black, South Asian		Smoker
ALB	BCTo	Q			L	Black	Multiparous	Smoker[b]

Sigma model

Variable	Distribution	GA	BMI	Weight	Age	Race	Parity	Smoking
ALP	BCTo		L[a]					
ALT	BCTo	C	L[a]				Multiparous	
AST	logSHASH	QN	L[a]				Multiparous	
ALT/AST	BCTo	C						
GGT	logSHASHo	C	L	L[a]		Black		
TBIL	logSHASHo	C						
ALB	BCTo	C						

Nu model

Variable	Distribution	GA	BMI	Weight	Age	Race	Parity	Smoking
ALP	BCTo							
ALT	BCTo							
AST	logSHASH							
ALT/AST	BCTo		L[b]			Black		
GGT	logSHASHo	L						
TBIL	logSHASHo	C	Q		L[a]			
ALB	BCTo	C[a]						

Tau model

Variable	Distribution	GA	BMI	Weight	Age	Race	Parity	Smoking
ALP	BCTo	L						
ALT	BCTo	L[c]				South Asian[c]		
AST	logSHASH	C						
ALT/AST	BCTo	L					Multiparous[a]	
GGT	logSHASHo					Black[a]		
TBIL	logSHASHo	Q[a]					Multiparous[b]	
ALB	BCTo	C[a]						

The μ model for ALP has an interaction of quadratic GA with linear BMI [ie, poly(GA, 2)*BMI].

BMI, body mass index; *C*, cubic; *GA*, gestational age; *L*, linear; *Q*, quadratic; *QN*, quintic (ie, polynomial order 5); *TBIL*, total bilirubin.

[a] Significant, $.001 < P \leq .01$; [b] Significant, $.01 < P \leq .05$; [c] Borderline, $.05 < P \leq .065$. All terms unmarked with superscript letters are significant at $P \leq .001$.

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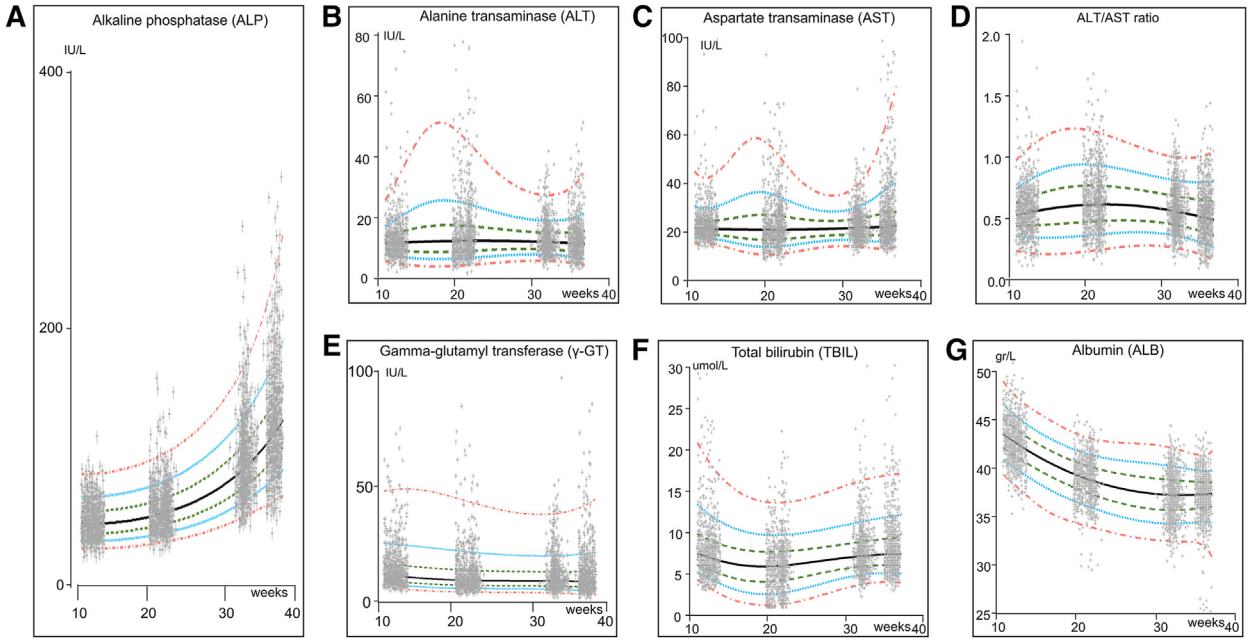
FIGURE 1
Gestational age–only model



Reference ranges for **A,** alkaline phosphatase, **B,** alanine transaminase, **C,** aspartate transaminase, **D,** alanine transaminase–aspartate transaminase ratio, **E,** gamma-glutamyl transferase, **F,** total bilirubin, and **G,** albumin.
Each graph demonstrates the raw data points at the 4 gestational age (GA) visits and their respective quantiles: 50th (*black line*), 25th and 75th (*green interrupted line*), 10th and 90th (*blue dotted line*), and 2nd and 98th (*red interrupted line*).

To facilitate inclusion of all graphs within the figure, 5 cases with GA >36 weeks and ALP >400 IU/L, 6 cases for ALT, 4 for AST, 2 for ALT-AST ratio, and 8 for GGT were omitted from the plot.

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theoretical distribution that has up to 4 distribution parameters (denoted μ, σ, ν and τ). Those distribution parameters usually represent the location, scale, and shape (skewness and kurtosis) of the distribution.

The main advantage of using GAMLSS for modeling a dependent variable is that, because all the parameters of the distribution of the response variable can be modeled as linear, polynomial, or smooth functions of the explanatory variables, the model can capture the change in location, scale, and shape of the distribution with respect to explanatory variables.

Distributions for liver function variables

We searched for the best fitting distribution for modeling each liver function variable. The final chosen distributions were the Box-Cox *t* (BCTo)[33] (for ALT, ALT/AST, ALP, and ALB), logSHASH (for AST), and the logSHASHo (for GGT and TBIL). Details about these distributions are provided in Supplemental Material A.

Calculation of z-scores

For new cases, their z-score for a liver function variable can be obtained as follows. First, the distribution parameters are estimated on the basis of the values of their explanatory variables. Then, given their value of the liver function variable, the probability of obtaining a liver function value below theirs is calculated using the cumulative distribution function (CDF). The inverse CDF of a standard normal distribution is then applied to obtain their z-score.

Results

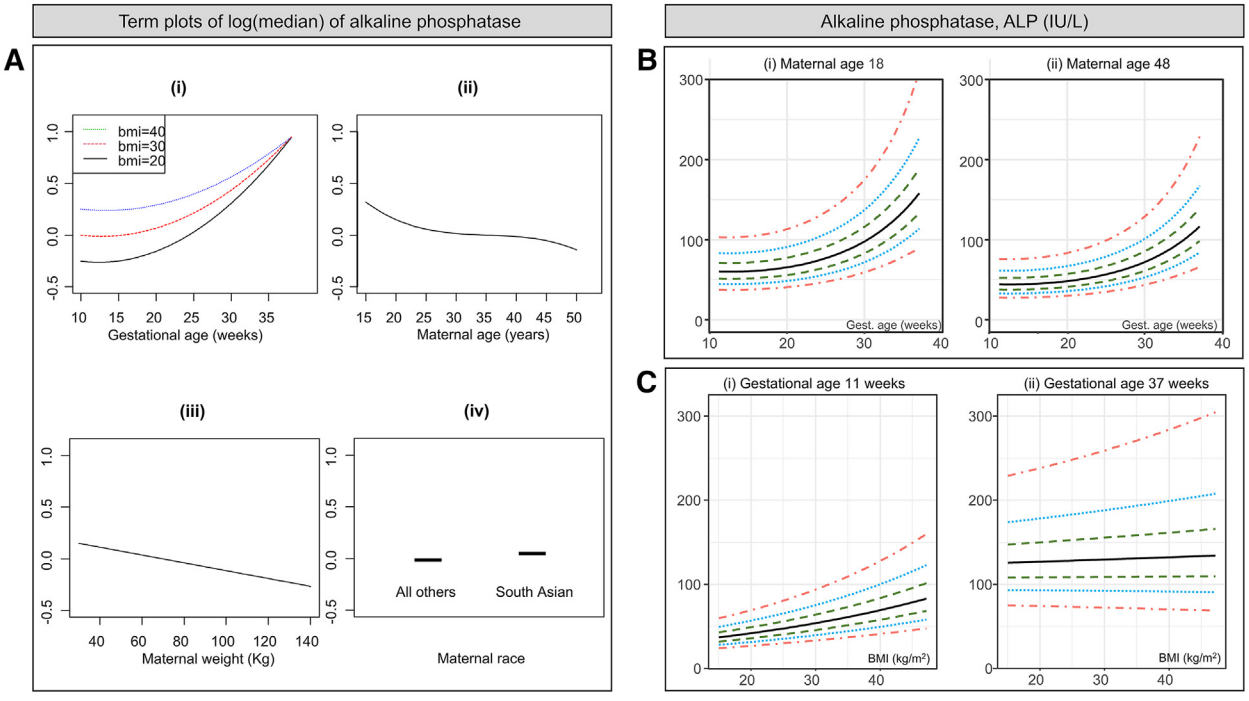
The distribution of the numerical data was assessed for normality using the Kolmogorov–Smirnov test. LFTs were nonnormally distributed and were therefore transformed to $\log_{10}$ values for summary statistics. Numerical variables are presented as median (interquartile range) and categorical variables as frequency (percentage) for each category value.

Between November 2009 and November 2017, 3686 women received routine antenatal care. In total, 235 women were excluded from further analysis because of an adverse pregnancy outcome or adverse medical history, as described above. Eventually, 3451 women were included in this study (Table 1). A total of 805 women were seen at 11^{+0} to 13^{+6} weeks (group 1), 860 at 19^{+0} to 24^{+6} weeks (group 2), 886 at 30^{+0} to 34^{+6} weeks (group 3), and 900 at 35^{+0} to 37^{+6} weeks (group 4).

Demographic characteristics and pregnancy outcomes (Table 1)

Among the 4 GA groups, there were small differences in age and height,

FIGURE 2
Full model for alkaline phosphatase



A, Term plots of log(median) of alkaline phosphatase: gestational age with interaction with BMI (i), maternal age (ii), maternal weight (iii), and maternal race (iv). **B,** Full model for alkaline phosphatase against gestational age for maternal age of 18 years (i) and 48 years (ii). **C,** Full model for alkaline phosphatase against BMI for gestational age of 11 weeks (i) and 37 weeks (ii).

BMI, body mass index.

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which were not clinically significant. There was a significant difference in weight and BMI, with an expected increase in weight and BMI with advancing GA. White, Black, and South Asian women were equally distributed among the 4 groups. However, group 4 had more women of mixed ethnicity, nulliparous women, and women who had undergone in vitro fertilization. Conversely, group 1 had more smokers, which could be explained by smoking cessation later in pregnancy.

Generalized additive model for location, scale, and shape for each liver function variable using gestational age only

In our study, it is important to distinguish between the "marginal" and "conditional" effects of GA on a liver variable. The "marginal" effect of GA on a liver variable is estimated by the GA-only model, whereas a "conditional" or "partial" effect (fixing the other explanatory variables) of GA on a liver variable is estimated by the model using all explanatory variables, which is discussed in the following section. In principle, the "marginal" and "conditional" effects of GA on a liver variable could be very different. However, in our study, we found that they are very similar.

The chosen GAMLSS models for each liver function variable (using GA only) are summarized in Table 2, together with a description of the models. The equations for the predictors of μ, σ, ν and τ (for the GA-only model) are provided in Supplemental Material B. The model selection procedure is also described in Supplemental Material B.

