

ReMAPP Aim 1 Report

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Abbreviation:

ReMAPP: Redefining Maternal Anemia in Pregnancy and Postpartum Study

FPR: Fractional polynomial regression

Version:

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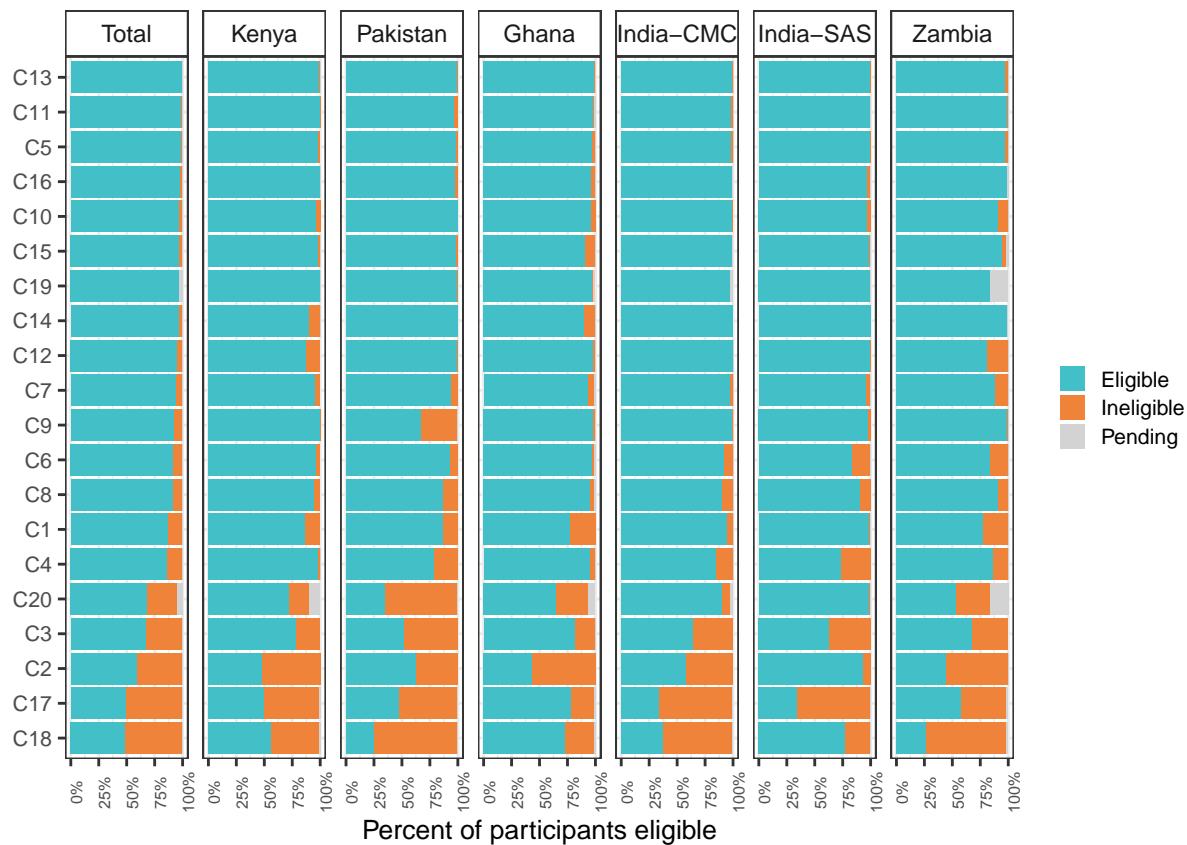
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1 Study data selection criteria and cleaning

1.1 Eligibility status and missingness of healthy cohort criteria

Figure shows the distribution of each healthy cohort criteria (C1-C20) among women enrolled in PRISMA between ReMAP launch and end date. Pending includes missing form, diagnosis, data, tests not performed or test results pending.

Note that the criteria are ranked by eligibility percentage, with the most commonly met criteria appearing at the top.



Description of eligibility criteria (C1-C20):

- C1: Aged 18 to 34 years;
- C2: Gestational age <14 weeks
- C3: Pre-pregnancy or early pregnancy body mass index (BMI) of >18.5 and <30 kg/m² AND mid-upper arm circumference (MUAC) >23cm
- C4: Height >=150 cm
- C5: Singleton pregnancy
- C6: No previous reported low birth weight delivery
- C7: No previous reported stillbirth
- C8: No previous reported unplanned cesarean delivery
- C9: No reported cigarette smoking, tobacco chewing, or betel nut use during pregnancy
- C10: No reported alcohol consumption during pregnancy

- C11: No known history or current chronic disease including cancer, kidney disease, and cardiac conditions
- C12: No known history or current HIV
- C13: Systolic blood pressure <140 mmHg and diastolic blood pressure <90 mmHg
- C14: No current malaria infection (per rapid diagnostic test)
- C15: No current Hepatitis B virus infection (per rapid diagnostic test)
- C16: No current Hepatitis C virus infection (per rapid diagnostic test)
- C17: Not iron deficient (serum ferritin >15 mcg/L adjusted for inflammation)
- C18: No subclinical inflammation (CRP<=5 mg/L and/or AGP<=1 g/L)
- C19: No hemoglobinopathies: SS, SC, SE, EE, CC, SD-Punjab, S^fthal, E^fthal, C^fthal, CD-Punjab, ED-Punjab, D-D-Punjab, D-Punjab^fthal, Thalassemia major, Thalassemia intermedia, or Alpha thalassemia
- C20: Normal glucose-6-phosphate dehydrogenase (>=6.1 U/g Hb)

1.2 Sample size and analytical subcohort

Table 1. Healthy cohort eligibility and data for FPR analysis

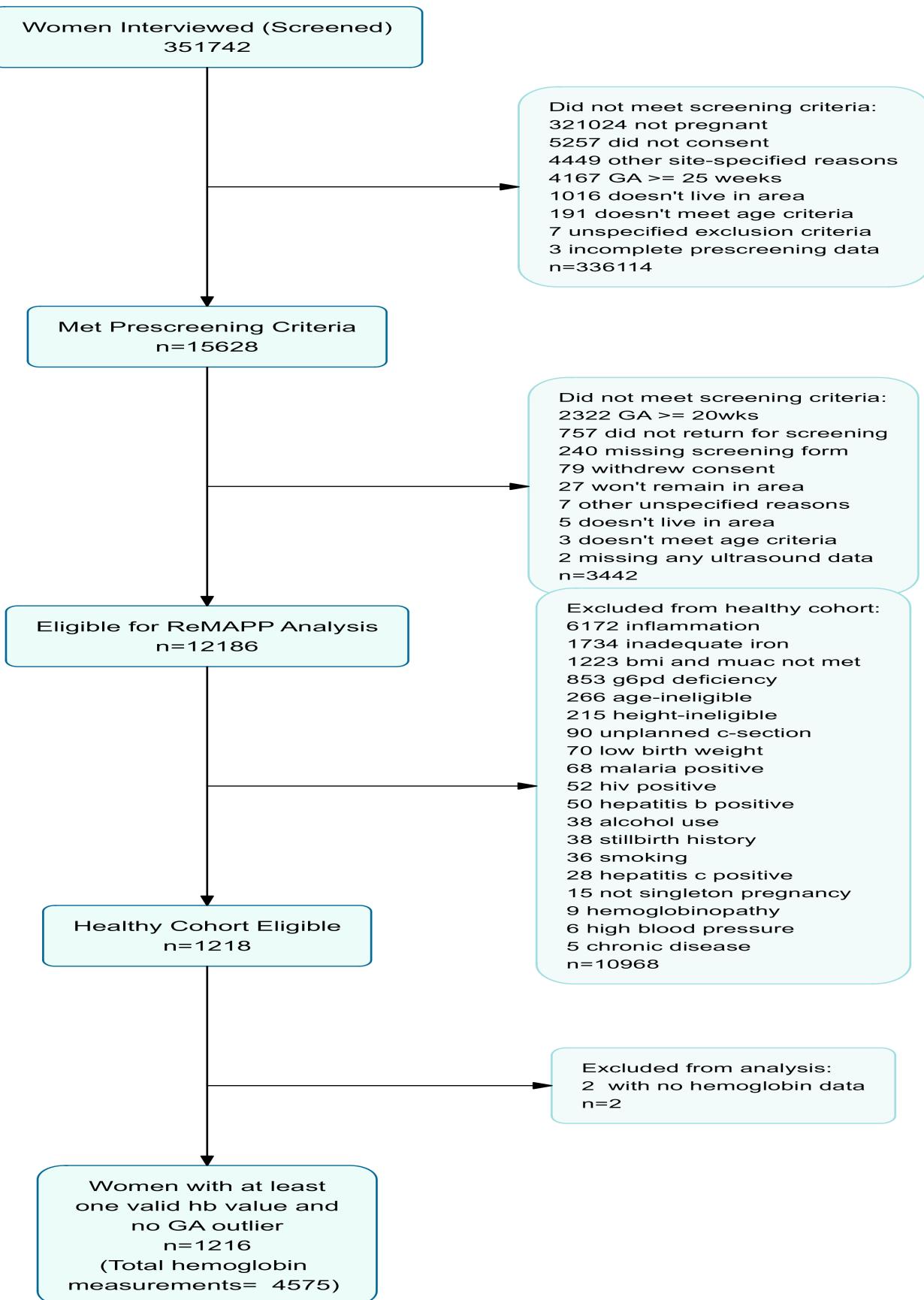
	Site						Total
	Ghana	India-CMC	India-SAS	Kenya	Pakistan	Zambia	
Total enrolled in PRISMA MNH since ReMAPP launch, N	1974	2000	2000	2003	2497	1713	12187
Eligible* for healthy cohort, n (%)	352 (17.83)	203 (10.15)	209 (10.45)	333 (16.63)	36 (1.44)	85 (4.96)	1218 (9.99)
Eligible for healthy cohort - G6PD excluded, n (%)	523 (26.49)	217 (10.85)	214 (10.70)	421 (21.02)	96 (3.84)	132 (7.71)	1603 (13.15)
Eligible for healthy cohort - GA Applied, n (%)	178 (9.02)	115 (5.75)	202 (10.10)	189 (9.44)	24 (0.96)	42 (2.45)	750 (6.15)
Healthy cohort with hemoglobin and gestational age data, n	352	202	209	333	36	84	1216
Hemoglobin data points in FPR analysis, n	1365	873	770	1166	143	258	4575

*Criteria of gestational age is not applied due to low first trimester enrollment. Pending value allowed for following criteria due to high pending rate: inflammation, iron deficiency, hemoglobinopathies and G6PD.

Note: Last two rows indicate the number of participants and number of HB data points included in the analysis

1.3 Flow diagram illustrating women included in the Healthy Cohort Analysis

The flowchart illustrates the selection process of female participants for inclusion in the Healthy Cohort analysis within the ReMAPP sample, detailing each stage from initial pre-screening to final eligibility determination.



1.4 Baseline characteristics of overall ReMAPP sample and healthy cohort in analysis

The table below describes the baseline characteristics and maternal and perinatal outcome data for women enrolled in the Redefining Anemia in Pregnancy and Postpartum (ReMAPP) study compared to those in the healthy cohort.