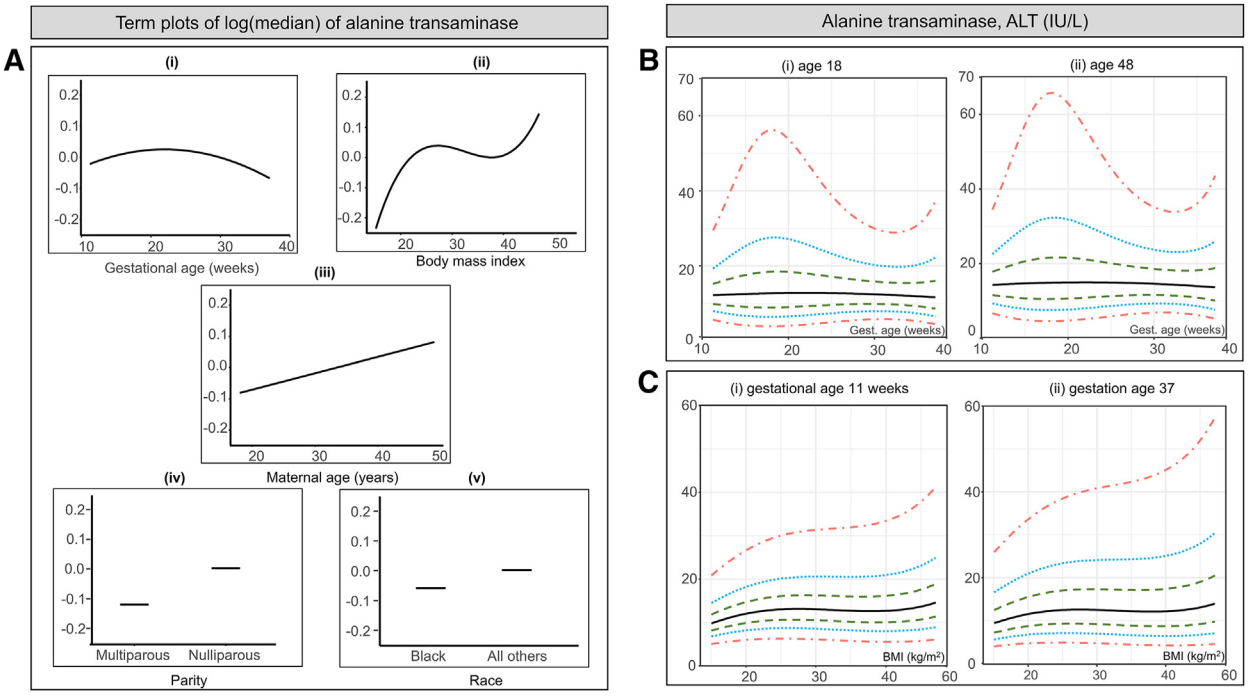
Figure 1, A shows the ALP centiles (2, 10, 25, 50, 75, 90, 98) plotted against GA (for the GA-only model), together with the observations. The median, other centiles, and semiinterquartile range of ALP all increase rapidly with GA. The distribution of ALP is highly positively skewed, and its kurtosis increases with GA.

Figure 1, B to G shows the corresponding centiles for the other 6 liver variables.

Generalized additive model for location, scale, and shape for each liver function variable using all explanatory variables

The chosen GAMLSS models for each liver function variable (using all explanatory variables) are summarized in Table 2. The first column lists the liver function variable, the second column shows the chosen distribution, and the subsequent columns present either the polynomial term in the continuous explanatory variable or the additional effect for a categorical variable level, chosen for the predictors of the distribution parameters μ, σ, ν and τ.

FIGURE 3
Full model for alanine transaminase



A, Term plots of log(median) of alanine transaminase: gestational age (i), BMI (ii), maternal age (iii), maternal weight (iii), maternal parity (iv), and maternal race (v). **B,** Full model for alanine transaminase against gestational age for maternal age of 18 years (i) and 48 years (ii). **C,** Full model for alanine transaminase against BMI for gestational age of 11 weeks (i) and 37 weeks (ii).

BMI, body mass index.

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The equations for the predictors of μ, σ, ν and τ are provided in the Supplemental Material C.

Figures 2 to 4 (ALP, ALT, AST) and Supplemental Figures 1 to 4 (ALT-AST ratio, GGT, TBIL, ALB) show the following for the 7 liver variables:

1. the term plots for the predictors of distribution parameter μ
2. the conditional centiles of the liver variable plotted against GA, separately for maternal age of (1) 18 years and (2) 48 years (for ALP, ALB, ALT, AST, and TBIL), and separately for different ethnicities (for GGT and ALT/AST)
3. the conditional centiles of the liver variable plotted against BMI, separately for fixed GA of (1) 11 weeks and (2) 37 weeks (for ALP, ALT, AST, ALT/AST, GGT, and TBIL), and against maternal age of (1) 18 and (2) 48 years (for ALB)

Supplemental Figures 5 to 7 show the term plots for the predictors of the other distribution parameters (ie, σ, ν and τ) for each of the 7 liver variables. The model selection procedure is described in the Supplemental Material C.

Table 3 presents the percentage change in the predicted median of the liver variables when each explanatory variable is changed, whereas the other explanatory variables are fixed at *any* value. For GGT and TBIL, the quantitative variables are fixed at their median values, and the categorical variables at their default level (ie, White, nulliparous, and nonsmoker for ethnicity, parity, and smoking status, respectively).

In Table 3, several percentage changes exceed 30%. Changing GA from 11 to 37 weeks decreases the median GGT by 34.5% but increases the median ALP by 204.4% (if BMI=20) and by 91.1% (if BMI=40) (Supplemental Figure 2 and Figure 2, respectively).

Changing BMI from 16 to 46 increases the median ALP by 113.6% (when GA=11 weeks), and increases the median ALT by 36.3%, but decreases the median TBIL by 36.3% (Figures 2 and 3 and Supplemental Figure 3, respectively).

Changing age from 18 to 48 years increases the median TBIL by 34.9% (Supplemental Figure 3).

Among individuals of Black ethnicity (relative to White and mixed ethnicities), the median GGT is 46.6% higher (Supplemental Figure 2).

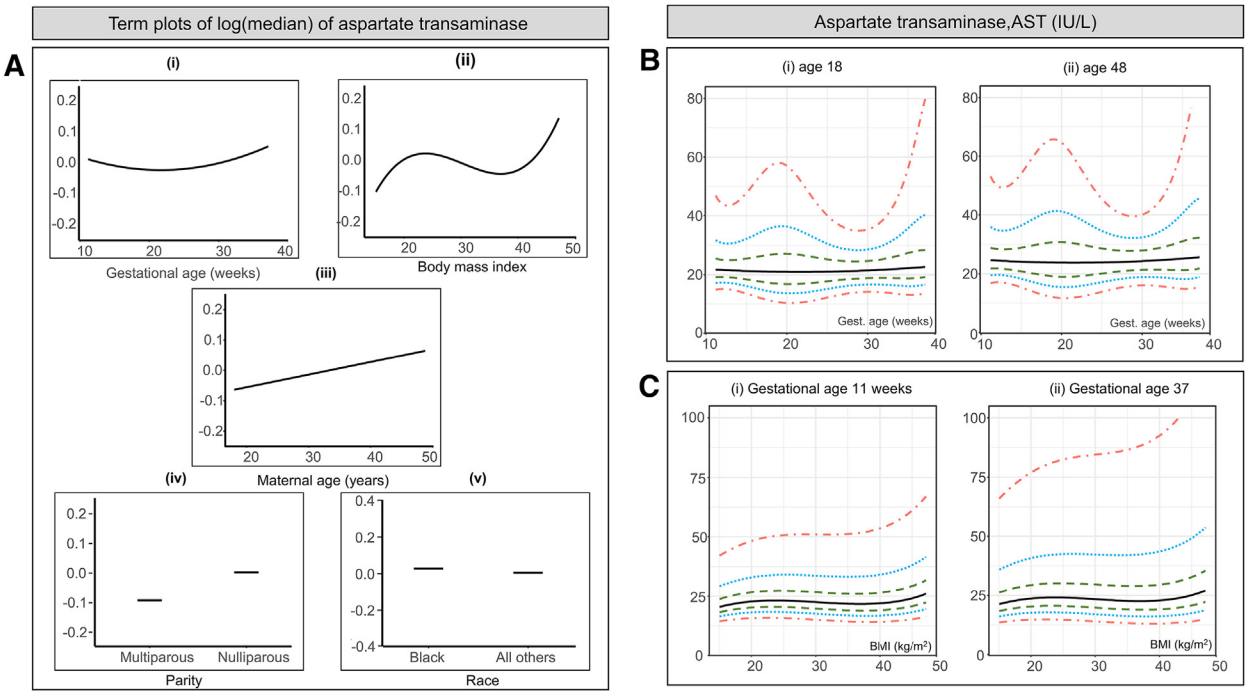
Calculation of z-scores

A web application was developed to obtain the z-scores after inputting the values of the liver function and their explanatory variables

(https://fetalmedicinefoundation.shinyapps.io/life/).

These are provided after controlling for GA only or all the explanatory variables.

FIGURE 4
Full model for aspartate transaminase



A, Term plots of log(median) of aspartate transaminase: gestational age (i), BMI (ii), maternal age (iii), maternal parity (iv), and maternal race (v). **B,** Full model for aspartate transaminase against gestational age for maternal age of 18 years (i) and 48 years (ii). **C,** Full model for aspartate transaminase against BMI for gestational age of 11 weeks (i) and 37 weeks (ii).

BMI, body mass index.

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Comment

Principal findings

This study assessed the distribution of maternal LFTs, focusing not only on the median of the distribution but also on measures of dispersion and shape. In addition, the study describes the effects of GA and maternal demographic characteristics on these measures of the distribution. Therefore, in this study, we provide not only the "marginal" centile plot of each liver variable against GA but also the "conditional" centile plot of each liver variable against GA while fixing the other explanatory variables. The models developed in this article can be used to obtain z-scores for the liver variables (based on either GA only or on all the explanatory variables). A web application is available to perform these calculations. An expanded version of this section is provided in Supplemental Material A.

Results in the context of what is known

Outside pregnancy, there is evidence on the effect of demographic characteristics on LFTs. Studies have demonstrated either a decline[34] or a U-shaped curve[8,35] between ALT or AST and age, and an increase in GGT levels with increasing age.[36] Higher BMI has been associated with higher levels of AST, ALT, and GGT,[11,12,37–40] and Black ethnicity has higher GGT[7] and lower TBIL[10] levels, compared with White populations.

Previous studies in pregnancy can only be compared with our GA-only model because they only examined the effect of GA, but not maternal demographic characteristics, on the liver variables. Since 1963, there have been 8 longitudinal[16,20,41–46] and 8 cross-sectional[15,17–19,21,47–49] studies that examined LFTs in pregnancy with conflicting results. These studies had small populations,[16–20,41–49] with the only large study reporting only on Chinese women.[15] Most studies did not treat numerical values as continuous and presented only values $<2.5^{th}$ and $>97.5^{th}$ centile.[15,16,19,42–44,46,48] As a consequence, they did not use regression analysis to examine the effect of GA on the distribution of these parameters during pregnancy.[15–20,41–45,47–49]

The changes in LFTs with GA result from the interplay between the hemodilution that characterizes pregnancy[50] and the potential upregulation of liver function caused by estrogen.[51] The decline in albumin has been considered to be the result of hemodilution in pregnancy,[45] and the rise in ALP has been attributed to the production of the placental isoenzyme with advancing gestation, in addition to the maternal levels.[47,52,53] This gradual production of the placental isoenzyme explains the

TABLE 3
Percentage change of liver variables according to maternal demographic parameters

Parameter	Gestational age (11–37 wk)	BMI (16–46)	Age (18–48 y)	Weight (40–130 kg)	Ethnicity (Black)	Ethnicity (South Asian)	Parity (parous)	Smoking (smoker)
ALP (Alkaline phosphatase), median	+204.4% (BMI=20)[a] +91.1% (BMI=40)[a]	+113.6% (GA=11 wk)[a] +6.3% (GA=37 wk)	−26.3%	−28.9%		+6.7%		
ALT (Alanine Aminotransferase), median	−4.2%	+36.3%[a]	+17.2%		−6.0%		−11.5%	
AST (Aspartate Aminotransferase), median	+4.0%	+18.2%	+13.3%		+2.4%		−9.1%	
ALT-AST ratio, median	−9.0%	+24.7%			−8.7%		−2.9%	
GGT (Gamma glutamyl transferase), location	−34.5%[a]	−7.7%	−1.4%		+46.6%[a]	+6.3%	−7.0%	
Total bilirubin (TBIL), location	+7.1%	−36.3%[a]	+34.9%[a]		+6.2%	−11.3%	+0.1%	−11.0%
ALB (albumin), median	−14.4%		−4.1%		−0.7%		−0.8%	+1.1%

Changes >30% are highlighted in red. For numerical demographic variables, the change is calculated from the lowest to the highest observed values (eg, from 11 to 37 weeks of gestation), and for categorical variables, relative to the control group ("All other" for race, nulliparous for parity, and nonsmokers for smoking status). Because of the interaction between GA and BMI for ALP, changes are shown for BMI 20 and BMI 40.

BMI, body mass index; *GA*, gestational age.

[a] Changes greater than 30%.

Judah. Liver function tests in pregnancy adjusted for maternal characteristics. Am J Obstet Gynecol 2025.

interaction between GA and BMI, wherein maternal BMI is the major determinant in early pregnancy but plays a minimal role after 34 weeks.

Clinical and research implications

Our model using all explanatory variables provides important information about how GA and maternal demographics affect the median, scale, and shape of the distribution of the liver variables. Modeling all the parameters of the distribution of a liver variable is essential for accurate z-score estimation (especially in the tails of the distribution). This methodology has the potential to provide more accurate adjusted z-scores and to better differentiate between women with escalating liver dysfunction and those with mildly elevated LFTs due to demographic variations such as age and ethnicity. More research and clinical work are needed to assess the impact of such models in screening and identifying disease early and thus improving perinatal outcomes.

Strengths and limitations

The strengths of this study include the large sample size with diverse ethnic representation and strict exclusion of complicated pregnancies. In addition, the use of a statistical method that addresses the impact of maternal characteristics not only on location but also on scale, skewness, and kurtosis of the distribution provides a comprehensive approach to assessing deviations of a patient's values from the predicted medians.

A limitation of this study is its cross-sectional design, with samples frozen and thawed for analysis, the impact of which is unknown. Secondly, this study recruited women at 4 GA intervals and not homogeneously across all gestational weeks; therefore, the centiles are less reliable during the gaps in GA. However, these 4 gestational "windows" cover the first, second, and early and late third trimester, at points when the pregnancy physiological changes are known to occur. Therefore, they should capture pertinent changes in LFTs. Finally, this study does not address the influence of metabolic dysfunction–associated steatotic liver

disease (MASLD), common medications,[54,55] respiratory viral infections,[56] and thyroid disorders[54] on LFTs. The global prevalence of MASLD is increasing to >30% currently.[57–59] The knowledge that LFTs can be affected by the metabolic syndrome and other extrahepatic conditions creates a methodological dilemma when planning a study on reference ranges in a population. One option is to report on an assumed healthy population after excluding known liver disease and medical or obstetrical causes of impaired liver function but allowing background causes such as obesity and MASLD to be represented, given that they may affect >30% of the population. The other option is to be very selective and recruit a population wherein all possible associations are excluded, thereby creating a "normal" range. However, the second option would exclude >40% of our pregnant population, and would therefore be counterproductive in screening for pregnancy complications. Indeed, a US study[60] demonstrated that applying very strict inclusion criteria when defining LFT ranges (such as low alcohol consumption, no diabetes, and normal BMI and waist circumference) would classify a third of the adult population as having abnormal levels. This raises questions regarding the cost-efficiency and side-effects of an approach with such a high screen-positive rate, which should be assessed in future prospective studies.

Conclusion

There is substantial variation in the median and measures of dispersion of all LFTs during pregnancy, related to GA and maternal demographic characteristics. Further research should investigate whether using the methodology applied in this study leads to enhanced detection of liver pathology and improved outcomes during pregnancy.

CRediT authorship contribution statement

Hannah R. Judah: Writing — original draft, Supervision, Investigation. **Robert A. Rigby:** Formal analysis, Methodology, Data curation. **Mikis D. Stasinopoulos:** Formal analysis, Data curation, Methodology. **Konstantinos Pateras:** Formal analysis, Data curation, Software. **Mussarat N. Rahim:** Methodology, Writing — review & editing. **Michael A. Heneghan:** Writing — review & editing, Methodology. **Kypros H. Nicolaides:** Investigation, Resources, Writing — review & editing, Supervision, Methodology, Funding acquisition. **Nikos A. Kametas:** Writing — review & editing, Methodology, Data curation, Writing — original draft, Formal analysis, Conceptualization. ■

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Supplementary Material A

The sections below are an extension of the relevant section of the main manuscript.

Methods

2.4 Measurement of liver function biomarker variables

Alkaline phosphatase (ALP)

The ADVIA Chemistry alkaline phosphatase (ALP-2) assay was used for measuring ALP. Alkaline phosphatase catalyses the transphosphorylation of p-nitrophenylphosphate (p-NPP) to p-nitrophenol (p-NP) in the presence of the transphosphorylating buffer, 2-amino-2- methyl-1-propanol (AMP). The reaction is enhanced through the use of magnesium and zinc ions. The change in absorbance is measured using a bichromatic rate technique (410/478 nm).

Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)

The ADVIA Chemistry Alanine Aminotransferase (ALTP5P) method was used for measuring ALT and the ADVIA Chemistry Aspartate Aminotransferase (ASTP5P) for measuring AST. Both assays used pyridoxal-5'-phosphate added to Reagent 1 of the ADVIA Chemistry ALT and AST method. The reaction was initiated by the addition of α-ketoglutarate as a second reagent. The concentration of NADH was then measured at 340/410 nm and the rate of absorbance decrease was proportional to ALT and AST activity.

Gamma glutamyl transferase (GGT)

GGT was measured using the ADVIA Chemistry Gamma glutamyl transferase method. In the reaction with synthetic substrate (L-γ-glutamyl-3-carboxy-4-nitroanilide), glycylglycine acts as an acceptor for the γ-glutamyl residue and 5-amino-2-nitro-benzoate (ANB) is liberated. The rate of formation was then measured at 410/478 nm.

Total bilirubin (TBIL)

The ADVIA Chemistry Total Bilirubin (TBIL-2) method was used and is based on a chemical oxidation method using vanadate as an oxidizing agent. The bilirubin was oxidized by vanadate to produce biliverdin. Both conjugated (direct) and unconjugated bilirubin are oxidized, and this oxidation reaction causes the decrease in the optical density of the yellow colour, which is specific to bilirubin. The decrease in optical density was measured at 451/545 nm and was proportional to the total bilirubin in the sample.

Albumin (ALB)

Albumin was measured using the ADVIA Chemistry Albumin BCP method, as adaptation of the bromocresol purple (BCP) dye-binding method. In this assay serum or plasma albumin quantitatively binds to BCP to form an albumin-BCP complex that is measured as an endpoint reaction at 596/694nm.

The sensitivities for ALT and AST were reported as $<$5.0 IU/L and for GGT, bilirubin, ALP and albumin as $<$0.0 IU/L, $<$1.03 μmol/L, $<$10 IU/L and $<$6.0 g/L, respectively.

Results

3.2 Distributions for liver function variables

We searched for the best fitting distribution for modelling each liver function variable. Our search comprised all explicit distributions on $(0, \infty)$ available in the gamlss.dist R package, and all explicit distributions on $(-\infty, \infty)$ available in the gamlss.dist R package exponentially transformed to be on $(0, \infty)$. The final chosen distributions are given below.

ALP, ALT, ALT/AST and ALB

The final chosen distribution in the GAMLSS models for ALP, ALT, ALT/AST and ALB was the BCTo(μ, σ, ν, τ) distribution.[32,34] Parameter μ for BCTo is approximately the median (m) [accurate if $\sigma<0.27$ and τ is not very small, which applies here to the fitted models]. Parameter σ for BCTo is approximately the centile coefficient of variation of the distribution (CCV) [provided τ is not very small, since $\sigma \cong$ CCV [33]], where CCV = (3*SIR)/(2*m) [35] and SIR is the semi-interquartile range. Hence SIR $\cong 2\mu^*\sigma/3$, and so SIR depends on both μ and σ. Parameter ν for BCTo is the skewness parameter where increasing ν decreases the skewness, while parameter τ is the kurtosis parameter where increasing τ decreases the kurtosis.

AST

The final chosen distribution in the GAMLSS model for AST was the exponentially transformed SHASH distribution, denoted logSHASH. Hence log(AST) has a sinh-arcsinh, SHASH(μ, σ, ν, τ), distribution,[32,34] where μ is the median. Hence μ is the log median of AST, while exp(μ) is the median of AST. Parameter σ is a scaling parameter for SHASH, so increasing σ increases its variation and hence the variation of logSHASH. Increasing parameter ν for SHASH decreases the left tail heaviness, while increasing parameter τ decreases the right tail heaviness, and similarly for logSHASH.

GGT and TBIL

The final chosen distribution in the GAMLSS models for GGT and TBIL was the logSHASHo distribution. Hence log(GGT) and log(TBIL) have a SHASHo(μ, σ, ν, τ) distribution,[32,34] where μ is a location shift parameter (not the median). Hence μ is the log location of GGT and TBIL, while exp(μ) is the corresponding location parameter of GGT and TBIL. Parameter σ is a scaling parameter for SHASHo, so increasing σ increases its variation and hence the variation of logSHASHo. Increasing parameter ν for SHASHo increases the skewness, while increasing parameter τ decreases the kurtosis, and similarly for logSHASHo.

Discussion

5.1 Principal findings

In our study, the location parameter (μ) of the distribution of the LFTs is independently predicted by gestational age and ethnicity for all variables, by maternal BMI for all variables except ALB, by maternal age for all variables except ALT/AST ratio and GGT, by maternal parity for all variables except TBIL and ALP, and by smoking for TBIL

and ALB, and by maternal weight for ALP.

Table 1 shows the changes in the median of the liver variables when each explanatory variable changes (while the others are fixed). Most notable are the increase in median ALP and decrease in the median of GGT, with gestational age. Also notable are the increase in median ALP with BMI (when ga=11), and the increase in median ALT and decrease in median TBIL, with BMI, the increase in median TBIL with maternal age, and the increase in median GGT with Black ethnicity.

The scale parameter (σ) of the distribution is also independently predicted by gestational age for all variables apart from ALP, maternal BMI for ALT, AST, GGT and ALP, maternal age and ethnicity for GGT and parity for ALT and AST. In contrast, the skewness and kurtosis of all liver variables have a non-uniform influence by gestational age and maternal BMI, ethnicity, age and parity.

5. 2 What is already known on this topic

Outside pregnancy, there is little and conflicting evidence on the effect of demographic characteristics on liver function tests. Some studies demonstrated a decline in ALT and AST with increasing age[1] and others a U-shaped curve,[2,3] whilst one study has demonstrated an increase in GGT levels with increasing age.[4]Higher BMI appears to have been associated with higher levels of AST, ALT and GGT.[5–10] possibly due to the liver steatosis changes that have been observed in obese patients.[5,6,8] In fact in one study, BMI was the single most important determinant of GGT.[10] There is limited evidence for the effect of ethnicity on liver function despite the fact that ethnicity is an independent risk factor for liver dysfunction with Bangladeshi and white patients at higher risk.[11] One study showed that black populations have higher GGT levels compared to white populations[12] and another demonstrated that black populations had the lowest bilirubin levels and Asians had the highest, compared to white and Latin-American populations.[13]

During pregnancy, previous studies have investigated the effect of pregnancy on liver function and attempted to develop reference ranges but none of them assessed the effect of maternal demographic characteristics on liver function parameters. Many of them examined a single parameter, such as AST,[14] alkaline phosphatase,[15–20] albumin[21–23] or both.[24] Since 1963, there have been 16 studies that examined a wide range of liver function tests, 8 of them longitudinal[25–32] and 8 cross-sectional.[33–40] These studies had small populations ranging between 29-565 women,[25–33,35–39] with the only large study reporting data only on Chinese women.[34] Many of these studies did not treat numerical values as continuous and they presented only values below the 2.5th and above the 97.5th centile.[26–29,31,34,37,39] As a consequence, they did not examine with regression analysis the effect of gestational age on the distribution of these parameters during pregnancy.[25–30,32–39]

The above mentioned studies have given conflicting results regarding changes of ALT and AST during pregnancy. Some have shown no change with pregnancy,[25,26,28,30,32,38–40] others a decline,[29,31,34] one study showed an increase[35] and four studies showed an increase in AST but not in ALT.[27,33,36,37] In our study, there is a substantial contribution of gestational age in the regression analysis, however the effect is low, with possibly not clinically significant changes across gestation. This mild effect of gestational age on change of transaminases during pregnancy, may be the reason for the conflicting results of the literature. There is also inconsistent evidence for the change of bilirubin with gestation, with some studies showing no change,[25,28,32,35,39] some a decrease[27,29,33,34,36,37] and only one study showing an increase in bilirubin with advancing gestation.[30] Our results, showing a mild quadratic relationship between bilirubin values and gestation, possibly explain the disagreement in the literature and concur with those of our previous work[40] and Girling et al.[36] In our study, gestational age had a mild effect on GGT with a decline after 20 weeks and an increase towards term. Again, there is disagreement in the literature with the majority of the studies showing values remaining unchanged,[25,27,30,32,33,36,40] two showing a decline[29,34] and one an increase with pregnancy.[26] On the contrary, there is agreement with our data and in the literature that albumin decreases[21–25,28–30,32–35,37,39] and alkaline phosphatase increases with advancing gestation.[15–20,24–30,32–35,37–40]

There is no data on the effect of parity on liver function. However, higher parity has been associated with increasing BMI,[41] which in turn has been implicated in liver disease.[5–10]

5.3 Physiology

The changes with gestation in liver function tests, are the result of the interplay between the hemodilution that characterises pregnancy[42] and the potential upregulation of liver function caused by oestrogen.[43] From early pregnancy, modifications in the regulation of arginine vasopressin and the renin − angiotensin − aldosterone system,[44–48] together with an increased extra-renal renin production due to the synthesis of angiotensinogen by the liver under the influence of oestrogen,[49] lead to an increase in maternal plasma volume.[42] Furthermore, in order to establish and support pregnancy, oestrogen and progesterone increase throughout gestation, with highest levels in the third trimester.[50,51] Oestrogens effect on the liver is achieved directly and indirectly. Direct effect would be through oestrogen receptors which are highly expressed in the liver and participate in liver metabolism.[51,52] Indirect effect of oestrogens on the liver would be through the increase in nitric oxide (NO) synthesis and levels of angiogenic factors, such as vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), and inhibition of TNF-α macrophage synthesis, which would lead to angiogenesis and vasodilation.[53–56] Although there are established oestrogen mediated adaptations in liver function during pregnancy,[50,51] these are mainly focusing on bilirubin,[57] glucose and lipid metabolism[50,51] with little known

on the effect of oestrogen on liver enzymes, and therefore current literature cannot fully explain our findings.