Baseline Characteristics and Perinatal Outcomes in Overall ReMAPP Sample vs. Healthy Cohort

Characteristic/Outcome	Healthy Cohort in Analysis, Overall ReMAPP cohort, N = 1216 ¹	N = 10970 ¹
Age (years) mean (SD)	25 ± 4	26 ± 6
Gestational age at first visit (weeks); mean (SD)	13.1 ± 3.7	13.3 ± 3.7
Body mass index (kg/m ²); mean (SD)	23.1 ± 2.7	23.6 ± 5.5
MUAC at first visit (cm); mean (SD) ²	26.9 ± 2.4	26.7 ± 4.3
Years of formal education; mean (SD)	10 ± 5	8 ± 5
Married or cohabiting; n (%)	1,100 (90%)	10,274 (94%)
Engaged in paid work; n (%)	419 (34%)	3,234 (29%)
Never gave birth before (no live birth & no stillbirth); n (%)	609 (50%)	3,427 (31%)
Previous miscarriage; n (%)	204 (17%)	2,281 (21%)
Live birth outcome; n (%)	1,064 (88%)	9,309 (85%)
Male; n (%)	562 (46%)	4,929 (45%)
Preterm (<37 weeks' gestation); n (%)	66 (5%)	1,204 (11%)
Birthweight (in kg); mean (SD)	2.97 ± 0.48	2.86 ± 0.52
Low birth weight (<2500g); n (%)	131 (11%)	1,787 (16%)
SGA Centile by INTERGROWTH Standards; mean (SD) ³	33 ± 26	31 ± 26
Neonatal mortality; n (%)	15 (1%)	253 (2%)

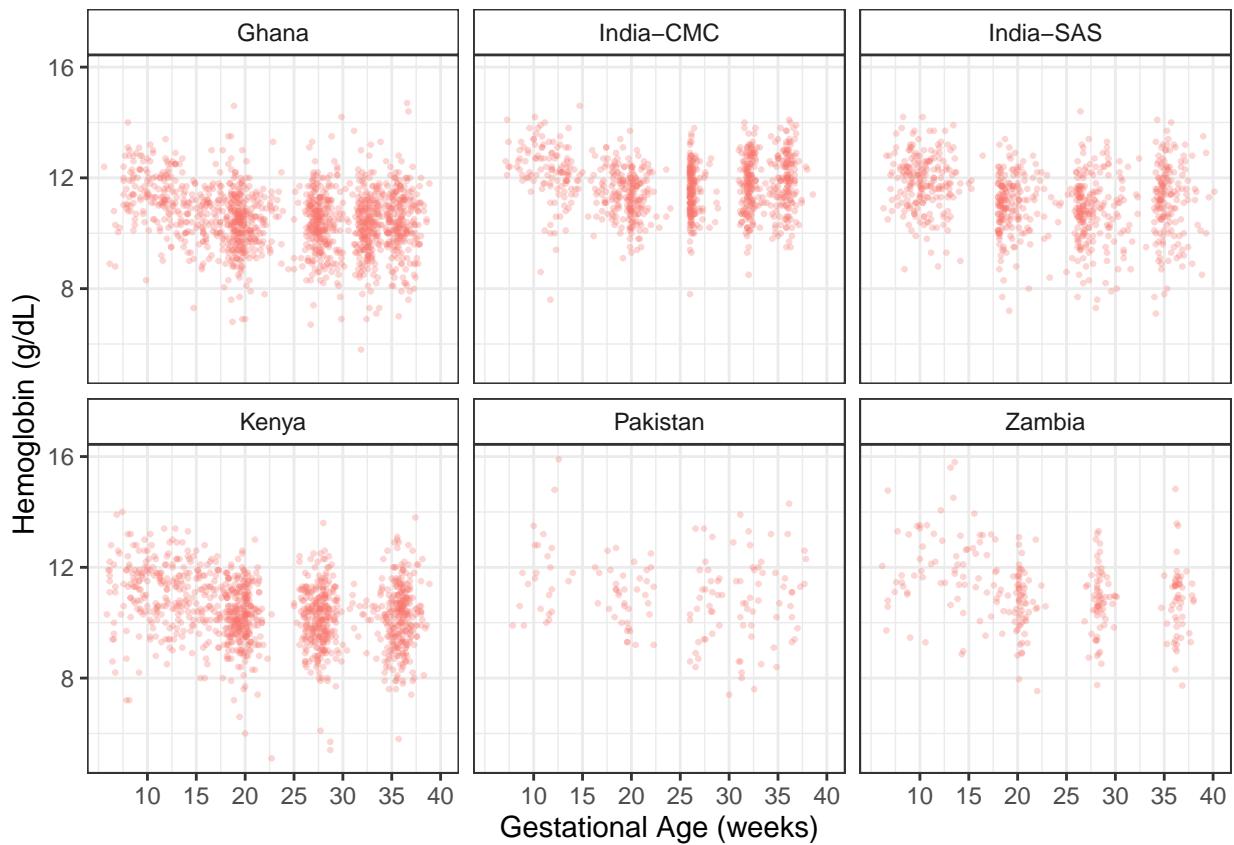
¹ Mean ± SD; n (%)

² MUAC = Mid-upper arm circumference

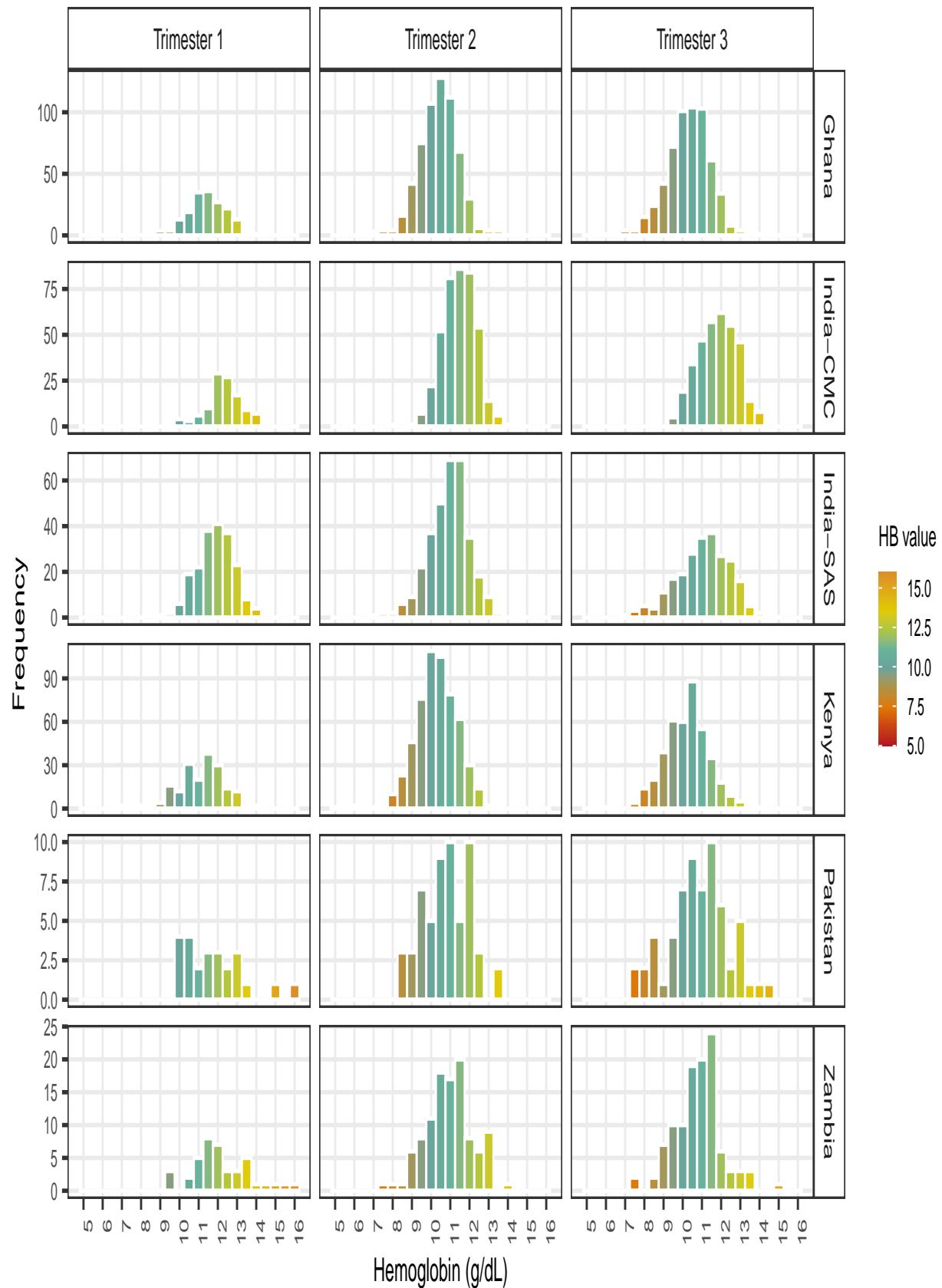
³ SGA = Small for Gestational Age

2 Description of study data

2.1 Scatterplot of hemoglobin by gestational age

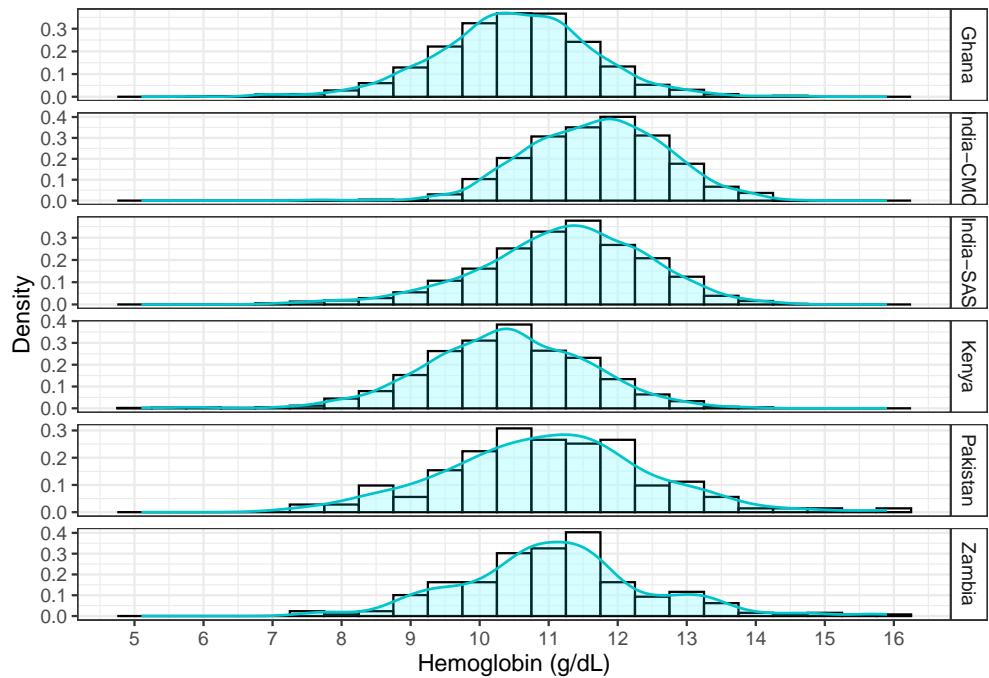


2.2 Hemoglobin by trimester and site



Note: Trimester1: 0-13 weeks; Trimester2: 14-27 weeks; Trimester3: 28-40 weeks.

2.3 Histograms of hemoglobin distribution



Note: The density plot will show whether the distribution is bell shaped or skewed.

2.4 Test of hemoglobin normality

Table 4. Test of Normality of Hemoglobin		
SITE	Statistic	Pvalue
Ghana	0.993593	<0.001
India-CMC	0.995056	0.006
India-SAS	0.987999	<0.001
Kenya	0.991197	<0.001
Pakistan	0.992549	0.661
Zambia	0.984373	0.006

Note: A p-value <0.05 will indicate non-normally distributed hemoglobin data.

2.5 Test of hemoglobin heterogeneity between sites

Table 5. Test of hemoglobin heterogeneity between sites			
Variance component	Estimates	Standard error	Proportion(%)
Var(subject within site)	0.757	0.87	49.407
Var(site)	0.235	0.484	15.311
Var(error)	0.541	0.735	35.283

Note: Heterogeneity in CBC-hemoglobin across sites is first assessed by comparing the percentages of the variance due to inter-subject and inter-site differences estimated by analysis-of-variance techniques. Variance component analysis is based on a liner mixed-effect model, with gestational age treated as fixed effect, and sites and individuals treated as random effects. We will test the significance of site effect in the model. We will compare the percentage of variance due to inter-site and that of inter-individual.

Table 6. Test the Effect of Site						
Term	Npar	Loglik	AIC	LRT	Df	Pvalue
	5	-6,189.43	12,388.87			
SITE	4	-6,319.01	12,646.01	259.14	1	<0.001
SITE:MOMID	4	-7,076.49	14,160.99		1	<0.001

We will also check the the significance of site effect. P value < 0.05 will indicate that the site effect cannot be ignored.

3 Regression model for the study

We will apply fractional polynomial regression on healthy cohort data. By assuming that the hemoglobin follows a normal distribution $N(\mu(t), \sigma^2(t))$ at given gestational week (t), the fractional polynomial regression (FPR) model fits regression models for both the mean parameter μ and standard deviation parameter σ . Then estimate the centiles by using the equation

$$C_{100\alpha}(t) = \mu(t) + Z_\alpha \sigma(t)$$

, where $C_{100\alpha}(t)$ is the expected hemoglobin centile 100α at given gestational week (t), and Z_α is the normal equivalent deviate of size α .

4 Fractional polynomial regression - Pooled

Assuming we have normally distributed data and the heterogeneity of CBC-hemoglobin across sites can be ignored, we pool the CBC-hemoglobin from all sites together, and estimate the 2.5th, 5th, 10th, 50th, 95th and 97.5th centiles of hemoglobin at given gestational weeks during pregnancy and 6-week postpartum.

The FPR model for μ and σ are

$$\mu = 10.7113709 + 2.20939109 - 1.83051332(GA/10) + 0.33554028(GA/10)^2$$

and

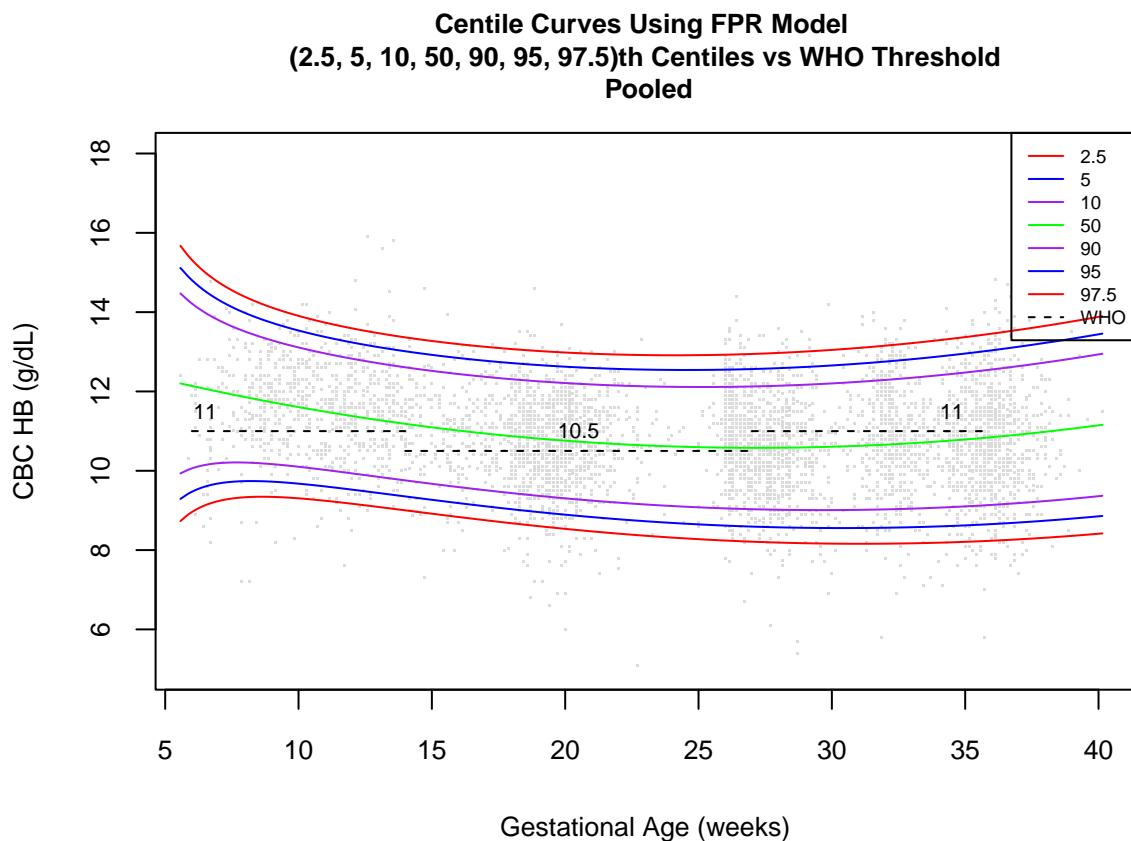
$$\log(\sigma) = 0.2386056 + 0.07206012 - 0.03983788(GA/10)^2 + 0.02760153(GA/10)^2 \log(GA/10)$$

respectively.

4.1 Centile plots of hemoglobin against gestational weeks

Given the fitted FPR model, we can plot the centile curves of hemoglobin against the gestational weeks. Also add the WHO thresholds to compare with.

4.1.1 Centile curves using FPR model (2.5, 5, 10, 50, 90, 95, 97.5)th centiles vs WHO threshold

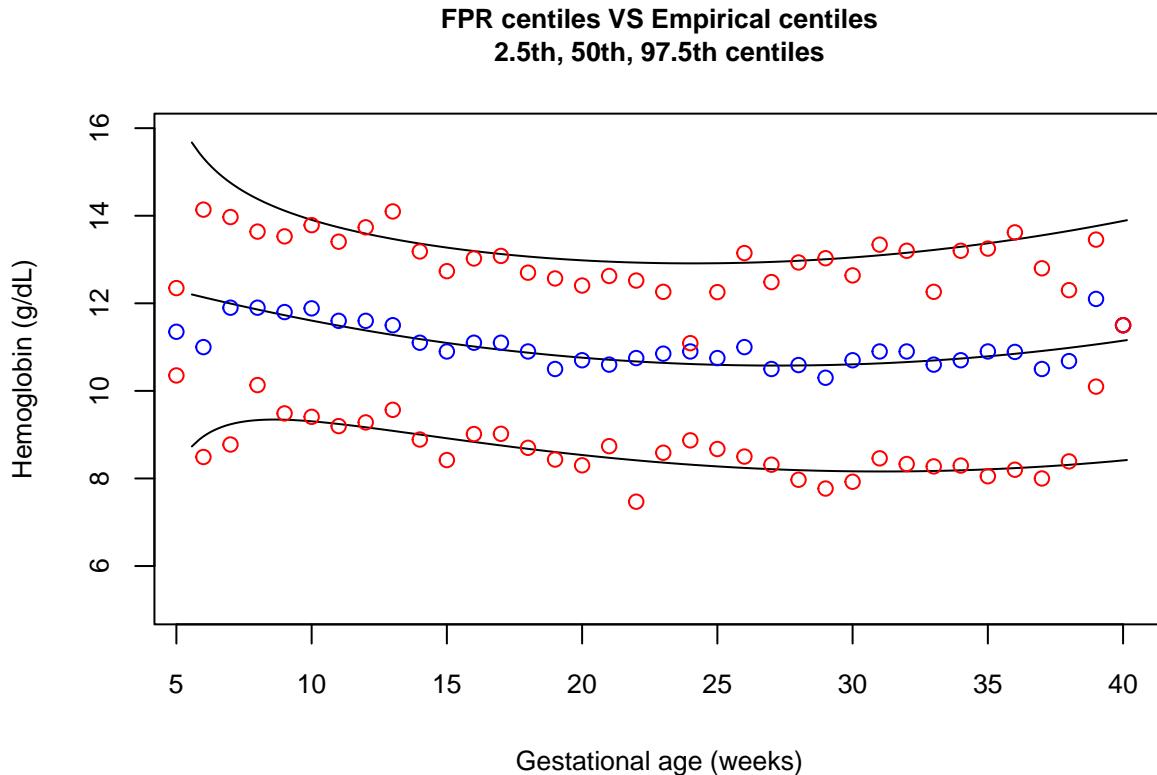


4.1.2 Centile table for maternal hemoglobin (in g/dL) by exact gestational age (in weeks)

Centiles for maternal haemoglobin (in g/dL)							
Gestational age (exact week)	2.5th centile	5th centile	10th centile	50th centile	90th centile	95th centile	97.5th centile
10 weeks + 0 days	9.31	9.68	10.1	11.61	13.11	13.54	13.91
11 weeks + 0 days	9.24	9.61	10.02	11.49	12.96	13.37	13.74
12 weeks + 0 days	9.17	9.52	9.93	11.38	12.83	13.24	13.59
13 weeks + 0 days	9.09	9.44	9.85	11.28	12.71	13.12	13.47
14 weeks + 0 days	9	9.35	9.76	11.18	12.61	13.01	13.36
15 weeks + 0 days	8.92	9.27	9.67	11.1	12.52	12.92	13.27
16 weeks + 0 days	8.83	9.18	9.59	11.01	12.44	12.84	13.19
17 weeks + 0 days	8.75	9.11	9.51	10.94	12.37	12.77	13.13
18 weeks + 0 days	8.68	9.03	9.44	10.87	12.31	12.72	13.07
19 weeks + 0 days	8.6	8.96	9.37	10.81	12.26	12.67	13.02
20 weeks + 0 days	8.54	8.89	9.3	10.76	12.21	12.62	12.98
21 weeks + 0 days	8.47	8.83	9.25	10.71	12.18	12.59	12.95
22 weeks + 0 days	8.41	8.78	9.2	10.67	12.15	12.57	12.93
23 weeks + 0 days	8.36	8.73	9.15	10.64	12.13	12.55	12.92
24 weeks + 0 days	8.32	8.69	9.11	10.61	12.12	12.54	12.91
25 weeks + 0 days	8.28	8.65	9.08	10.6	12.11	12.54	12.92
26 weeks + 0 days	8.24	8.62	9.05	10.58	12.12	12.55	12.93
27 weeks + 0 days	8.21	8.59	9.03	10.58	12.13	12.56	12.95
28 weeks + 0 days	8.19	8.57	9.02	10.58	12.14	12.59	12.97
29 weeks + 0 days	8.17	8.56	9.01	10.59	12.17	12.62	13.01
30 weeks + 0 days	8.16	8.56	9.01	10.61	12.2	12.66	13.05
31 weeks + 0 days	8.16	8.56	9.01	10.63	12.24	12.7	13.1
32 weeks + 0 days	8.16	8.56	9.03	10.66	12.29	12.75	13.15
33 weeks + 0 days	8.17	8.58	9.04	10.69	12.35	12.81	13.22
34 weeks + 0 days	8.19	8.6	9.07	10.74	12.41	12.88	13.29
35 weeks + 0 days	8.21	8.62	9.1	10.79	12.48	12.96	13.37
36 weeks + 0 days	8.24	8.66	9.14	10.85	12.55	13.04	13.46
37 weeks + 0 days	8.27	8.7	9.18	10.91	12.64	13.13	13.55
38 weeks + 0 days	8.31	8.74	9.24	10.98	12.73	13.22	13.65
39 weeks + 0 days	8.36	8.79	9.29	11.06	12.83	13.33	13.76
40 weeks + 0 days	8.41	8.85	9.36	11.15	12.93	13.44	13.88

4.1.3 Diagnostic plot

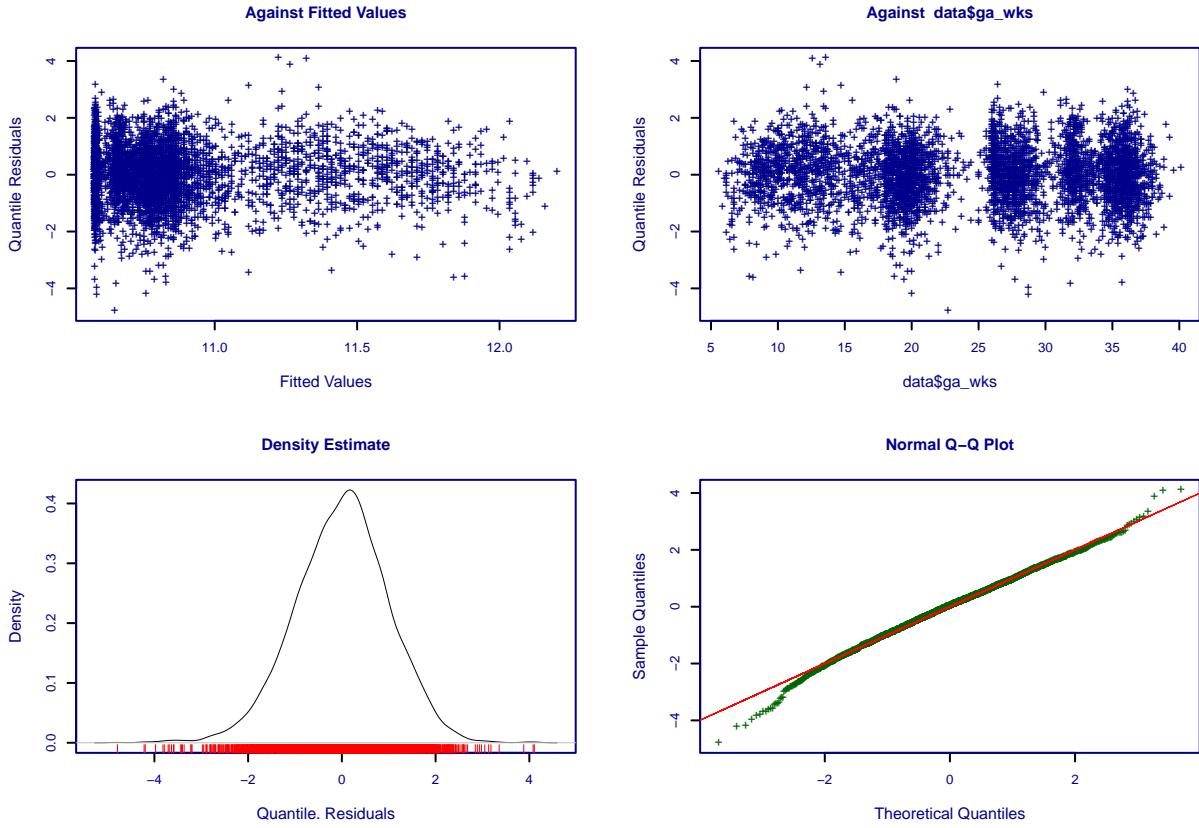
We use diagnostic plots to examine if the FPR model is a good fit to the hemoglobin centile curves.



In the $\hat{}$ FPR centiles VS Empirical centiles $\hat{}$ plot, we will observe if the FPR curves are consistent with empirical curves.

In addition, we use four plots of normalized quantile residuals to check the adequacy of the fitted fractional polynomial regression model. If the residuals display the behavior of following a standard normal distribution, the model is a good fit. The four plots are:

- top left: residuals against the fitted values of the mean parameter.
- top right: residuals against the specified covariate.
- bottom left: a kernel density estimate of the residuals.
- bottom right: a QQ-normal plot of the residuals.



The residuals behave well if the top two plots show a random scatter around the horizontal line at 0, and the kernel density estimate of the residuals is approximately normal and the normal QQ-plot is approximately linear.