The interplay between hemodilution and upregulated liver function, demographic differences and suboptimal methodologies could be the reason for the literature inconsistencies in the changes with gestation of ALT, AST, GGT and bilirubin. On the contrary, the consistent decline of albumin with pregnancy has been considered to be the result of hemodilution in pregnancy[30] and the rise in ALP has been attributed to the production of the placental and bone isoenzyme.[18,35,58]

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Supplementary Material B

Models for each of the seven liver variables using gestational age only.

B.1 TABLE 1

Terms in gestational age for the predictors of μ, σ, ν and τ, for each of the seven liver variables.

Var	Dist	mu	sigma	nu	tau
ALP	BCTo	Q	1	1	L
ALT	BCTo	Q **	C	1	L *
AST	logSHASH	Q	QN	1	C
ALT/AST	BCTo	Q	C	1	L
GGT	logSHASHo	C	C	L	1
TBIL	logSHASHo	L *	C	C	Q *
ALB	BCTo	Q	C	C **	C **

ALL terms are *** significant, $p \leq 0.001$, except for

** significant, $0.001 < p \leq 0.01$

* significant, $0.01 < p \leq 0.05$

Polynomials in gestational age (ga) for the predictors of the distribution parameters mu, sigma, nu and tau for each of the seven liver function variables.

1 indicates a constant for the distribution parameter. L, Q, C and QN indicate linear, quadratic, cubic and quintic polynomials in ga (respectively) for the predictor of the distribution parameter.

In Table 1, The first column gives the liver function variable, the second column gives the chosen distribution, and the columns headed mu, sigma, nu and tau give the polynomial term in gestational age chosen for the predictors of the distribution parameters μ, σ, ν and τ, respectively. [The predictor is μ itself for logSHASH and logSHASHo, and ν itself for BCTo and logSHASHo, otherwise the predictor is the log function. The significance of each term is also given in Table 2. Only terms significant * or ** are highlighted. All other terms are significant ***.]

B.2 Results of the chosen models for each of the seven liver variables.

ALP

The GAMLSS model chosen for ALP (for the ga only model) was a BCTo distribution with a quadratic, constant, constant and linear term in gestational age for the predictors log μ, log σ, ν and log τ, of the BCTo distribution parameters μ, σ, ν and τ, respectively. Hence the approximate log median of ALP increases quadratically with ga, and the approximate log centile coefficient of variation of ALP is constant, the skewness parameter is a negative constant so the distribution of ALP is highly positively skewed, and the log kurtosis parameter decreases linearly with ga so the kurtosis *increases* with ga,

ALT, AST and ALT/AST

The medians of both ALT and AST change slowly with ga. The two upper centiles (90% and 98%) of both ALT and AST show a first peak around ga=20 and then a second peak at ga=38. The distributions of ALT and AST are highly positively skewed. The kurtosis of ALT decreases with ga, while the right tail heaviness of AST is higher for both low ga and high ga. The centiles of ALT/AST change slowly with ga. The median and upper centiles of ALT/AST have a gently peak around ga=20. The distribution of ALT/AST is moderately positively skewed and its kurtosis decreases with ga.

GGT

The median and other centiles of GGT generally decrease with ga. The distribution of GGT is highly positively skewed.

TBIL

The median and other centiles of TBIL have a trough around ga=20. The distribution of TBIL is positively skewed. TBIL has a lower skewness but higher kurtosis around ga=20.

ALB

The median and other centiles of ALB generally decrease rapidly with ga from ga=11 to ga=25. The distribution of ALB is roughly symmetrical (except for very low and very high ga, where it is positively and negatively skewed, respectively).

B.3 Equations for the predictors of μ, σ, ν, and τ, for each of the seven liver variables

Equations for models with ga only

1) **ALP**

```
# ALP has a BCTo distribution
# the fitted parameter predictors for mu, sigma, nu and tau are:
mu_p <- 4.086893689 - (0.032521837*ga) + (0.001446202*(ga-2))
sigma_p <- - 1.360177
nu_p <- - 0.3212401
tau_p <- 10.5096636 - (0.2344702*ga)
# NOTE BCTo used log link functions for mu, sigma and tau
# BUT identity link function for nu
# HENCE the fitted parameters for mu, sigma, nu and tau are:
mu <- exp(mu_p)
sigma <- exp(sigma_p)
nu <- nu_p
tau <- exp(tau_p)

################################################################
# the z-scores are:
z <- qNO(pBCTo(ALP,mu,sigma,nu,tau))
```

2) **ALT**

```
# ALT has a BCTo distribution
# the fitted parameter predictors for mu, sigma, nu and tau are:

################################################################
# BELOW ARE NEW 03_07_2022

################################################################
```

```
mu_p <- 2.2492607651 + (0.0224727376*ga) - (0.0004756784*(ga^2))
sigma_p <- - 5.9465263036 + (0.69605577427*ga) - (0.0296731062*(ga^2)) + (0.0003946408*(ga^3))
nu_p <- - 0.1881983
tau_p <- 1.19461814 + (0.03338011*ga)
# NOTE BCTo used log link functions for mu, sigma and tau
# BUT identity link function for nu
# HENCE the fitted parameters for mu, sigma, nu and tau are:
mu <- exp(mu_p)
sigma <- exp(sigma_p)
nu <- nu_p
tau <- exp(tau_p)
##############################################################
# the z-scores are:
z <- qNO(pBCTo(ALT,mu,sigma,nu,tau))
```

3) **AST**

```
# AST has a logSHASH distribution
# the fitted parameter predictors for mu, sigma, nu and tau are:
mu_p <- 3.1588189250 - (0.0107754708*ga) + (0.0002594616*(ga^2))
sigma_p <- 28.50128 - (7.803575*ga) + (0.7490228*(ga^2)) - (0.03366822*(ga^3)) +
(0.0007167982*(ga^4)) - (0.0000058379 81*(ga^5))
nu_p <- - 0.09433653
tau_p <- 0.0080481075 - (0.1111508939*ga) + (0.0072179551*(ga^2)) - (0.0001255725*(ga^3))
# NOTE logSHASH used identity link functions for mu
# BUT identity link function for sigma, nu and tau
# HENCE the fitted parameters for mu, sigma, nu and tau are:
mu <- mu_p
sigma <- exp(sigma_p)
nu <- exp(nu_p)
tau <- exp(tau_p)
##############################################################
# the z-scores can be calculated by:
z <- qNO(plogSHASH(AST,mu,sigma,nu,tau))
# ALTERNATIVELY the z-scores can also be calculated by
z2 <- qNO(pSHASH(log(AST),mu,sigma,nu,tau))
```

4) **ALT/AST**

```
# ALT/AST has a BCTo distribution
# the fitted parameter predictors for mu, sigma, nu and tau are:
mu_p <- - 1.078553639 + (0.051331640*ga) - (0.001123857*(ga^2))
sigma_p <- - 4.2104842856 + (0.4291338622*ga) - (0.0187073688*(ga^2)) + (0.0002582967*(ga^3))
nu_p <- 0.4074968
tau_p <- 0.45989120 + (0.09776133*ga)
# NOTE BCTo used log link functions for mu, sigma and tau
# BUT identity link function for nu
# HENCE the fitted parameters for mu, sigma, nu and tau are:
mu <- exp(mu_p)
sigma <- exp(sigma_p)
nu <- nu_p
tau <- exp(tau_p)
##############################################################
# the z-scores are:
z <- qNO(pBCTo(ALT/AST,mu,sigma,nu,tau))
```

5) **GGT**

```
# GGT has a logSHASHo distribution
# the fitted parameter predictors for mu, sigma, nu and tau are:
mu_p <- 3.305602 - (0.1429979*ga) + (0.005284184*(ga^2)) - (0.000006439701*(ga^3))
sigma_p <- - 2.4826692319 + (0.1828827534*ga) - (0.0077353559*(ga^2)) + (0.0001078294*(ga^3))
nu_p <- 0.528136893 - (0.008478866*ga)
tau_p <- - 0.2407418
# NOTE logSHASHo used idntity link functions for mu and nu,
# BUT log link function for sigma and tau
# HENCE the fitted parameters for mu, sigma, nu and tau are:
mu <- mu_p
sigma <- exp(sigma_p)
nu <- nu_p
tau <- exp(tau_p)
##############################################################
# the z-scores can be calculated by:
z <- qNO(plogSHASHo(GGT,mu,sigma,nu,tau))
# The z-scores can also be calculated by
z2 <- qNO(pSHASHo(log(GGT),mu,sigma,nu,tau))
```

6) **TBIL**

```
# TBIL has a logSHASHo distribution
# the fitted parameter predictors for mu, sigma, nu and tau are:
mu_p <- 1.845530179 + (0.002581006*ga)
sigma_p <- - 3.3422130398 + (0.3323645286*ga) - (0.0158106030*(ga^2)) + (0.0002259245*(ga^3))
nu_p <- 4.4727925487 - (0.5804387837*ga) + (0.0224008152*(ga^2)) - (0.0002660718*(ga^3))
tau_p <- 0.411389598 - (0.063528181*ga) + (0.001332236*(ga^2))
# NOTE logSHASHo used identity link functions for mu and nu,
# BUT log link function for sigma and tau
# HENCE the fitted parameters for mu, sigma, nu and tau are:
mu <- mu_p
sigma <- exp(sigma_p)
nu <- nu_p
tau <- exp(tau_p)
##############################################################
# the z-scores are:
z <- qNO(plogSHASHo(TBIL,mu,sigma,nu,tau))
# NOTE the z-scores can also be calculated by
z2 <- qNO(pSHASHo(log(TBIL),mu,sigma,nu,tau))
```

7) **ALB**

```
# ALB has a BCTo distribution
# the fitted parameter predictors for mu, sigma, nu and tau are:
mu_p <- 3.9723198588 - (0.0213200140*ga) + (0.0003193823*(ga^2))
sigma_p <- - 1.1296257854 - (0.3043774527*ga) + (0.0148304598*(ga^2)) - (0.0002169953*(ga^3))
```

```
nu_p <- - 31.314926762 + (4.57
0682864*ga) - (0.199654810*(ga^2)) +
(0.002755424*(ga^3))
tau_p <- 20.557187510 - (2.75
6260644*ga) + (0.129003301*(ga^2)) -
(0.001851761*(ga^3))
# NOTE BCTo used log link functions
for mu, sigma and tau
# BUT identity link function for nu
# HENCE the fitted parameters for
mu, sigma, nu and tau are:
mu <- exp(mu_p)
sigma <- exp(sigma_p)
nu <- nu_p
tau <- exp(tau_p)
##############################
######################
# the z-scores are:
z <- qNO(pBCTo(ALB,mu,
sigma,nu,tau))
```

B.4 Model selection procedure

Methodology for selecting a model for one of the the seven liver function variables (Y), using gestational age (ga) as the only explanatory variable, (in order to obtain centiles and z-scores).

Summary

Selection of the model for each liver function variable using gestational age only

Extreme outlier values were removed from the data set. Then, for each liver function variable, the predictor of each distribution parameter (μ, σ ν, τ) was initially modelled using a smooth function of gestational age. We then searched for the best fitting distribution. Then the best fitting polynomial term (up to quintic) in gestational age was selected [using a generalized Akaike information criterion with penalty 4 for each extra parameter, i.e. GAIC(4)] in turn for the predictors of each of the four distribution parameters, μ, σ, ν and τ, (to replace the smooth function). Polynomials were used in order to provide an explicit model that could then easily be programmed in shiny (or EXCEL). The modelling was performed using the gamlss package in R. Full details of the model selection procedure are given in Supplemental materials C.

STEP 1) Remove extreme outliers

STEP 2) Choose the distribution.

Model each distribution parameter (μ, σ, ν and τ) using a smooth function of ga, then

(a) use the chooseDist() function with argument: type="realplus" in the gamlss R package to select the best distribution for Y in the range (0, ∞) according to the generalised Akaike information criterion (GAIC) with penalty 4 for each effective degree of freedom (i.e. each effective parameter) used in each distribution model, i.e. criterion GAIC(4),

(b) use the chooseDist() function with argument: type="realline" in the gamlss R package to select the best distribution for log(Y) in the range ($-\infty$, ∞) according to criterion GAIC(4).