4.2 Estimate the centiles at given gestational weeks

We estimate the 2.5th, 50th, and 97.5th centiles at 14, 20, 28, 32, 36 gestational weeks using predictions from the FPR curves. Also compare the predicted hemoglobin value with empirical percentile at each time point.

Table 7. FPR predicted centiles & Empirical percentile - Pooled

GA	FPR-2.5	Empir-2.5	FPR-50	Empir-50	FPR-97.5	Empir-97.5
12	9.169	9.28	11.381	11.6	13.592	13.736
14	9.003	8.889	11.183	11.1	13.364	13.184
20	8.535	8.3	10.759	10.7	12.982	12.406
28	8.19	7.97	10.581	10.59	12.972	12.933
32	8.162	8.33	10.658	10.9	13.154	13.2
36	8.236	8.2	10.847	10.89	13.458	13.62

4.3 Sensitivity analysis

4.3.1 Excluding G6PD (Glucose-6-Phosphate Dehydrogenase) as a healthy criteria

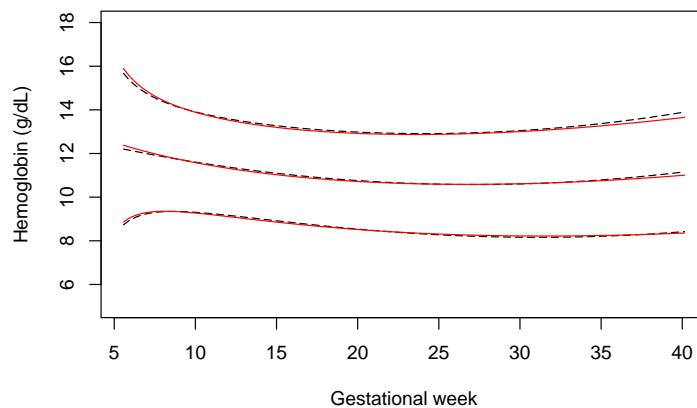
Although G6PD deficiency can predispose individuals to hemolysis under oxidative stress, its effect on hemoglobin levels during pregnancy is variable and context-dependent. Most women with G6PD deficiency remain clinically asymptomatic and do not experience chronic hemolysis or persistent anemia in the absence of specific triggers (e.g., certain medications, infections, or fava bean exposure). Therefore, excluding all G6PD-deficient women from the “healthy” analytic cohort may be overly conservative and could remove individuals whose hemoglobin levels are physiologically normal.

To assess the robustness of the hemoglobin distribution to this assumption, we conducted a sensitivity analysis in which G6PD deficiency was not used as an exclusion criterion. This allows us to evaluate:

- Whether the inclusion of G6PD-deficient women meaningfully alters the estimated hemoglobin distribution,
- The extent to which G6PD deficiency contributes to lower hemoglobin values in this population, and

This approach ensures that the final hemoglobin reference distribution is not unduly influenced by assumptions about the clinical impact of G6PD deficiency, and it helps determine whether excluding these women is necessary or whether their inclusion provides a more representative estimate of hemoglobin levels in the general pregnant population.

Sensitivity analysis: FPR Excluding G6PD Deficinecy as a Healthy Crit

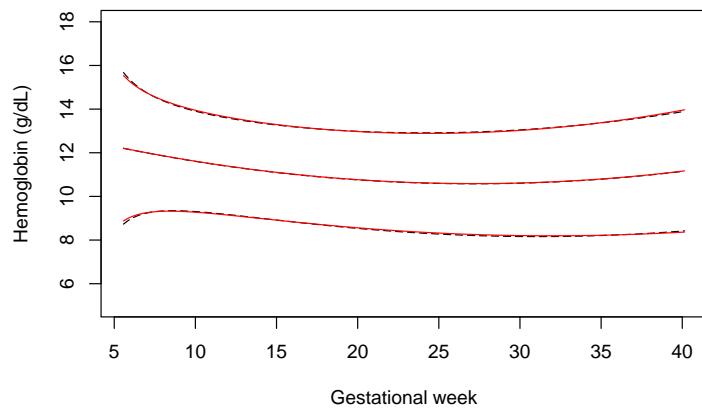


4.3.2 Excluding the subgroup of subjects with adverse outcomes.

In order to see if participants with adverse outcome has different hemoglobin distribution, we will compare sample under each subsample (red line) with all enrolled (black line). We need discuss more subgroups.

- Sensitivity analysis by excluding the subgroup of subjects with preterm delivery

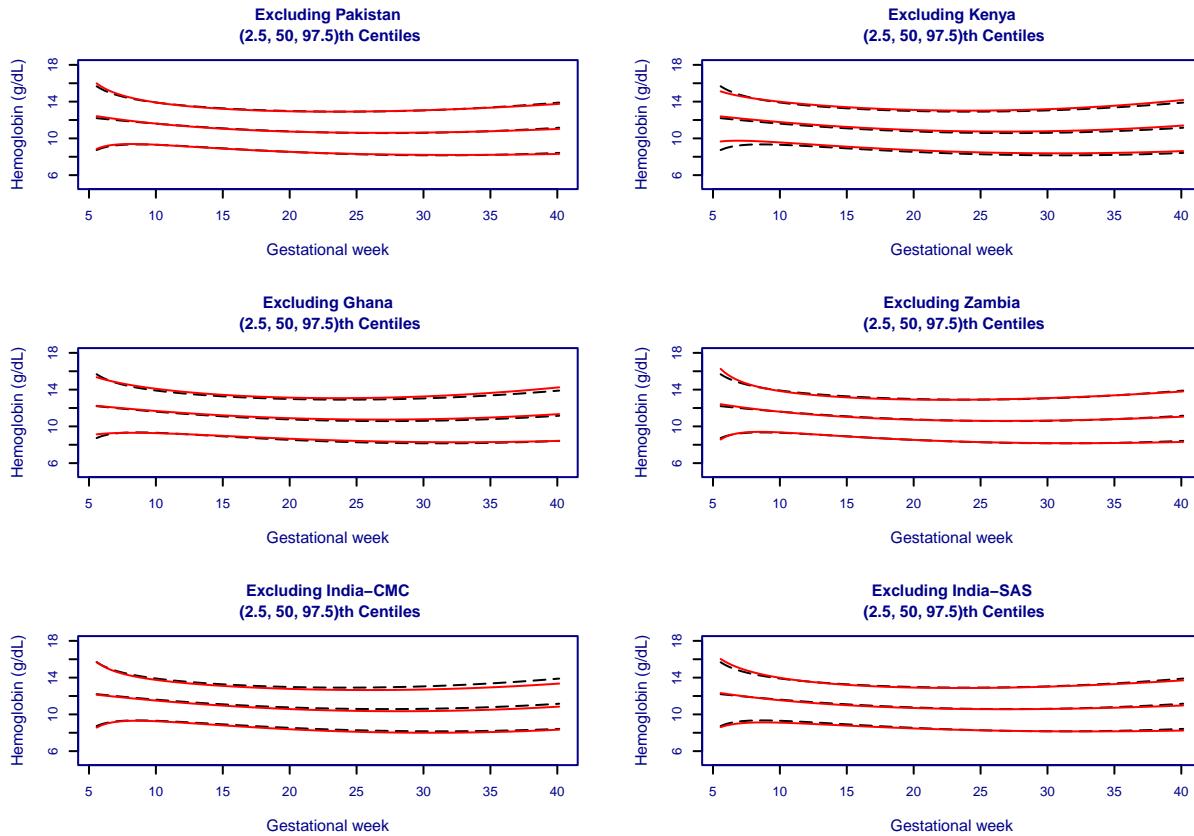
Excluding preterm37_mom



Check whether the centile plots are sensitive to removing the group with preterm delivery.

4.3.3 Excluding one site at a time

4.3.3.1 Plots: Sensitivity analysis plots excluding one site at a time Compare sample by excluding one site at a time (red line) with pooled (black line).



Check whether the centile plots are sensitive to removing one site at a time.

4.3.3.2 Tables: Sensitivity analysis tables excluding one site at a time

Centile Comparison - Excluding Pakistan										
	GA	Pooled 2.5th	Excl 2.5th	Diff 2.5th	Pooled 50th	Excl 50th	Diff 50th	Pooled 97.5th	Excl 97.5th	Diff 97.5th
12		9.17	9.15	-0.02	11.38	11.35	-0.03	13.59	13.56	-0.03
14		9	8.97	-0.03	11.18	11.14	-0.04	13.36	13.31	-0.05
28		8.19	8.24	0.05	10.58	10.61	0.03	12.97	12.98	0.01
32		8.16	8.2	0.04	10.66	10.68	0.02	13.15	13.16	0
36		8.24	8.22	-0.01	10.85	10.82	-0.03	13.46	13.41	-0.05
40		8.41	8.31	-0.11	11.15	11.02	-0.12	13.88	13.74	-0.14

Centile Comparison - Excluding Kenya										
	GA	Pooled 2.5th	Excl 2.5th	Diff 2.5th	Pooled 50th	Excl 50th	Diff 50th	Pooled 97.5th	Excl 97.5th	Diff 97.5th
12		9.17	9.37	0.21	11.38	11.54	0.16	13.59	13.71	0.12
14		9	9.19	0.19	11.18	11.34	0.16	13.36	13.49	0.13
28		8.19	8.4	0.21	10.58	10.75	0.17	12.97	13.09	0.12
32		8.16	8.38	0.22	10.66	10.84	0.18	13.15	13.3	0.15
36		8.24	8.45	0.21	10.85	11.05	0.21	13.46	13.66	0.2
40		8.41	8.61	0.2	11.15	11.39	0.24	13.88	14.16	0.28

Centile Comparison - Excluding Ghana										
	GA	Pooled 2.5th	Excl 2.5th	Diff 2.5th	Pooled 50th	Excl 50th	Diff 50th	Pooled 97.5th	Excl 97.5th	Diff 97.5th
12		9.17	9.16	-0.01	11.38	11.47	0.09	13.59	13.77	0.18
14		9	9.04	0.03	11.18	11.28	0.1	13.36	13.53	0.17
28		8.19	8.33	0.14	10.58	10.74	0.16	12.97	13.16	0.19
32		8.16	8.28	0.11	10.66	10.83	0.17	13.15	13.39	0.23
36		8.24	8.31	0.07	10.85	11.02	0.18	13.46	13.74	0.29
40		8.41	8.42	0.01	11.15	11.32	0.18	13.88	14.23	0.35

Centile Comparison - Excluding Zambia									
GA	Pooled 2.5th	Excl 2.5th	Diff 2.5th	Pooled 50th	Excl 50th	Diff 50th	Pooled 97.5th	Excl 97.5th	Diff 97.5th
12	9.17	9.18	0.01	11.38	11.34	-0.05	13.59	13.49	-0.11
14	9	9	0	11.18	11.12	-0.06	13.36	13.25	-0.12
28	8.19	8.21	0.02	10.58	10.6	0.02	12.97	13	0.03
32	8.16	8.17	0.01	10.66	10.68	0.02	13.15	13.19	0.03
36	8.24	8.21	-0.03	10.85	10.83	-0.02	13.46	13.45	0
40	8.41	8.31	-0.11	11.15	11.05	-0.1	13.88	13.79	-0.09

Centile Comparison - Excluding India-CMC									
GA	Pooled 2.5th	Excl 2.5th	Diff 2.5th	Pooled 50th	Excl 50th	Diff 50th	Pooled 97.5th	Excl 97.5th	Diff 97.5th
12	9.17	9.09	-0.07	11.38	11.26	-0.12	13.59	13.43	-0.16
14	9	8.9	-0.1	11.18	11.05	-0.13	13.36	13.2	-0.17
28	8.19	8.02	-0.17	10.58	10.34	-0.24	12.97	12.67	-0.31
32	8.16	8.01	-0.15	10.66	10.4	-0.26	13.15	12.78	-0.37
36	8.24	8.11	-0.12	10.85	10.56	-0.29	13.46	13.01	-0.45
40	8.41	8.33	-0.08	11.15	10.83	-0.31	13.88	13.34	-0.54

Centile Comparison - Excluding India-SAS									
GA	Pooled 2.5th	Excl 2.5th	Diff 2.5th	Pooled 50th	Excl 50th	Diff 50th	Pooled 97.5th	Excl 97.5th	Diff 97.5th
12	9.17	8.99	-0.18	11.38	11.29	-0.09	13.59	13.59	0
14	9	8.85	-0.15	11.18	11.09	-0.1	13.36	13.32	-0.05
28	8.19	8.19	0	10.58	10.57	-0.01	12.97	12.94	-0.03
32	8.16	8.15	-0.01	10.66	10.63	-0.03	13.15	13.11	-0.04
36	8.24	8.17	-0.07	10.85	10.77	-0.08	13.46	13.37	-0.09
40	8.41	8.24	-0.17	11.15	10.96	-0.18	13.88	13.69	-0.19

Difference values are highlighted in **light green** (absolute value < 0.5) or **light red** (absolute value > 0.5) to indicate potential clinically meaningful variations in hemoglobin percentiles when excluding each site.

4.3.4 Assessing potential in-site differences in hemoglobin distributions

This analysis used the Wilcoxon Rank-Sum Test to compare the hemoglobin distribution of each individual site against the combined data from all other sites. For each test, one site was excluded from the dataset, and its hemoglobin values were compared to the pooled distribution of the remaining sites. These comparisons were performed across all trimesters pooled, as well as separately for each trimester, to identify whether any site shows significantly different hemoglobin distribution compared to the collective data from all other locations.

Wilcoxon Test for All Trimesters					
Site	Statistic	N.site.	N.others.	p.value	
Ghana	1648189.5	1365	3210	<0.001	
India-CMC	2408831	873	3702	<0.001	
India-SAS	1778454.5	770	3805	<0.001	
Kenya	1364694.5	1166	3409	<0.001	
Pakistan	330626	143	4432	0.377	
Zambia	602105.5	258	4317	0.028	

Wilcoxon Test for 1st Trimester					
Site	Statistic	N.site.	N.others.	p.value	
Ghana	41693	176	570	<0.001	
India-CMC	50617	115	631	<0.001	
India-SAS	63681	202	544	<0.001	
Kenya	35169.5	188	558	<0.001	
Pakistan	8444.5	24	722	0.833	
Zambia	17350	41	705	0.031	

Wilcoxon Test for 2nd Trimester					
Site	Statistic	N.site.	N.others.	p.value	
Ghana	341690.5	605	1471	<0.001	
India-CMC	515260.5	410	1666	<0.001	
India-SAS	336415	332	1744	<0.001	
Kenya	293187.5	565	1511	<0.001	
Pakistan	60843	57	2019	0.459	
Zambia	118355.5	107	1969	0.031	

Wilcoxon Test for 3rd Trimester					
Site	Statistic	N.site.	N.others.	p.value	
Ghana	249563.5	583	1168	<0.001	
India-CMC	388461	348	1403	<0.001	
India-SAS	211212.5	235	1516	<0.001	
Kenya	184726.5	413	1338	<0.001	
Pakistan	55260	62	1689	0.458	
Zambia	92411.5	110	1641	0.674	

A *p*-value < 0.05 indicates that the hemoglobin distribution at a given site is significantly different from the combined distribution of all other sites.

5 Fractional polynomial regression - Pakistan

Assuming we have normally distributed data and the heterogeneity of CBC-hemoglobin across sites cannot be ignored, we will do site specific analysis. We will use Pakistan data in this section and estimate the 2.5th, 5th, 10th, 50th, 95th and 97.5th centiles of hemoglobin at given gestational weeks during pregnancy and 6-week postpartum.

The FPR model for μ and σ are

$$\mu = 10.9536555 + 1.4449204 - 0.75360485(GA/10)^2 + 0.5105301(GA/10)^2 \log(GA/10)$$

and

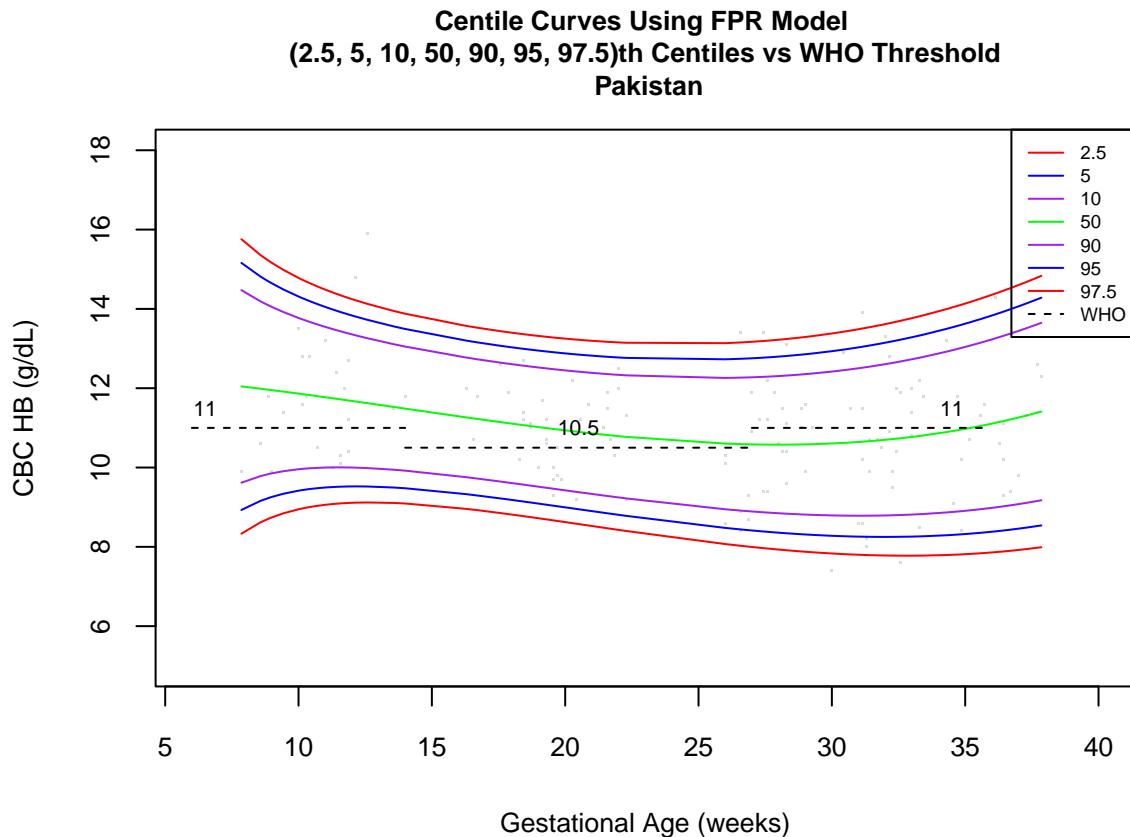
$$\log(\sigma) = 0.2368345 - 0.0465253 + 0.06017977(GA/10)^{-2} + 0.2584217(GA/10)^{-2} \log(GA/10)$$

respectively.

5.1 Centile plots of hemoglobin against gestational weeks

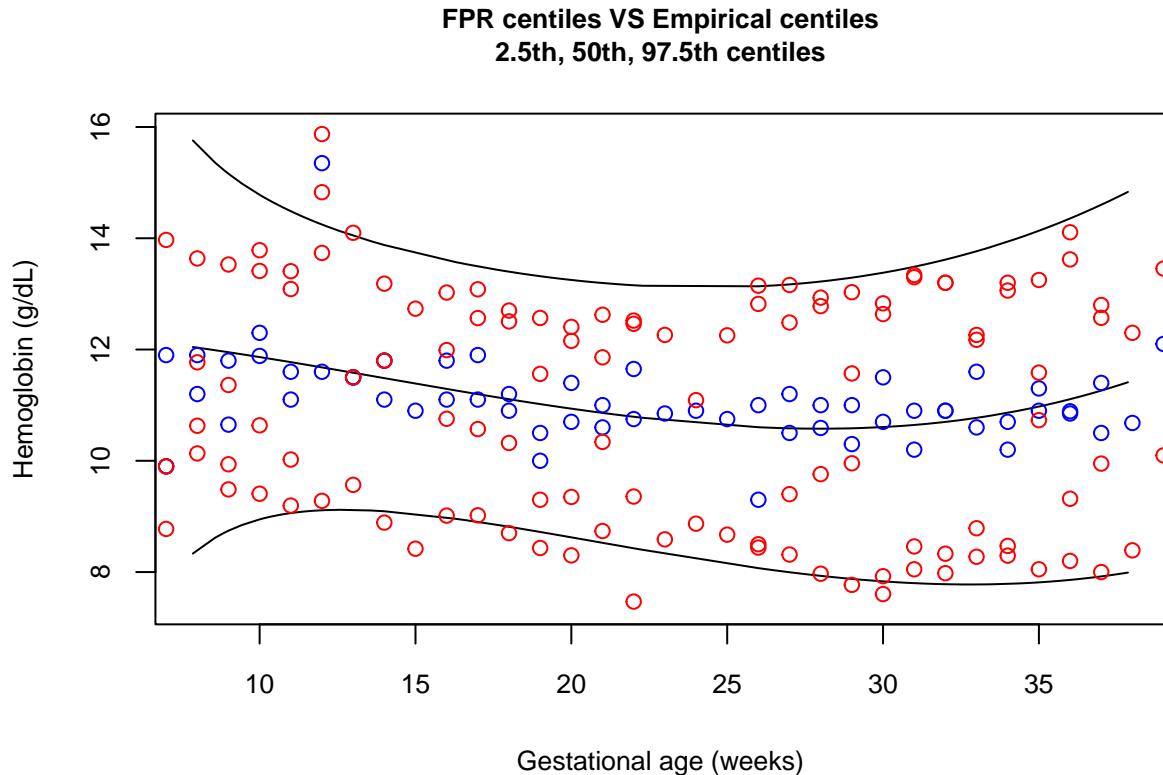
Given the fitted FPR model, we can plot the centile curves of hemoglobin against the gestational weeks. Also add the WHO thresholds to compare with.

5.1.1 Centile curves using FPR model (2.5, 5, 10, 50, 90, 95, 97.5)th centiles vs WHO threshold



5.1.2 Diagnostic plot

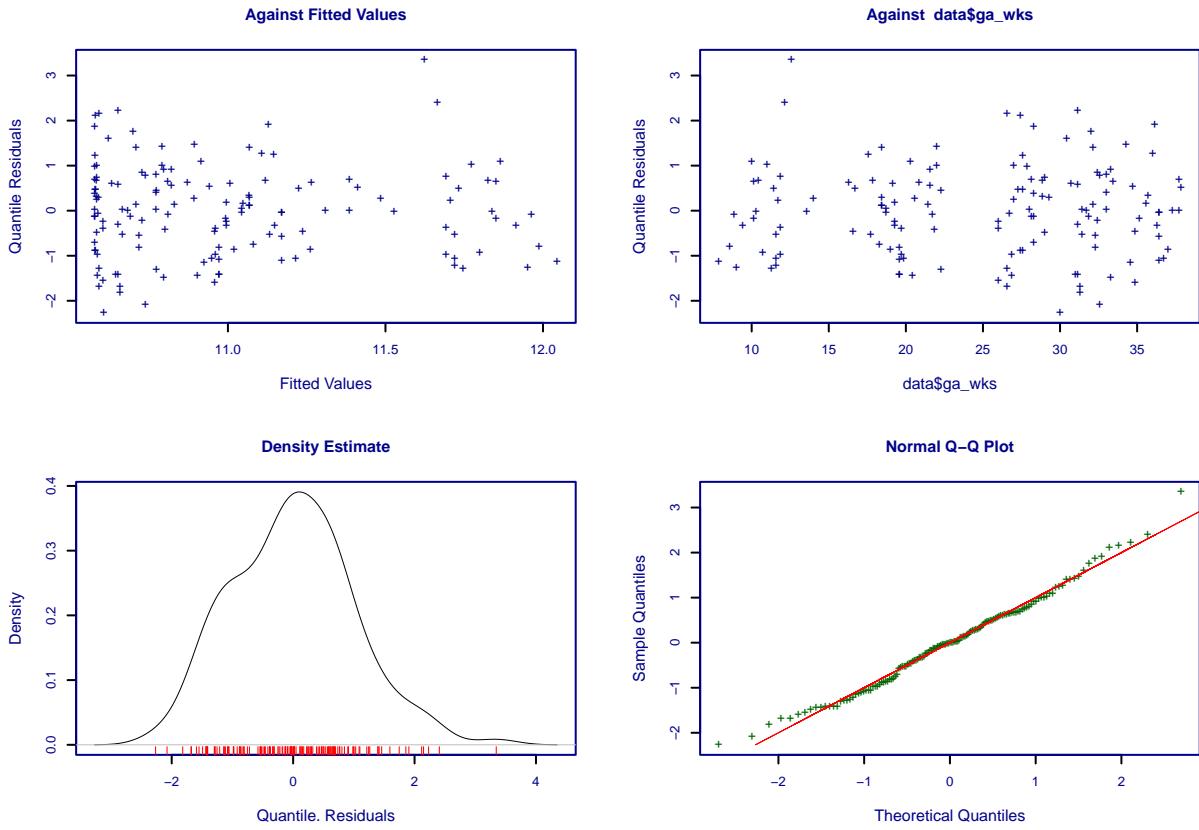
We use diagnostic plots to examine if the FPR model is a good fit to the hemoglobin centile curves.



In the $\hat{}$ FPR centiles VS Empirical centiles $\hat{}$ plot, we will observe if the FPR curves are consistent with empirical curves.

In addition, we use four plots of normalized quantile residuals to check the adequacy of the fitted fractional polynomial regression model. If the residuals display the behavior of following a standard normal distribution, the model is a good fit. The four plots are:

- top left: residuals against the fitted values of the mean parameter.
- top right: residuals against the specified covariate.
- bottom left: a kernel density estimate of the residuals.
- bottom right: a QQ-normal plot of the residuals.