Create the corresponding best distribution for Y using gen.Family() with argument: type="log" and then fit the distribution to Y.

(c) choose between the best distributions for Y in (a) and (b) using GAIC(4).

STEP 3) Select the polynomial terms in ga (to replace the smooth functions in ga) for the distribution parameters (μ, σ, ν and τ).

For the chosen distribution from STEP 2), use the stepGAICAll.A() function in the gamlss R package to select the best polynomial term in ga for each of the distribution parameters, according to criterion GAIC(4):

(i) set the start and lower models of stepGAICAll.A() to be constants (for all distribution parameters)

(ii) set the upper model of stepGAICAll.A() to include all polynomial terms in ga up to quintic [i.e. constant, linear, quadratic, cubic, quartic or quintic, i.e. models 1, ga, poly(ga,2),…,poly(ga,5)], (for all distribution parameters)

(iii) apply stepGAICAll.A() using criterion GAIC(4).

STEP 4) Checking the model diagnostics: single and multiple worm plots

(c) For the chosen model for STEP 3),

(i) obtain the single worm plot (i.e. detrended QQ plot) of the (normalized quantile) residuals,

(ii) obtain the multiple worm plots of the residuals split into 4 (or 9) ranges of gestational age

(b) Check the residual worm plots.

The worm plots (i.e. detrended QQ plots) in STEP 4)(a) should be roughly horizontal, with approximately 95% of points within the approximate 95% confidence bands.

Focusing on the important tail of the distribution of the liver variable (Y),

(i) check the worm plots in STEP 4)(a) are OK.

(ii) If the single worm plot in 4)(a)(i) is not OK, this may indicate an inadequate distribution.

If the multiple worm plot in 4)(a)(ii) is not OK, this indicates that a term in gestational age (ga) is not adequate (a higher polynomial in ga may be needed, e.g. a quadratic term in ga instead of a linear term in ga, for a distribution parameter, e.g. μ).

(iii) check whether the worm plots in STEP 4)(a) are still OK if the model is simplified by removing borderline terms.

Supplementary Material C

C.1 Models for each of the seven liver variables using all the explanatory variables.

Results for ALP

Summary

The GAMLSS model chosen for ALP was a BCTo distribution.[33] The median of ALP depends most strongly on the interaction of gestational age (ga) and body mass index (bmi). For fixed bmi, the median of ALP increases strongly with ga, while for lower fixed values of ga only, the median of ALP increases strongly with bmi (see Figure 2A and Table 4). Similarly, the semi-interquartile range of ALP increases strongly with ga, and also strongly with bmi for lower values of ga (see Figures 2B

and 2C). The distribution of ALP is highly positively skewed, while the kurtosis of ALP *increases* sharply with ga (from supplementary materials E, Figure 9).

Details

The model for the log median (i.e. log μ) of ALP was the sum of the following terms: an interaction of a quadratic in gestational age (ga) with a linear in BMI, a cubic term in age, a linear term in maternal weight (wt), and an extra effect for Asian race, (Table 2 and Figure 2A).

Figure 2A(i) shows that for a fixed of BMI, the log median of ALP generally increases with ga. From Table 3, column 2, when ga increases from 11 to 37 weeks, the median of ALP increases by 204.4% when BMI=20, [since the log median of ALP increases by 1.113, so the median of ALP changes by a factor 3.044 = exp(1.11303), i.e. a 204.4% increase], but only 91.1% when BMI=40.

Figure 2A(i) also shows how gestational age (ga) affects the contribution of BMI to the log median (i.e. log μ) of ALP. When ga is 11, changing the BMI from 20 to 40 increases considerably the log median of ALP, but the effect of BMI diminishes to zero when ga=38. From Table 1, column 3, when BMI increases from 16 to 46, the median of ALP increases by 113.6% when ga = 11, but only 6.3% when ga = 37. Figure 2A(ii) shows that the log median of ALP decreases with maternal age. From Table 3, changing from age 18 to 48 decreases the median ALP by 26.3%.

Figure 2A(iii) shows that the log median of ALP decreases linearly with weight (for fixed BMI). Hence for two women with the same BMI, the one with the higher weight has a lower median ALP. From Table 3, changing from weight 40 to 130 kg (for a fixed BMI) decreases the median ALP by 28.9%. Figure 2A(iv) shows that the log median of ALP increases if the race is South Asian. From Table 3, the median ALP increases by 6.7% if the race is South Asian (relative to all the other races, i.e. White, Black and Mixed).

Supplemental Figure 5 shows that the log of the scale parameter (i.e. log σ), i.e. the log of the approximate centile coefficient of variation, of ALP increases linearly with BMI. The skewness parameter ν of ALP was a negative constant, so the distribution of ALP is highly positively skewed (Supplemental Figure 6). Supplemental Figure 7 shows that the log of the kurtosis parameter (i.e. log τ) of ALP decreases sharply with ga, so the kurtosis of ALP *increases* sharply with ga.

Figures 2B(i) and 2B(ii) show the ALP centiles (2, 10, 25, 50, 75, 90, 98)% plotted against gestational age, fixing maternal age=18 and age=48 respectively, [from the full model, fixing the BMI and weight at their median values, and fixing race at All Others (i.e. White, Black or Mixed)]. Comparing Figures 2B(i) and 2B(ii) shows that the ALP centiles are much higher when age=18.

Figures 2C(i) and 2C(ii) show the ALP centiles (2, 10, 25, 50, 75, 90, 98)% plotted against BMI, fixing gestational age at 11 weeks and 37 weeks respectively, [from the full model, fixing the maternal age and weight at their median values, and fixing race at All Others (i.e. White, Black or Mixed)] Comparing Figures 2C(i) and 2C(ii) shows that the ALP centiles are much higher when ga=37 than ga=11, and change rapidly with BMI when ga=11 but more slowly with BMI when ga=37.

Results for ALT, AST

Summary

The GAMLSS model chosen for ALT was a BCTo distribution and for AST a logSHASH distribution. The models for the medians of ALT and AST were very similar, with the medians changing most strongly with bmi, with a sharp decrease below bmi 20 and a sharp increase above bmi 40, while the medians change only slowly with ga, (see Figures 3A and 4A and Table 3).

The models for the scale parameter (i.e. σ) for ALT and AST were very similar, (see supplementary Figure 5). The semi-interquartile ranges of ALT and AST increase with bmi (see Figures 3C and 4C). The distribution of ALT is highly positively skewed (since its skewness parameter ν is a negative constant). The kurtosis of ALT decreases with ga (since its kurtosis parameter τ *increases* with ga, see supplementary Figure 7).

The right tail of AST is heaviest for low and high ga (since its parameter τ is *lower* for low and high ga, see supplementary Figure 7).

Details

The models for the log median (i.e. log μ) of ALT and AST were very similar, and both were the sum of the following terms: a quadratic in gestational age (ga), a cubic in bmi, a linear term in age, an extra effects for black race and multiple parity, [see Table 2 and Figures 3A and 4A].

Figures 3A(i) and 4A(i) show that gestational age has only a slow effect on the log median of ALT and AST, respectively.

Figures 3A(ii) and 4A(ii) show that bmi has the strongest effect on the log median of ALT and AST, respectively, with a sharp decrease below bmi 20 and a sharp increase above bmi 40. Changing the bmi from 16 to 46 increases the medians of ALT and AST by 36.3% and 18.2 %, respectively (see Table 3). Figures 3A(iii) and 3A(iii) show that the log medians of ALT and AST increase linearly with maternal age. Changing from age 18 to 48 increases the median ALT and AST by 17.2 % and 13.3%, respectively (see Table 3). Figures 3A(iv) and 4A(iv) and Table 3 show that the medians of ALT and AST decrease by 6.0 % and increase by 2.4 %, respectively, if the race is Black. Figures 3A(v) and 3A(v) and Table 3 show that the medians of ALT and AST decrease by 11.5 % and 9.1 %, respectively, if the parity is multiple.

Figures 3B(i) and 3B(ii) show the ALT quantiles, and Figures 4B(i) and 4B(ii) show the AST centiles, (0.02, 0.10, 0.25, 0.50, 0.75, 0.90, 0.98), plotted against gestational age, fixing maternal age at 18 and 48, respectively, (for the full model, fixing bmi at its median value, and fixing race at All Others and parity at Nulliparous). Comparing Figures 3B(i) and 3B(ii) shows that the ALT quantiles are slightly higher when maternal age is 48. Similarly for AST.

Figures 3(C)(i) and 3(C)(ii) show the ALT quantiles, and Figures 4C(i) and 4C(ii) show the AST centiles, (0.02, 0.10, 0.25, 0.50, 0.75, 0.90, 0.98), plotted against bmi, fixing gestational age at 11

and 37 weeks, respectively, (for the full model, fixing maternal age at its median value, and fixing race at All Others and parity at Nulliparous). Comparing Figures 3C(i) and 3C(ii) shows that the ALT quantiles generally increase with bmi. Similarly for the AST centiles.

Supplementary Figure 5 shows that the models for log σ for ALT and AST were very similar. They show that gestational age, bmi and parity affect log σ. Figure 5 shows that log σ increases with bmi, but decreases if parity is multiple.

Results for ALT/AST

Summary

The GAMLSS models chosen for ALT/AST was the BCTo distribution. The median of ALT/AST increases with bmi, but changes only slowly with ga. The approximate centile coefficient of variation (i.e. σ) of ALT/AST changes slowly with ga. The semi-interquartile ranges of ALT/AST increases slowly with bmi (see supplementary Figure 1C). The distribution of ALT/AST is generally moderately positively skewed. The kurtosis of ALT/AST *increases* sharply with ga (since its kurtosis parameter τ *increases* with ga).

Details

The model for the log of the median μ (i.e. log μ) of ALT/AST was the sum of the following terms: a quadratic in gestational age (ga), a linear term in bmi, and extra effects for black race and multiple parity (see Table 2 and supplementary Figure 1A). Supplementary Figure 1A shows that gestational age has a slow quadratic effect on the log median of ALT/AST, with a maximum around 22 weeks. The median of ALT/AST increases slowly with bmi, but decreases by 8.7% if the race is black, and decreases by 2.9% if the parity is multiple (see supplementary Figure 1A and Table 3).

Supplementary Figure 5 shows that gestational age has a cubic effect on the log of the approximate coefficient of variation (i.e. log σ) of ALT/AST, with a sharp decrease for ga below 18 weeks and a sharp increase for ga above 33 weeks.

Results for GGT

Summary

The GAMLSS model chosen for GGT was a logSHASHo distribution. The median of GGT increases strongly for race Black, but decreases with ga (see Table 3). The semi-interquartile range of GGT increases strongly for race Black, but slowly with bmi (see Supplementary Figure 2B). The skewness of GGT decreases with ga (since its skewness parameter μ decreases with ga).

Details

Parameter μ is the log of the location parameter for GGT. The model for μ was the sum of the following terms: a cubic in gestational age (ga), a cubic in bmi, and extra effects for black race, South Asian race and multiple parity (see Table 2 and Supplementary Figure 2A). Supplementary Figure 2A shows that μ decreases with gestational age, especially from 11 to 14 weeks (and Table 3 shows that the median of GGT decreases by 34.5% when ga changes from 11 to 37 weeks). Variable bmi has a cubic effect on μ, with an increase for bmi below 20 and a decrease for bmi above 40. Also μ increases sharply by 42.2% if the race is black, increases slightly by 6.3% if the race is south Asian (both relative to All others, i.e. while or mixed race), but decreases by 7.0% if the parity is multiple. The scale parameter (i.e. σ) increases with ga and bmi.

Results for TBIL (using all explanatory variables).

Summary

The GAMLSS model chosen for TBIL was a logSHASHo distribution. The median of TBIL decreases strongly with bmi, but increases strongly with maternal age (see Table 3). The semi-interquartile range of TBI decreases with bmi (see supplementary Figure 3C). The distribution of TBIL has a lower skewness but higher kurtosis around ga 20 weeks (since its ν and τ parameters both decrease around ga 20 weeks).

Details

Parameter μ is the log of the location parameter for GGT. The model for μ was the sum of the following terms: inear terms in each of gestational age (ga), bmi and age, and extra effects for black race, south asian race and smoker (see Table 2 and Supplementary Figure 5A). Supplementary Figure 3A shows that μ increases linearly with ga and age, but decreases linearly with bmi. Table 3 shows that the median of TBIL increases by 6.2% if the race is black, but decreases by 11.3% if the race is south Asian (both relative to other, i.e. white or mixed race), and decreases by 11.0% for a Smoker.