The residuals behave well if the top two plots show a random scatter around the horizontal line at 0, and the kernel density estimate of the residuals is approximately normal and the normal QQ-plot is approximately linear.

5.2 Estimate the centiles at given gestational weeks

We estimate the 2.5th, 50th, and 97.5th centiles at 14, 20, 28, 32, 36 gestational weeks using predictions from the FPR curves. Also compare the predicted hemoglobin value with empirical percentile at each time point.

Table 8. FPR predicted centiles & Empirical percentile - Pakistan

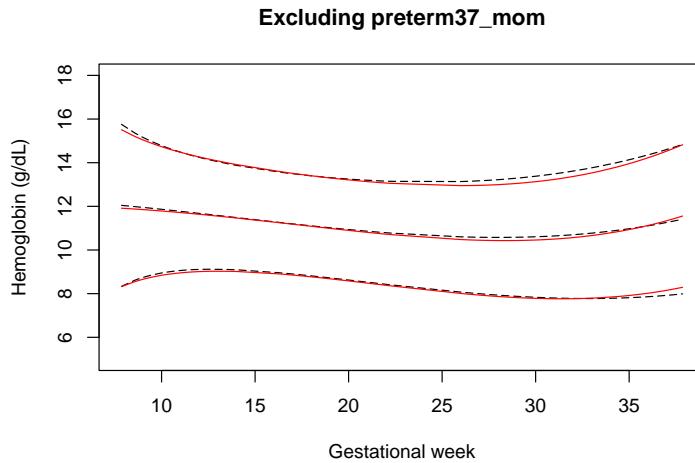
GA	FPR-2.5	Empir-2.5	FPR-50	Empir-50	FPR-97.5	Empir-97.5
12	9.111	14.828	11.679	15.35	14.247	15.873
14	9.093	11.8	11.485	11.8	13.878	11.8
20	8.624	9.35	10.937	11.4	13.25	12.155
28	7.932	9.76	10.577	11	13.222	12.78
32	7.782	7.98	10.699	10.9	13.616	13.2
36	7.859	9.318	11.107	10.85	14.356	14.108

5.3 Sensitivity analysis

5.3.1 Sensitivity analysis by excluding the subgroup of subjects with adverse outcomes.

Compare sample under each subsample (red line) with all enrolled (black line).

- Sensitivity analysis by excluding the subgroup of subjects with preterm delivery



Check whether the centile plots are sensitive to removing the group with preterm delivery.

6 Fractional polynomial regression - Kenya

Assuming we have normally distributed data and the heterogeneity of CBC-hemoglobin across sites cannot be ignored, we will do site specific analysis. We will use Kenya data in this section and estimate the 2.5th, 5th, 10th, 50th, 95th and 97.5th centiles of hemoglobin at given gestational weeks during pregnancy and 6-week postpartum.

The FPR model for μ and σ are

$$\mu = 10.4276666 + 2.0555622 - 1.66938838(GA/10) + 0.29597244(GA/10)^2$$

and

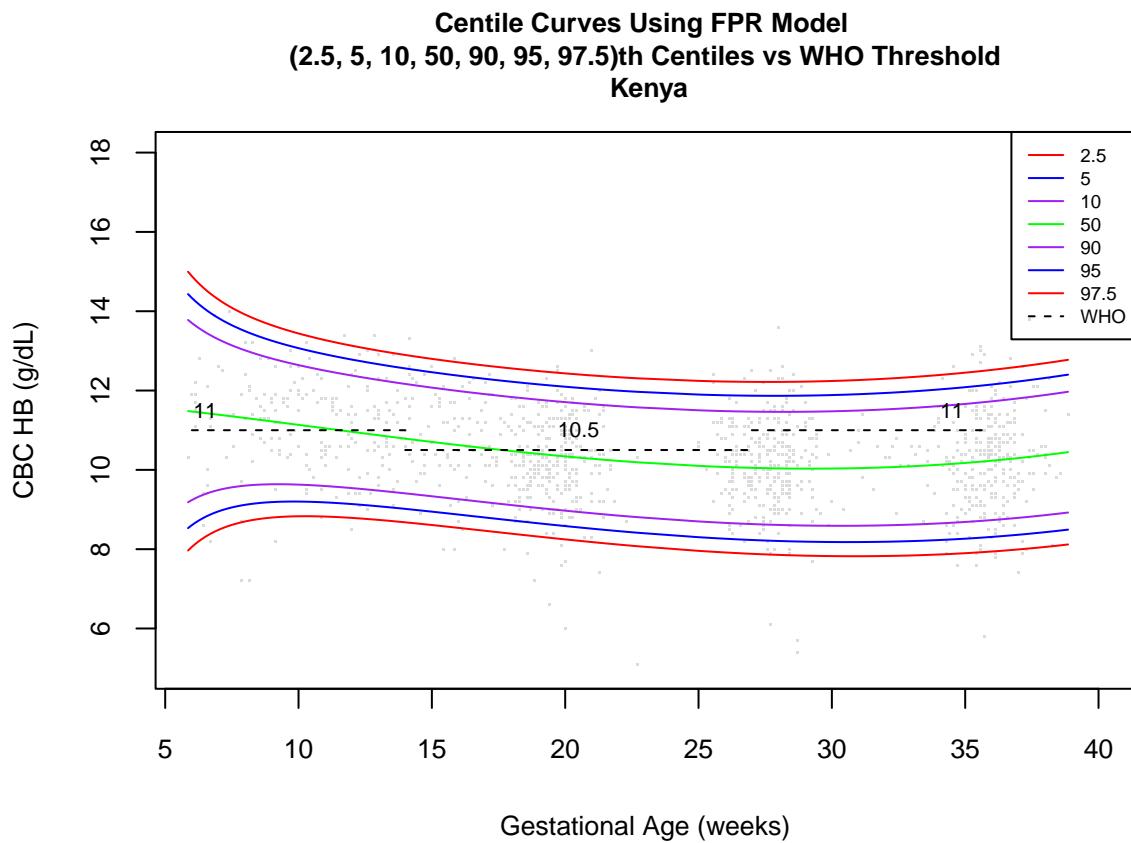
$$\sigma = 0.2085309 + 0.1891124 - 0.04334507(GA/10)^3 + 0.02963553(GA/10)^3 \log(GA/10)$$

respectively.

6.1 Centile plots of hemoglobin against gestational weeks

Given the fitted FPR model, we can plot the centile curves of hemoglobin against the gestational weeks. Also add the WHO thresholds to compare with.

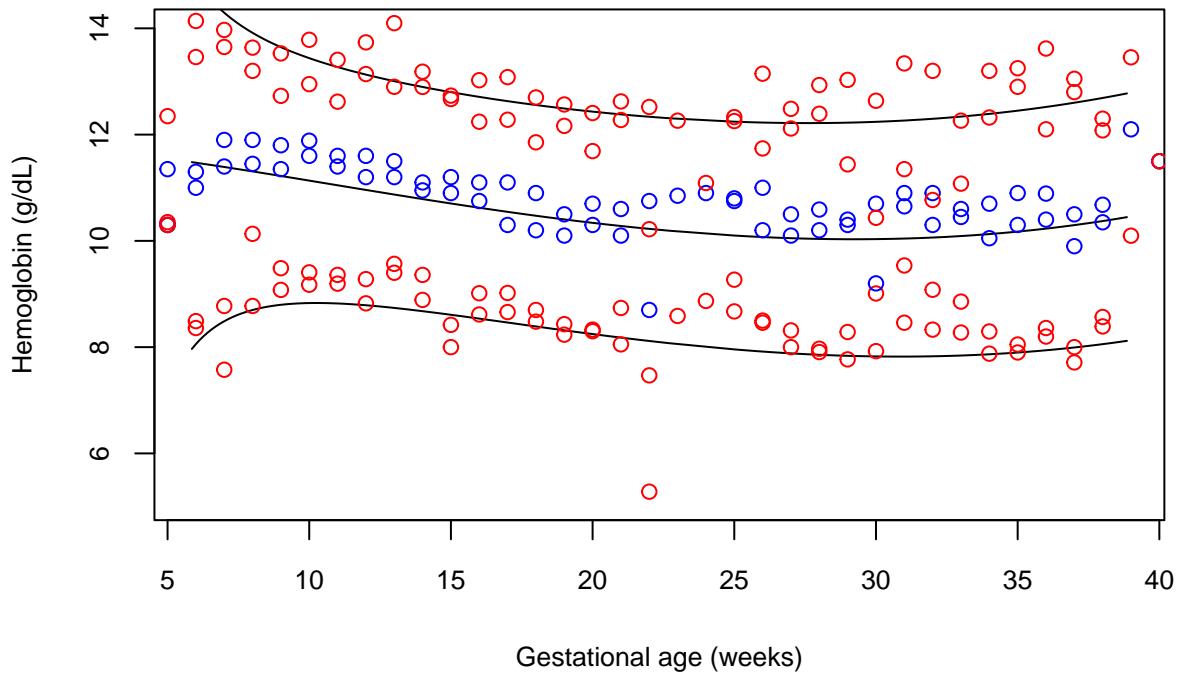
6.1.1 Centile curves using FPR model (2.5, 5, 10, 50, 90, 95, 97.5)th centiles vs WHO threshold



6.1.2 Diagnostic plot

We use diagnostic plots to examine if the FPR model is a good fit to the hemoglobin centile curves.

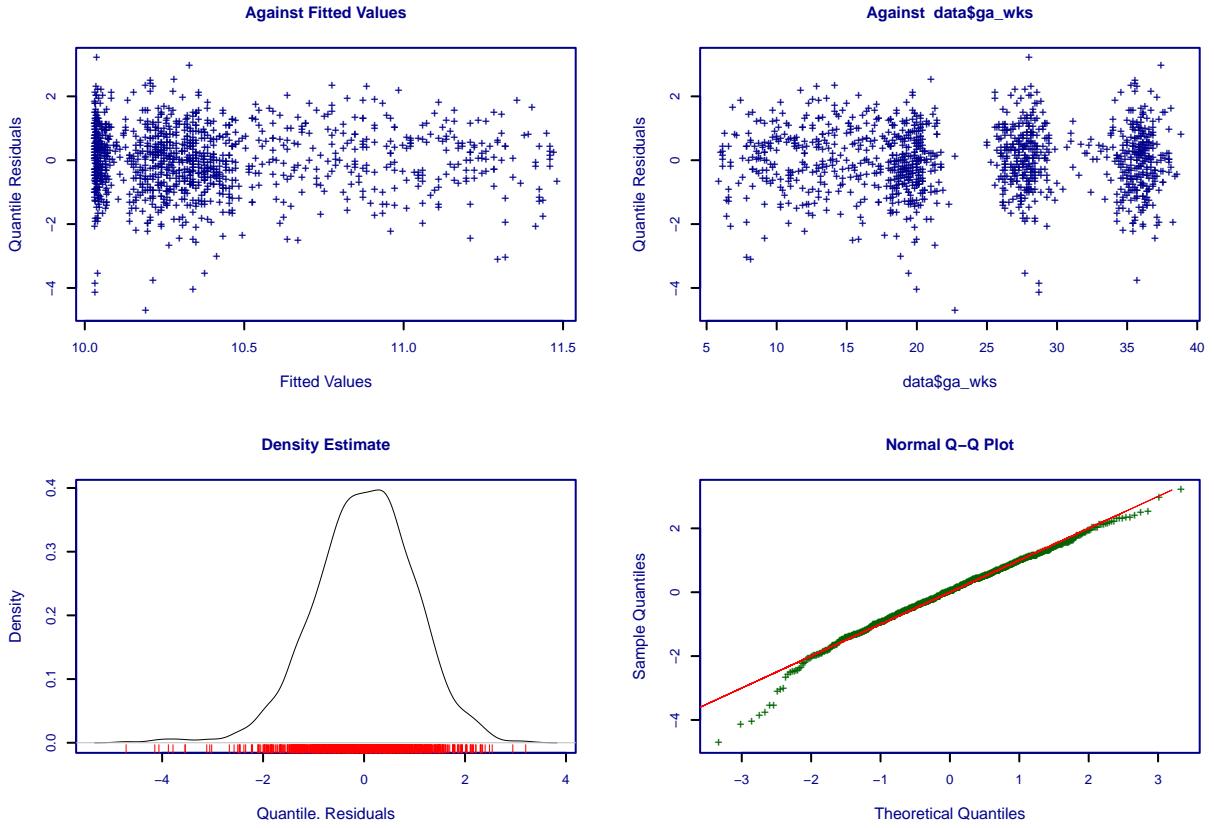
FPR centiles VS Empirical centiles
2.5th, 50th, 97.5th centiles



In the $\hat{}$ FPR centiles VS Empirical centiles $\hat{}$ plot, we will observe if the FPR curves are consistent with empirical curves.

In addition, we use four plots of normalized quantile residuals to check the adequacy of the fitted fractional polynomial regression model. If the residuals display the behavior of following a standard normal distribution, the model is a good fit. The four plots are:

- top left: residuals against the fitted values of the mean parameter.
- top right: residuals against the specified covariate.
- bottom left: a kernel density estimate of the residuals.
- bottom right: a QQ-normal plot of the residuals.