Results for ALB (using all explanatory variables).

Summary

The GAMLSS model chosen for ALB was a BCTo distribution. The median of ALB decreases with ga (see Supplementary Figure 4A and Table 3), but changes very little with the other explanatory variables. The semi-interquartile range of ALB changes very little with the explanatory variables. The kurtosis of ALB increases sharply above ga 35 weeks (since its kurtosis parameter τ *decreases* sharply above ga 35 weeks).

Details

The model for the log median (i.e. log μ) of ALB was the sum of the following terms: a quadratic in gestational age (ga), a linear term in age, and extra effects for black race, multiple parity and smoker (see Table 2 and Supplementary Figure 4A). Supplementary Figure 4A shows that only gestational age has much effect on the log median of ALB. The median ALB decreases by 14.1% when gestational age changes from 11 to 37 weeks,(see Table 3). The other variables have only a small effect on the median ALB.

C.2 Equations for the predictors of μ, σ, ν, and τ, for each of the seven liver variables

Equations for models with all X variables

1) **ALP**

```
# ALP has a BCTo distribution
# the fitted parameter predictors for mu, sigma, nu and tau are:
mu_p <- 5.377496 - (0.06015644*ga) + (0.002518065*(ga^2)) +
(0.02140362*bmi) + (0.0007258407*ga*bmi) - (0.00003376952*(ga^2)*bmi) -
```

```
(0.1266759*age) + (0.00353910
7*(age^2)) - (0.00003353082*(age^3)) -
(0.003786953*wt) + (0.06469994*
ifelse(race_new=="South Asian",1,0))
sigma_p <- - 1.65035844 + (0.008
81468*bmi)
nu_p <- - 0.2742018
tau_p <- 6.9594177 - (0.1359355*ga)
# NOTE BCTo used log link functions
for mu, sigma and tau
# BUT identity link function for nu
# HENCE the fitted parameters for
mu, sigma, nu and tau are:
mu <- exp(mu_p)
sigma <- exp(sigma_p)
nu <- nu_p
tau <- exp(tau_p)
##############################
#######################
# the z-scores are:
z <- qNO(pBCTo(ALP,mu,sigma,
nu,tau))
```

2) **ALT**

```
# ALT has a BCTo distribution
# the fitted parameter predictors for
mu, sigma, nu and tau are:
mu_p <- 0.04141243 + (0.017
87777*ga) - (0.0004070535*(ga^2)) +
(0.2127753*bmi) - (0.006683781*
(bmi^2)) + (0.00006819463*(bmi^3)) +
(0.005282731*age) - (0.1217854*ifel-
se(parity=="Multip",1,0)) - (0.0619
1635*ifelse(race_B=="Black",1,0))
sigma_p <- - 5.7709295247 +
(0.6545800148*ga) - (0.0282035272*
(ga^2)) + (0.0003770237*(ga^3)) - (0.12
95681864*ifelse(parity=="Multip",1,0)) +
(0.0091125074*bmi)
nu_p <- - 0.1748747
tau_p <- 1.36743023 + (0.02487
922*ga)
# NOTE BCTo used log link functions
for mu, sigma and tau
# BUT identity link function for nu
# HENCE the fitted parameters for
mu, sigma, nu and tau are:
mu <- exp(mu_p)
sigma <- exp(sigma_p)
nu <- nu_p
tau <- exp(tau_p)
##############################
#######################
# the z-scores are:
z <- qNO(pBCTo(ALT,mu,sigma,
nu,tau))
```

3) **AST**

```
# AST has a logSHASH distribution
# the fitted parameter predictors for
mu, sigma, nu and tau are:
mu_p <- 1.706184 - (0.0139
7961*ga) + (0.0003229808*(ga^2)) +
(0.1522253*bmi) - (0.005266160*
(bmi^2)) + (0.00005786314*(bmi^3)) +
(0.0041605751*age) - (0.09502892
*ifelse(parity=="Multip",1,0)) + (0.02
332516*ifelse(race_B=="Black",1,0))
sigma_p <- 28.06858 - (7.72
5966*ga) + (0.7414985*(ga^2)) - (0.033
34406*(ga^3)) +
(0.0007099831*(ga^4)) - (0.00000578
0393*(ga^5)) - (0.1289892*ifelse
(parity=="Multip",1,0)) + (0.00850
4816*bmi)
nu_p <- - 0.1127009
tau_p <- - 0.0969439483 - (0.09
12068991*ga) + (0.0062566848*
(ga^2)) - (0.0001119908*(ga^3)) -
(0.0508417384*ifelse(r-
ace_SA=="South Asian",1,0))
# NOTE logSHASH used identity link
functions for mu
# BUT identity link function for
sigma, nu and tau
# HENCE the fitted parameters for
mu, sigma, nu and tau are:
mu <- mu_p
sigma <- exp(sigma_p)
nu <- exp(nu_p)
tau <- exp(tau_p)
##############################
#########################
# the z-scores can be calculated by:
z <- qNO(plogSHASH(AST,mu,
sigma,nu,tau))
# ALTERNATIVELY the z-scores can
also be calculated by
z2 <- qNO(pSHASH(log(AST),
mu,sigma,nu,tau))
```

4) **ALT/AST**

```
# ALT/AST has a BCTo distribution
# the fitted parameter predictors for
mu, sigma, nu and tau are:
mu_p <- - 1.196857850 + (0.04
9254419*ga) - (0.001100915*(ga^2)) +
(0.007344188*bmi) - (0.0291722
42*ifelse(parity=="Multip",1,0)) - (0.0
90501514*ifelse(race_B=="Black",1,0))
sigma_p <- - 4.0145069969 +
(0.4004021397*ga) - (0.01753
28628*(ga^2)) + (0.0002431356*(ga^3))
nu_p <- 0.04017033 + (0.01
783978*bmi) - (0.33059866*ifelse
(race_B=="Black",1,0))
tau_p <- 0.20397514 + (0.091
38889*ga) + (0.62563020*ifelse
(parity=="Multip",1,0))
# NOTE BCTo used log link functions
for mu, sigma and tau
# BUT identity link function for nu
# HENCE the fitted parameters for
mu, sigma, nu and tau are:
mu <- exp(mu_p)
sigma <- exp(sigma_p)
nu <- nu_p
tau <- exp(tau_p)

##################################
####################
# the z-scores are:
z <- qNO(pBCTo(ALT/AST,mu,-
sigma,nu,tau))
```

5) **GGT**

```
# GGT has a logSHASHo distribution
# the fitted parameter predictors for
mu, sigma, nu and tau are:
mu_p <- 5.457007 - (0.1726
892*ga) + (0.006502069*(ga^2)) - (0.0
0008108264*(ga^3)) -
(0.2358648*bmi) + (0.0088048
68*(bmi^2)) - (0.0001008965*(bmi^3)) -
(0.07226004*ifelse(parity==
"Multip",1,0)) + (0.3518561
*ifelse(race_new=="Black",1,0)) +
(0.06071507*ifelse(r-
ace_new=="South Asian",1,0))
sigma_p <- - 2.619330 + (0.158
3799*ga) - (0.006793622*(ga^2)) + (0.0
0009692623*(ga^3)) +
(0.01265221*bmi) - (0.007292244*
age) + (0.5542374*ifelse(race_B==
"Black",1,0))
nu_p <- 0.433044441 - (0.008
289355*ga)
tau_p <- - 0.2777979 + (0.17312
48*ifelse(race_B=="Black",1,0))
# NOTE logSHASHo used identity
link functions for mu and nu,
```

```
# BUT log link function for sigma and tau
# HENCE the fitted parameters for mu, sigma, nu and tau are:
mu <- mu_p
sigma <- exp(sigma_p)
nu <- nu_p
tau <- exp(tau_p)
##########################
##########################
# the z-scores can be calculated by:
z <- qNO(plogSHASHo(da2$GGT, mu,sigma,nu,tau))
# The z-scores can also be calculated by
z2 <- qNO(pSHASHo(log(da2$GGT), mu,sigma,nu,tau))
```

6) **TBIL**

```
# TBIL has a logSHASHo distribution
# the fitted parameter predictors for mu, sigma, nu and tau are:
mu_p <- 1.805897593 + (0.004860683*ga) -
(0.014354208*bmi) + (0.012274651*age) - (0.116758776*ifelse(smoking=="Smoker",1,0)) +
(0.060534931*ifelse(race_new=="Black",1,0)) -
(0.119460272*ifelse(race_new=="South Asian",1,0))
sigma_p <- - 3.5648859765 + (0.3606436371*ga) - (0.0173634740 *(ga^2)) + (0.0002501788*(ga^3))
nu_p <- 6.4612639831 - (0.6019507146*ga) + (0.0231576488*(ga^2)) - (0.0002728793*(ga^3)) -
(0.1019429413*bmi) + (0.0016097413*(bmi^2)) - (0.0085782271*age)
tau_p <- 0.56537244 - (0.07675210*ga) + (0.00158353*(ga^2)) - (0.03473982*ifelse(parity=="Multip",1,0))
# NOTE logSHASHo used identity link functions for mu and nu,
# BUT log link function for sigma and tau
# HENCE the fitted parameters for mu, sigma, nu and tau are:
mu <- mu_p
sigma <- exp(sigma_p)
nu <- nu_p
tau <- exp(tau_p)
##########################
##########################
# the z-scores are:
z <- qNO(plogSHASHo(TBIL, mu,sigma,nu,tau))
# NOTE the z-scores can also be calculated by
z2 <- qNO(pSHASHo(log(TBIL), mu,sigma,nu,tau))
```

7) **ALB**

```
# ALB has a BCTo distribution
# the fitted parameter predictors for mu, sigma, nu and tau are:
mu_p <- 4.0222118224 - (0.0213657598*ga) + (0.0003207592*(ga^2)) -
(0.0013942882*age) - (0.0076485211*ifelse(parity=="Multip",1,0)) - (0.0074160088*ifelse(race_B=="Black",1,0)) + (0.0105793891*ifelse(smoking=="Smoker",1,0))
sigma_p <- - 0.9753259273 - (0.3280439428*ga) + (0.0158287349*(ga^2)) - (0.0002302735*(ga^3))
nu_p <- - 30.019689147 + (4.453739060*ga) - (0.196894158*(ga^2)) + (0.002741265*(ga^3))
tau_p <- 21.717810306 - (2.894110962*ga) + (0.133897405*(ga^2)) - (0.001907536*(ga^3))
# NOTE BCTo used log link functions for mu, sigma and tau
# BUT identity link function for nu
# HENCE the fitted parameters for mu, sigma, nu and tau are:
mu <- exp(mu_p)
sigma <- exp(sigma_p)
nu <- nu_p
tau <- exp(tau_p)
##########################
##########################
# the z-scores are:
z <- qNO(pBCTo(ALB,mu, sigma,nu,tau))
```

C.3 Figures 1 to 4 (for ALP, ALT, AST, ALT/AST, GGT, TBIL and ALB, respectively)

: Aterm plot for μ,
B: conditional centiles against ga, for maternal age=18 and age=48 (for ALP, ALT, AST, TBIL and ALB), and for different races (for ALT/AST and GGT)
C: conditional centiles against bmi, for ga=11 and ga=37

C.4 Figures 5 to 7: term plots for the predictors of σ, ν, and τ, respectively (A for ALP, B for the other six liver variables)

C.5 Model selection procedure

Methodology for selecting a model for one of the the seven liver function variables (Y), using all the explanatory variables, (in order to obtain centiles and z-scores).

Summary

For each liver function variable, the initial distribution was the one selected in section 3.3, and polynomial terms (up to quintic) in each continuous explanatory variable were chosen using a stepwise selection procedure [using criterion GAIC(4)] in turn for the predictors of each of the four distribution parameters (μ, σ, ν, τ). Interaction terms were then selected, but using criterion GAIC(10) to avoid overfitting due to the large number of possible interaction terms. We then searched for the best fitting distribution. Finally, the model was checked using residuals diagnostic plots (and, if necessary amended). Full details of the model selection procedure are given in materials D.

STEP 1) Remove extreme outliers

STEP 2) Start with the distribution previously chosen for the liver variable using gestational age only

STEP 3) Selecting the main effects terms for the distribution parameters (μ, σ, ν and τ).

Use stepGAICAll.A() to select the best main effects models [for all the distribution parameters (μ, σ, ν and τ)]

(i) set the start and lower models of stepGAICAll.A() to be constants (for all distribution parameters)
(ii) set the upper model of stepGAICAll.A() to include all polynomial terms in ALL the continuous explanatory variables up to quintic [i.e. constant, linear, quadratic, cubic, quartic or quintic], and binary indicator factors for all the levels of ALL the categorical variables (with the least frequent level of each factor omitted), (for all distribution parameters)

(iii) apply stepGAICAll.A() using criterion GAIC(4).

STEP 4) Selecting the interaction terms for the distribution parameters (μ, σ, ν and τ).

(a) Selecting interaction terms for μ using stepGAIC():
  (i) Fix the model terms for σ, ν and τ from STEP 3).
  (ii) Set the start μ model in stepGAIC() to include all linear, quadratic and cubic terms (i.e. x, x^2, and x^3) in ALL the continuous explanatory variables, and also the quartic and quintic terms (i.e. x^4 and x^5) if poly(x,4) or poly(x,5) was chosen for x in STEP 3), and also to include all binary indicator factors for ALL the categorical variables (with the least frequent level of each factor omitted),
  (iii) Set the lower μ model in stepGAIC() to be the μ model from STEP 3).
  (iv) Set the upper μ model in stepGAIC() to be all interactions of μ terms in (ii).
  (v) Apply stepGAIC() using GAIC(10). Penalty 10 was used to avoid overfitting due to the large number of possible interaction terms. [Note to reduce the time taken to search for interactions, stop the search at the first step of stepGAIC() if no interaction improves the value of GAIC(10), and choose the μ model from STEP 3).]

(b) If any interaction terms are clearly selected (not borderline) in (a), then use stepGAIC() to reselect the main effects terms for μ using criterion GAIC(4).
  (i) Fix the model terms for σ, ν and τ from STEP 3).
  (ii) Set the start and lower μ models of stepGAIC() to be the interaction terms selected in 4)(a)(v).
  (iii) Set the upper μ model of stepGAIC() to be the same as in 3)(a)(ii).
  (iii) Apply stepGAIC() for the μ model using criterion GAIC(4).

(c) Select the interaction terms for σ using stepGAIC():
  (i) Fix the the μ model to be the μ model from STEP 4)(b), and the model terms for ν and τ from STEP 3).
  (ii) If any interaction terms were chosen for mu, then reselect the main effect terms for σ using criterion GAIC(4) to use as the start model for σ in stepGAIC(), otherwise set the start model for σ to be the σ model from STEP 3).
  (iii) Set the lower σ model in stepGAIC() to be the σ model from STEP 4)c)(ii).
  (iv) Set the upper σ model in stepGAIC() to be all interactions of σ terms in STEP 4)c)(ii).
  (v) Apply stepGAIC() using GAIC(10). Penalty 10 was used to avoid overfitting due to the large number of possible interaction terms. [Note to reduce the time taken to search for interactions, stop the search at the first step of stepGAIC() if no interaction improves the value of GAIC(10), and choose the μ model from STEP 3).]
  (vi) If any interaction terms are clearly selected (not borderline) above in STEP 4(c), then use stepGAIC() to reselect the main effects terms for σ using criterion GAIC(4) in an equivalent approach to STEP 4(b).
  (vii) If any interactions were chosen for μ, check if the same interactions improve the σ model.

(d) Similarly repeat STEP 4)(c) for selection of the interaction terms for ν.

(e) Similarly repeat STEP 4)(c) for selection of the interaction terms for τ.

STEP 5) Checking the distribution.

(d) Fix the chosen model terms for μ and σ from STEP 4), and for each of ν and τ include all the combined ν and τ model terms from STEP 4), [since for some distributions ν and τ are skewness and kurtosis parameters respectively, while for other distributions ν and τ are left and right tail heaviness parameters],

(e) use the chooseDist() function with argument: type="realplus" in the gamlss R package to select the best distribution for Y in the range $(0, \infty)$ according to criterion GAIC(4),

(f) use the chooseDist() function with argument: type="realline" in the gamlss R package to select the best distribution for log(Y) in the range $(-\infty, \infty)$ according to criterion GAIC(4).

Create the corresponding best distribution for Y using gen.Family() with argument: type="log" and then fit the distribution to Y.

(d) choose between the best distributions for Y in (b) and (c) using GAIC(4).

STEP 6) Checking the model diagnostics: single and multiple worm plots

(a) Fix the chosen model from STEP 5),
  (i) obtain the single worm plot (i.e. detrended QQ plot) of the (normalized quantile) residuals,
  (ii) obtain the multiple worm plots of the residuals split into 4 (or 9) ranges of each continuous explanatory variable (x),
  (iii) obtain the multiple worm plots of the residuals split into the levels of each categorical explanatory variable (x),
  (iv) obtain the 2-dimensional multiple worm plots of the

residuals with a 2-dimensional split into ranges or levels of 2 explanatory variables (x1 and x2).

(b) Check the residual worm plots.

The worm plots (i.e. detrended QQ plots) in STEP 6)(a) should be roughly horizontal, with approximately 95% of points within the approximate 95% confidence bands.

Focusing on the important tail of the distribution of the liver variable (Y),

(i) check the worm plots in STEP 6)(a) are OK.

(ii) If the single worm plot in 6)(a)(i) is not OK, this may indicate an inadequate distribution.

If the multiple worm plot in 6)(a)(ii) is not OK, this indicates that a term in x is not adequate (a higher polynomial in x may be needed, e.g. a quadratic term in x instead of a linear tem in x, for a distribution parameter, e.g. μ).

If the multiple worm plot in 6)(a)(iii) is not OK, this indicates that the categorical variable is needed in the model for a distribution parameter, e.g. μ.

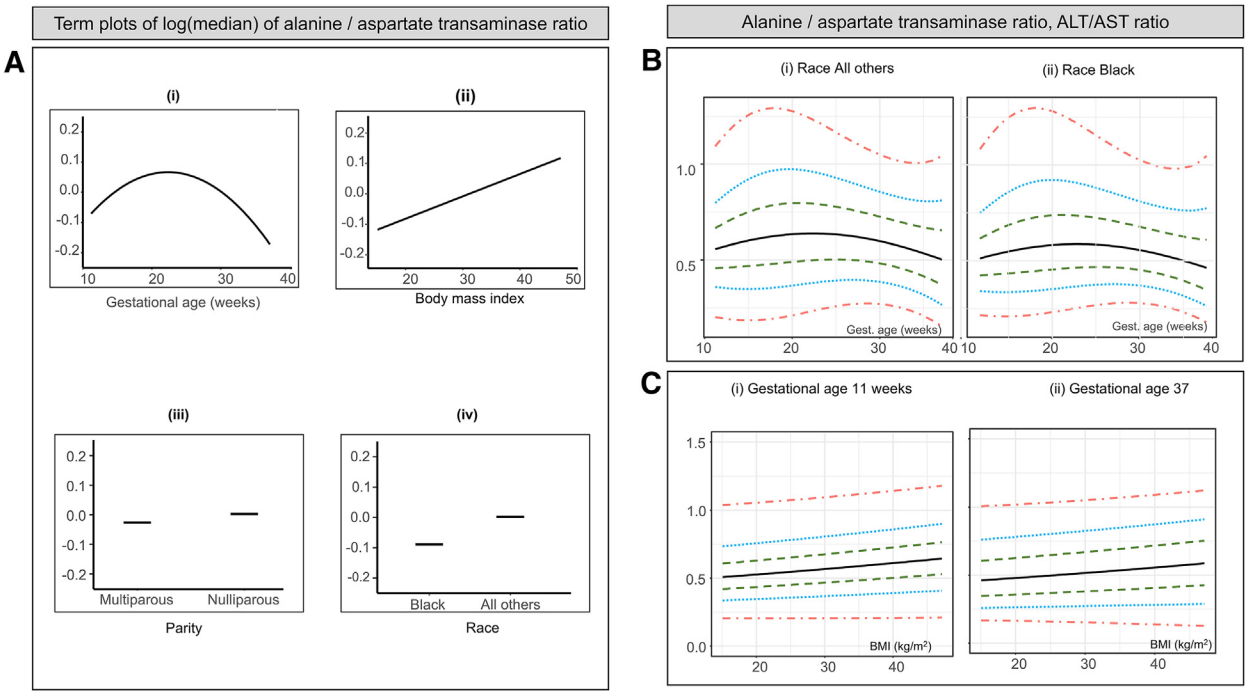
If the multiple worm plot in 6)(a)(iv) is not OK, this indicates that an interaction term in x1 and x2 is needed for a distribution parameter, e.g. μ.

(iii) check whether the worm plots in STEP 6)(a) are still OK if the model is simplified by removing borderline terms.

Appendix: Summary Table of Supplemental Materials

A	Expanded material on Methods, Results and Discussion.
B	Models for each of the seven liver variables using gestational age only. 1. Table of terms in gestational age for the predictors of μ, σ, ν, and τ, for each of the seven liver variables. 2. Results of the chosen models using gestational age only. 3. Equations for the predictors of μ, σ, ν, and τ, for each of the seven liver variables. 4. Model selection procedure.
C	Models for each of the seven liver variables using all the explanatory variables. 1. Results of the chosen models for the liver variables. 2. Equations for the predictors of μ, σ, ν, and τ, for each of the seven liver variables. 3. Supplemental Figures 1 to 4 (for ALT/AST, GGT, TBIL and ALB, respectively) A: term plot for μ, B: conditional centiles against ga, for maternal age=18 and age=48 (for TBIL and ALB), and for different ethnicities (for ALT/AST and GGT) C: conditional centiles against BMI, for ga=11 and ga=37. 4. Supplemental Figures 5 to 7: term plots for the predictors of σ, ν, and τ, respectively (A for ALP, B for the other six liver variables). 5. Model selection procedure.
D	Reference ranges for non-pregnant populations

SUPPLEMENTAL FIGURE 1
Full model for alanine transaminase / aspartate transaminase ratio

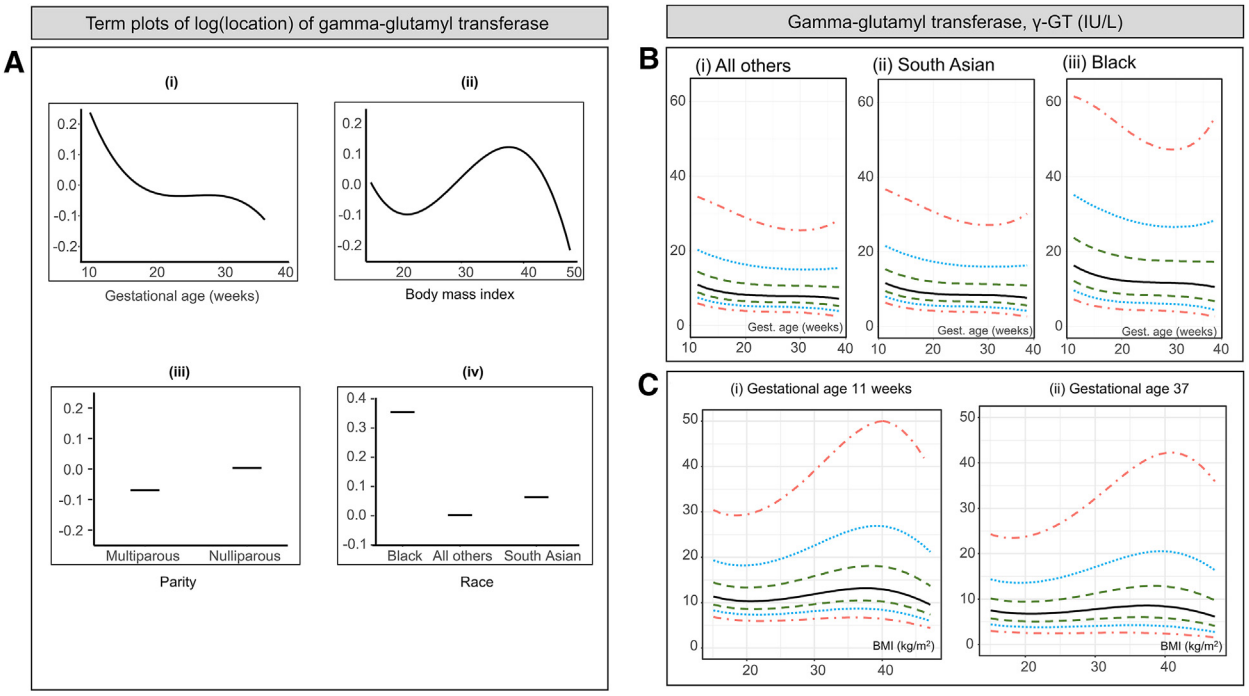


A: Term plots of log(median) of alanine / aspartate transaminase ratio: gestational age (i), body mass index (ii), maternal parity (iii), maternal race (iv).
B: Full model for alanine / aspartate transaminase ratio against gestational age for maternal all other maternal races (i) against maternal race Black (ii).

C: Full model for alanine / aspartate transaminase ratio against body mass index for gestational age 11 weeks (i) and gestational age 37 weeks (ii).