The residuals behave well if the top two plots show a random scatter around the horizontal line at 0, and the kernel density estimate of the residuals is approximately normal and the normal QQ-plot is approximately linear.

6.2 Estimate the centiles at given gestational weeks

We estimate the 2.5th, 50th, and 97.5th centiles at 14, 20, 28, 32, 36 gestational weeks using predictions from the FPR curves. Also compare the predicted hemoglobin value with empirical percentile at each time point.

Table 9. FPR predicted centiles & Empirical percentile - Kenya

GA	FPR-2.5	Empir-2.5	FPR-50	Empir-50	FPR-97.5	Empir-97.5
12	8.789	8.825	10.959	11.2	13.13	13.14
14	8.677	9.36	10.787	10.95	12.897	12.898
20	8.246	8.33	10.34	10.3	12.433	11.69
28	7.856	7.907	10.037	10.2	12.218	12.392
32	7.828	9.08	10.063	10.3	12.298	10.77
36	7.941	8.36	10.231	10.4	12.52	12.1

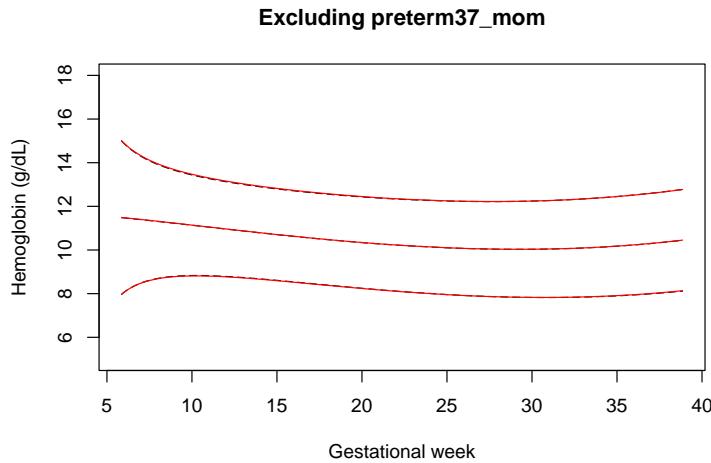
Note: No 32 gestational week data for Kenya. ANC-32 visit happened at or after 33 gestational week.

6.3 Sensitivity analysis

6.3.1 Sensitivity analysis by excluding the subgroup of subjects with adverse outcomes.

Compare sample under each subsample (red line) with all enrolled (black line).

- Sensitivity analysis by excluding the subgroup of subjects with preterm delivery



Check whether the centile plots are sensitive to removing the group with preterm delivery.

7 Fractional polynomial regression - Ghana

Assuming we have normally distributed data and the heterogeneity of CBC-hemoglobin across sites cannot be ignored, we will do site specific analysis. We will use Ghana data in this section and estimate the 2.5th, 5th, 10th, 50th, 95th and 97.5th centiles of hemoglobin at given gestational weeks during pregnancy and 6-week postpartum.

The FPR model for μ and σ are

$$\mu = 10.4470733 + 6.9103503 - 8.5317526(GA/10)^{0.5} + 2.5440287(GA/10)$$

and

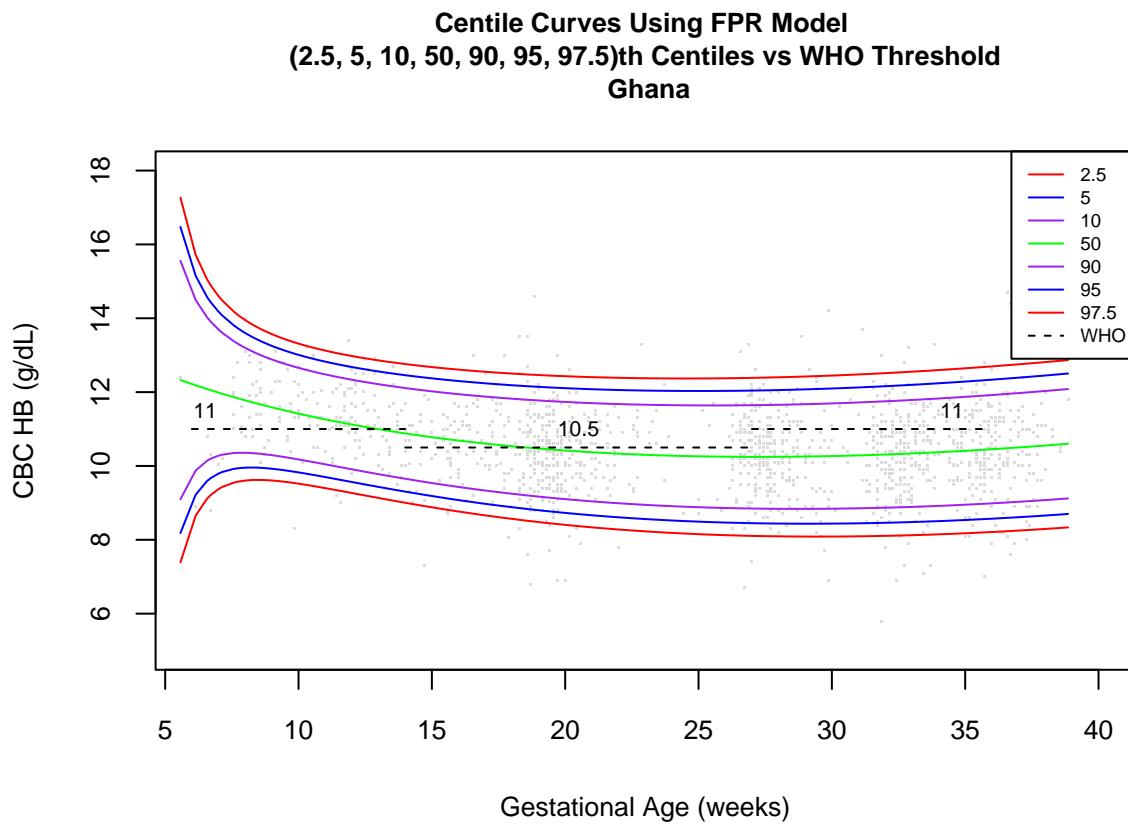
$$\log(\sigma) = 0.1404536 + 0.1794717 - 0.3228972(GA/10)^{-2} - 0.7546436(GA/10)^{-2}\log(GA/10)$$

respectively.

7.1 Centile plots of hemoglobin against gestational weeks

Given the fitted FPR model, we can plot the centile curves of hemoglobin against the gestational weeks. Also add the WHO thresholds to compare with.

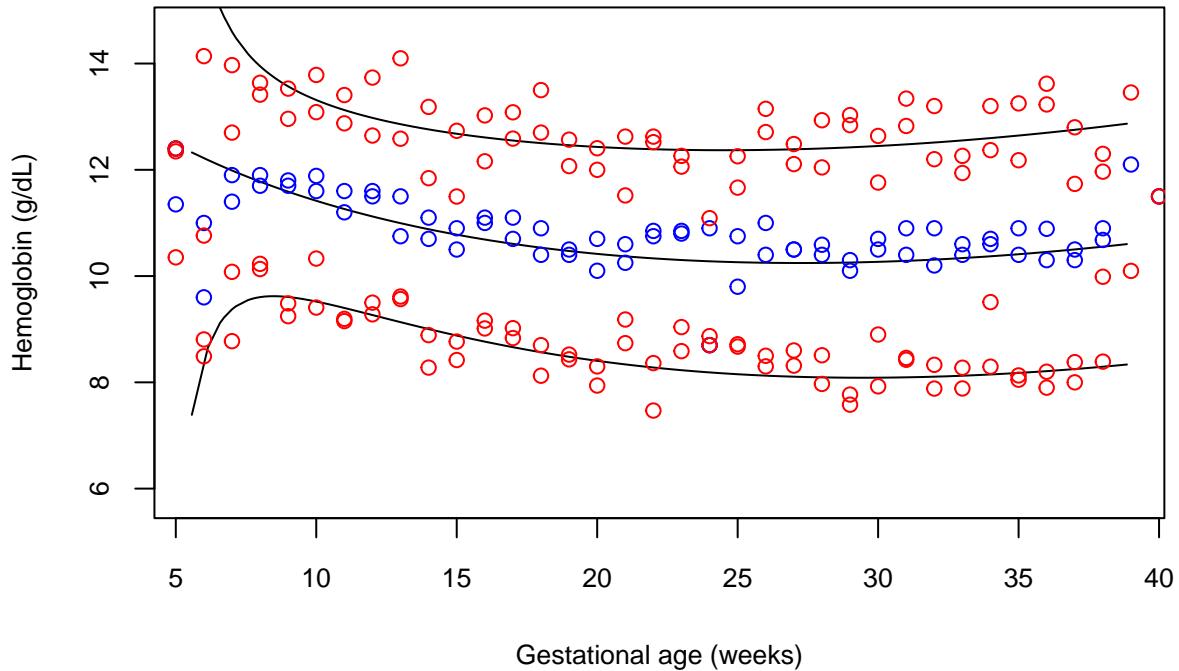
7.1.1 Centile curves using FPR model (2.5, 5, 10, 50, 90, 95, 97.5)th centiles vs WHO threshold



7.1.2 Diagnostic plot

We use diagnostic plots to examine if the FPR model is a good fit to the hemoglobin centile curves.

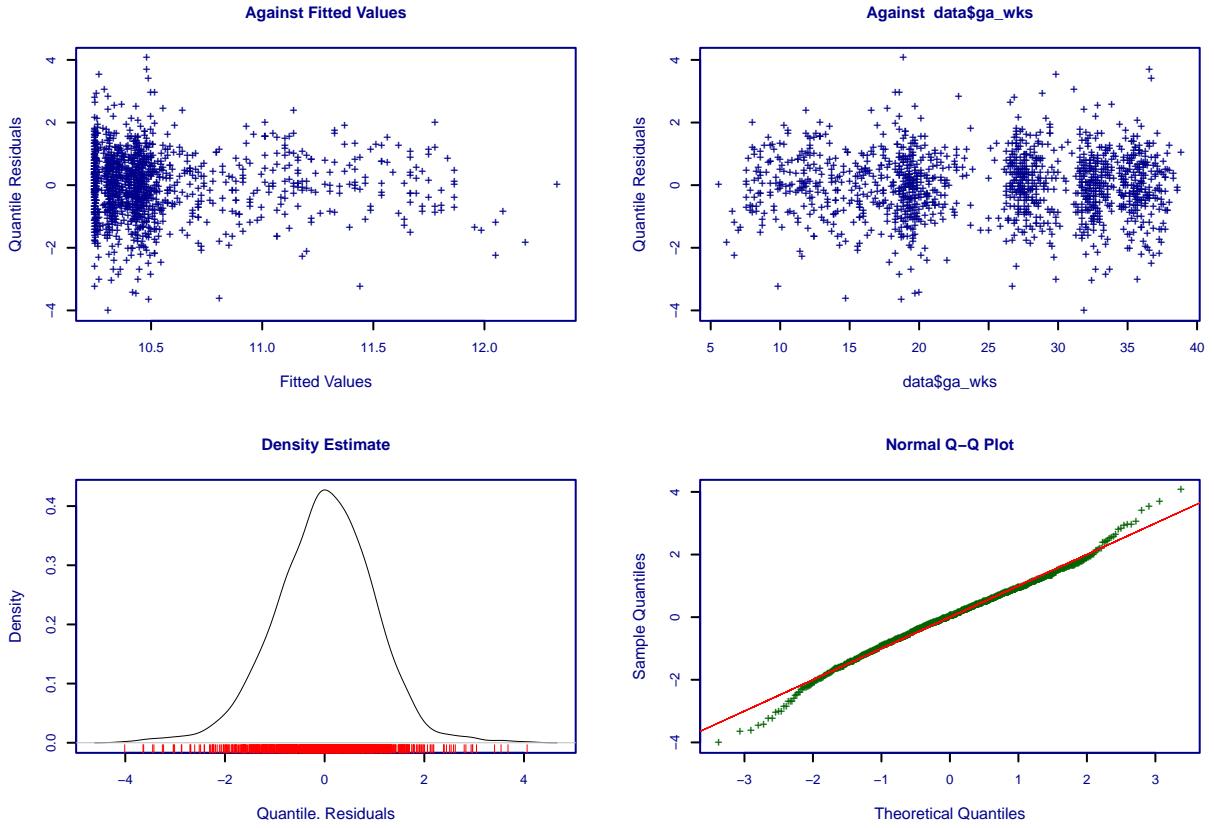
FPR centiles VS Empirical centiles
2.5th, 50th, 97.5th centiles



In the $\hat{}$ FPR centiles VS Empirical centiles $\hat{}$ plot, we will observe if the FPR curves are consistent with empirical curves.