SUPPLEMENTAL FIGURE 2

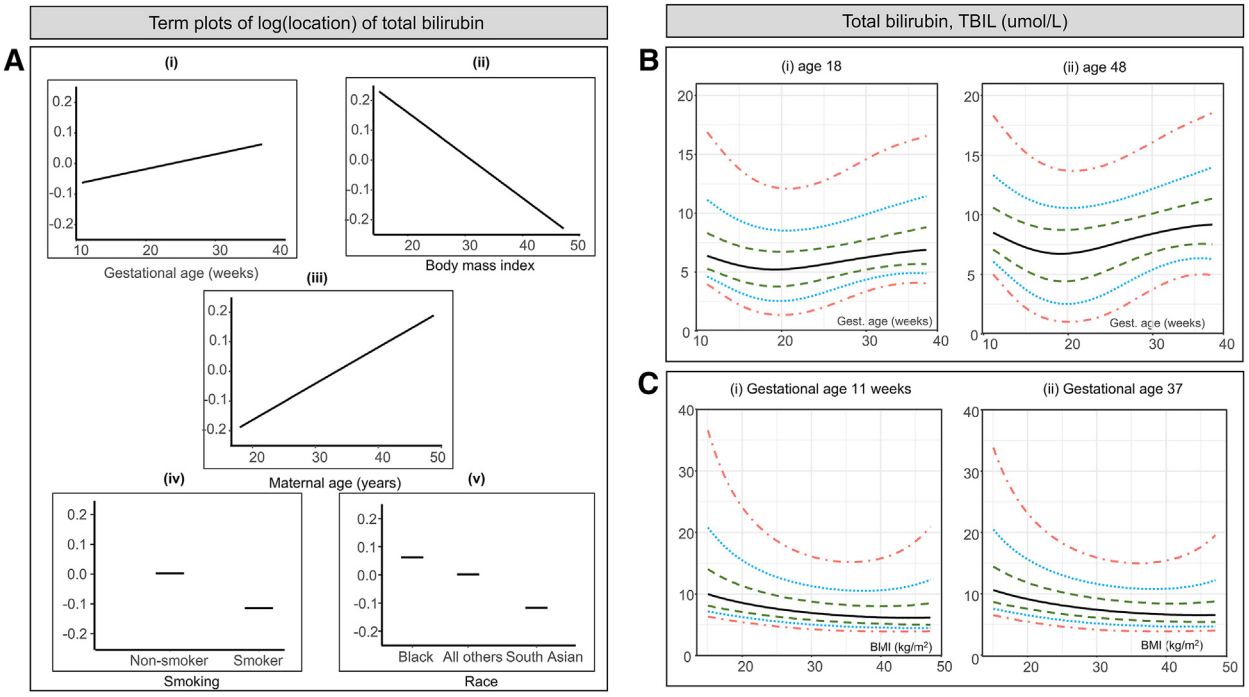
Full model for gamma-glutamyl transferase



A: Term plots of log(location) of gamma-glutamyl transferase: gestational age (i), body mass index (ii), maternal parity (iii), maternal race (iv).
B: Full model for gamma-glutamyl transferase against gestational age for maternal all other races (i) against South Asian (ii) and Black race (iii).

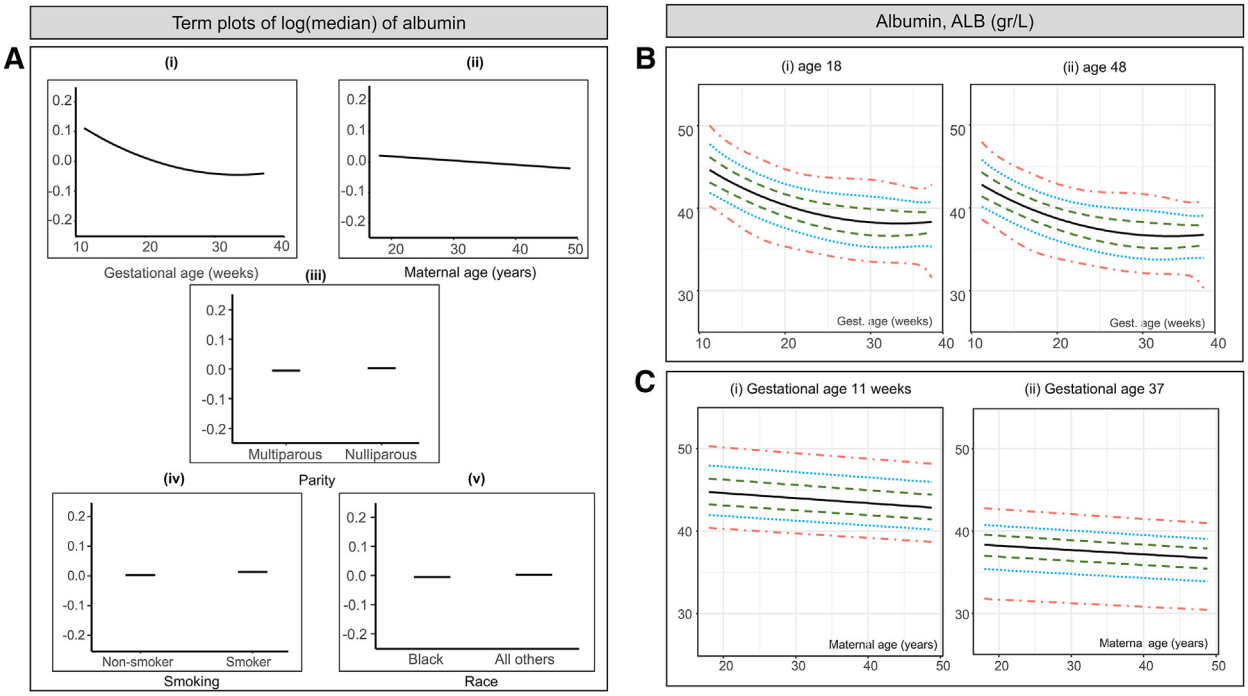
C: Full model for gamma-glutamyl transferase against body mass index for gestational age 11 weeks (i) and gestational age 37 weeks (ii).

SUPPLEMENTAL FIGURE 3
Full model for total bilirubin



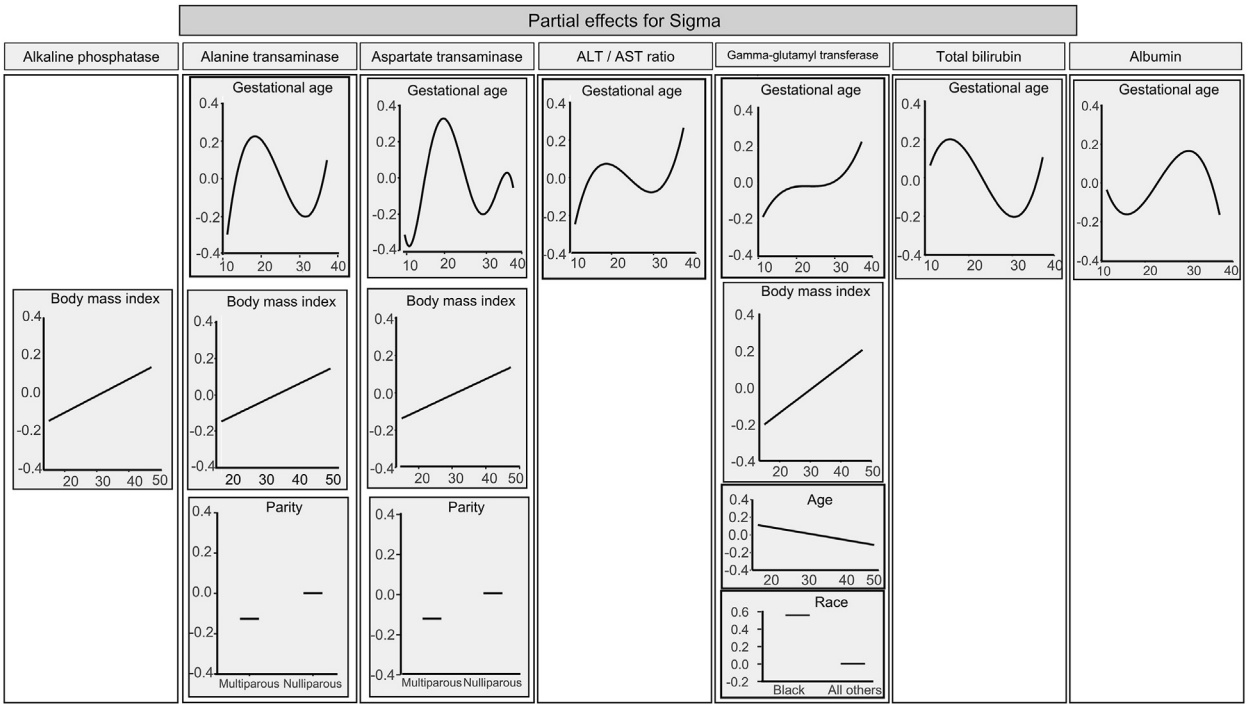
A: Term plots of log(location) of total bilirubin: gestational age (i), body mass index (ii), maternal age (iii), smoking (iv), maternal race (v).
B: Full model for total bilirubin against gestational age for maternal age 18 (i) and maternal age 48 (ii).
C: Full model for total bilirubin against body mass index for gestational age 11 weeks (i) and gestational age 37 weeks (ii).

SUPPLEMENTAL FIGURE 4
Full model for albumin



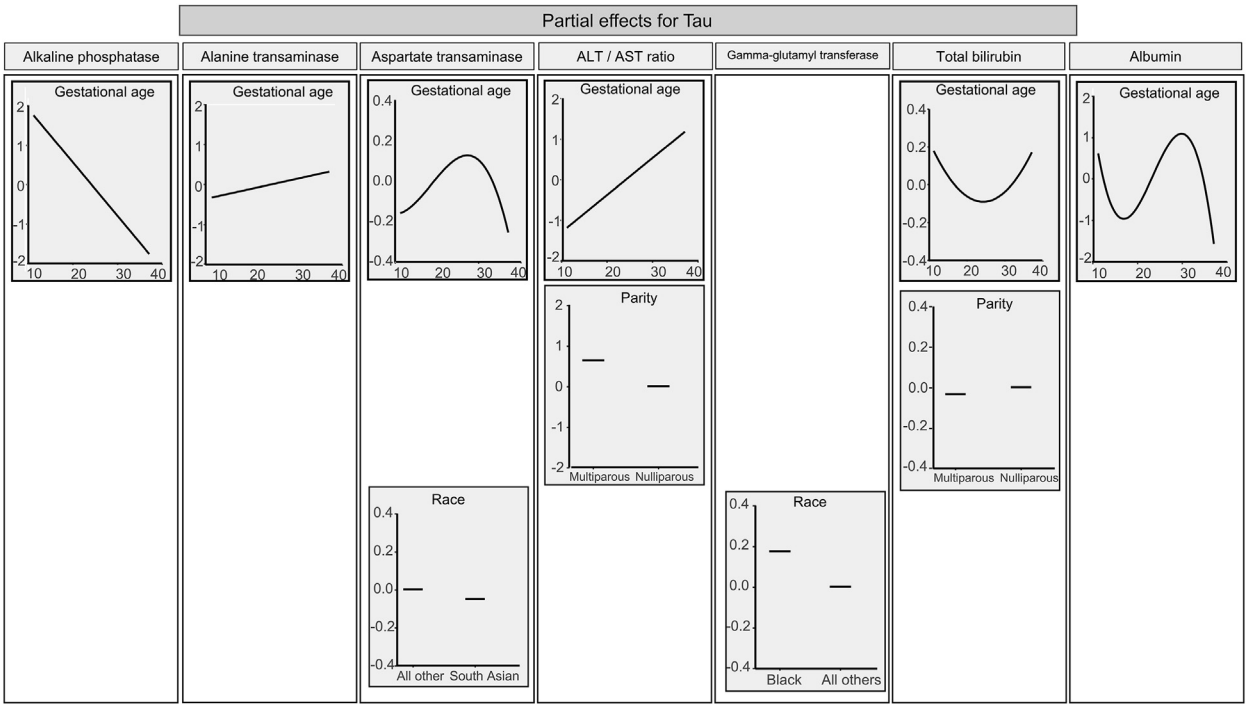
A: Term plots of log(median) of albumin: gestational age (i), maternal age (ii), parity (iii), smoking (iv), maternal race (v).
B: Full model for albumin against gestational age for maternal age 18 (i) and maternal age 48 (ii).
C: Full model for albumin against maternal age for gestational age 11 weeks (i) and gestational age 37 weeks (ii).

SUPPLEMENTAL FIGURE 5
Partial effects for Log of Sigma for all liver function tests



SUPPLEMENTAL FIGURE 6

Partial effects for the predictor of Nu for all liver function tests



SUPPLEMENTAL FIGURE 7

Partial effects for the predictor of Tau for all liver function tests