In addition, we use four plots of normalized quantile residuals to check the adequacy of the fitted fractional polynomial regression model. If the residuals display the behavior of following a standard normal distribution, the model is a good fit. The four plots are:

- top left: residuals against the fitted values of the mean parameter.
- top right: residuals against the specified covariate.
- bottom left: a kernel density estimate of the residuals.
- bottom right: a QQ-normal plot of the residuals.



The residuals behave well if the top two plots show a random scatter around the horizontal line at 0, and the kernel density estimate of the residuals is approximately normal and the normal QQ-plot is approximately linear.

7.2 Estimate the centiles at given gestational weeks

We estimate the 2.5th, 50th, and 97.5th centiles at 14, 20, 28, 32, 36 gestational weeks using predictions from the FPR curves. Also compare the predicted hemoglobin value with empirical percentile at each time point.

Table 10. FPR predicted centiles & Empirical percentile - Ghana

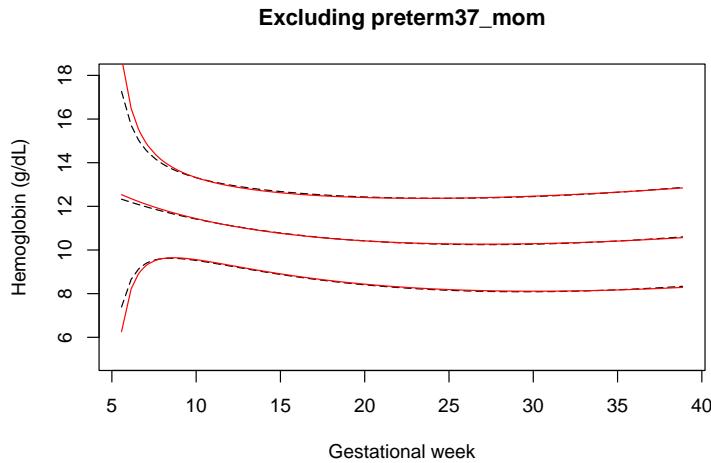
GA	FPR-2.5	Empir-2.5	FPR-50	Empir-50	FPR-97.5	Empir-97.5
12	9.267	9.5	11.122	11.5	12.977	12.645
14	9.003	8.278	10.882	10.7	12.76	11.843
20	8.405	7.94	10.418	10.1	12.431	12
28	8.094	8.51	10.248	10.4	12.402	12.045
32	8.107	7.883	10.31	10.2	12.514	12.2
36	8.211	7.9	10.454	10.3	12.697	13.23

7.3 Sensitivity analysis

7.3.1 Sensitivity analysis by excluding the subgroup of subjects with adverse outcomes.

Compare sample under each subsample (red line) with all enrolled (black line).

- Sensitivity analysis by excluding the subgroup of subjects with preterm delivery



Check whether the centile plots are sensitive to removing the group with preterm delivery.

8 Fractional polynomial regression - Zambia

Assuming we have normally distributed data and the heterogeneity of CBC-hemoglobin across sites cannot be ignored, we will do site specific analysis. We will use Zambia data in this section and estimate the 2.5th, 5th, 10th, 50th, 95th and 97.5th centiles of hemoglobin at given gestational weeks during pregnancy and 6-week postpartum.

The FPR model for μ and σ are

$$\mu = 11.1138539 + 1.1919510 - 0.6064498(GA/10)^2 + 0.3984395(GA/10)^2 \log(GA/10)$$

and

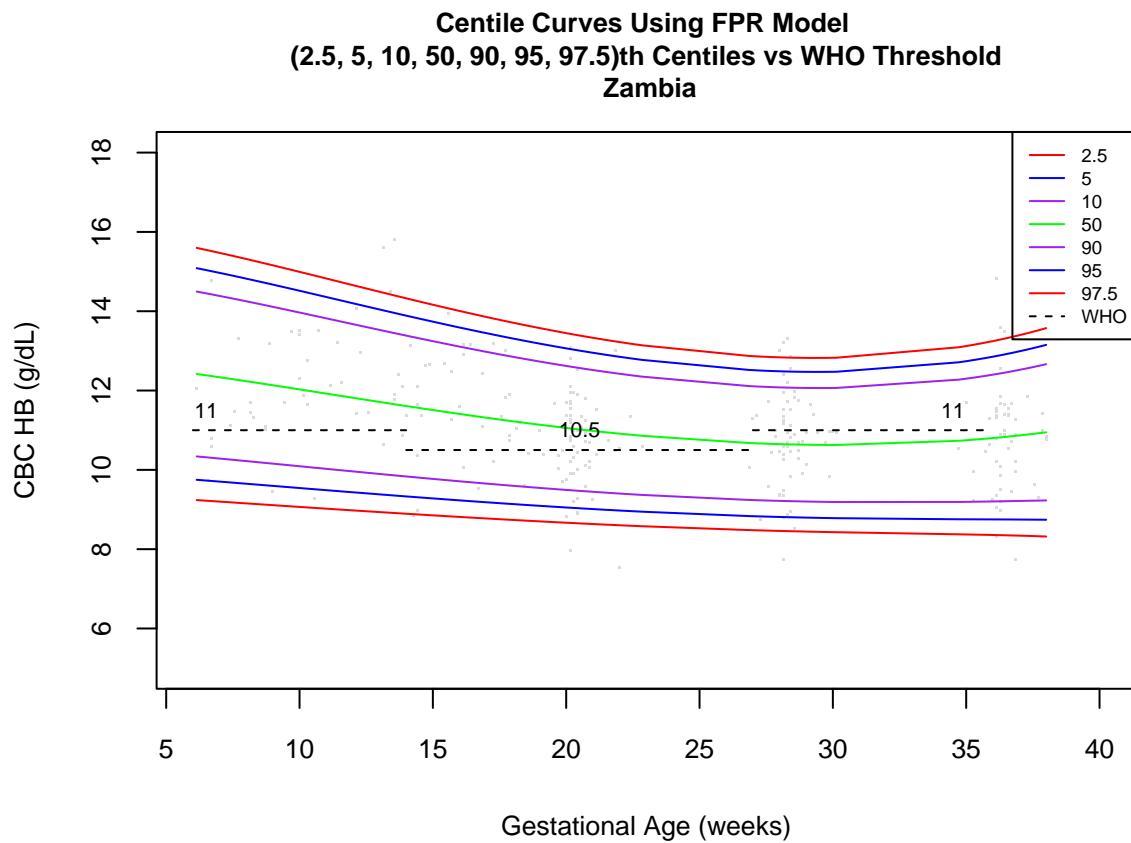
$$\log(\sigma) = 0.2936178 + 0.0175724 + 0.3880845(GA/10)^{-2} - 0.2931737(GA/10)^{-1}$$

respectively.

8.1 Centile plots of hemoglobin against gestational weeks

Given the fitted FPR model, we can plot the centile curves of hemoglobin against the gestational weeks. Also add the WHO thresholds to compare with.

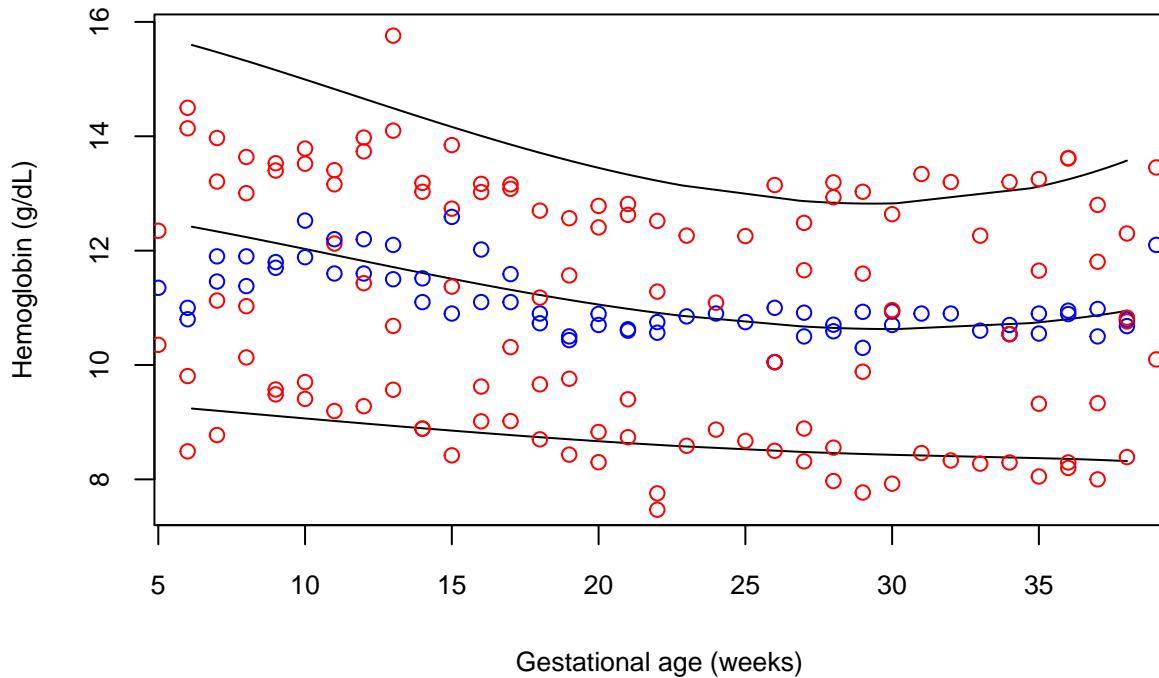
8.1.1 Centile curves using FPR model (2.5, 5, 10, 50, 90, 95, 97.5)th centiles vs WHO threshold



8.1.2 Diagnostic plot

We use diagnostic plots to examine if the FPR model is a good fit to the hemoglobin centile curves.

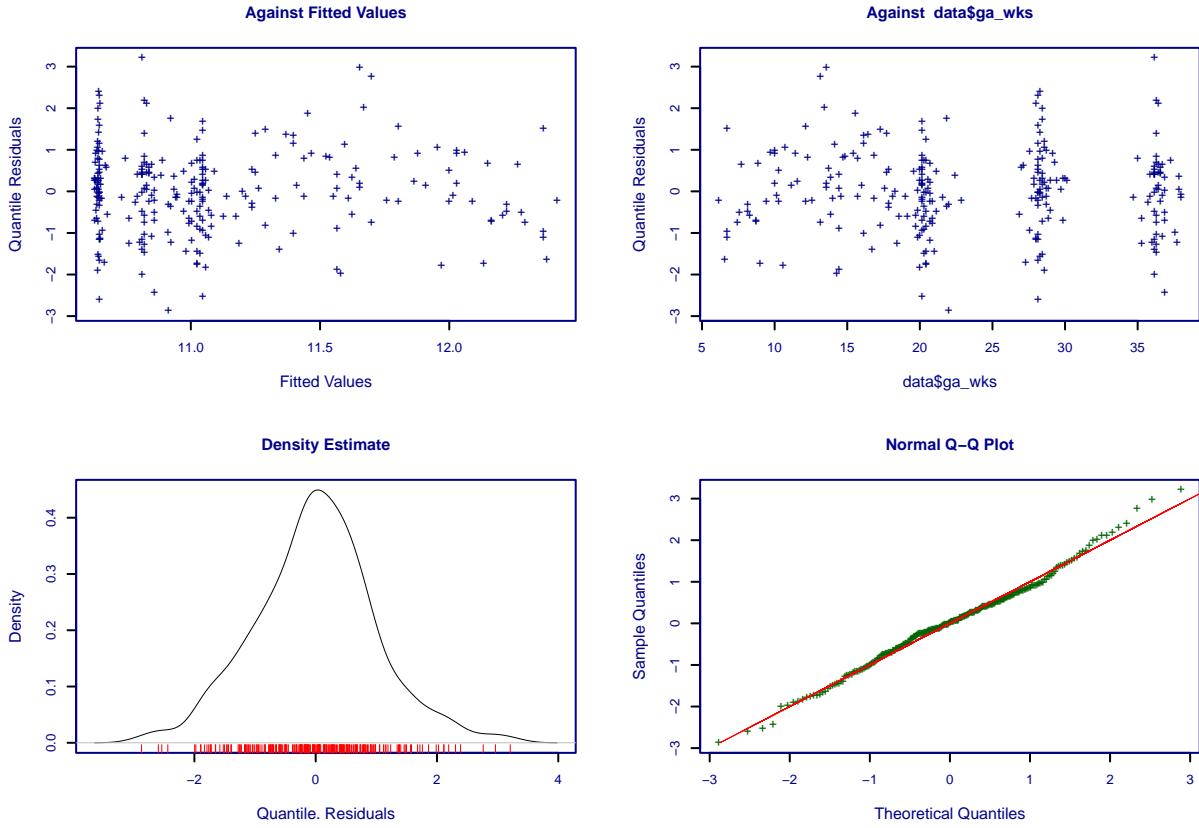
FPR centiles VS Empirical centiles
2.5th, 50th, 97.5th centiles



In the $\hat{}$ FPR centiles VS Empirical centiles $\hat{}$ plot, we will observe if the FPR curves are consistent with empirical curves.

In addition, we use four plots of normalized quantile residuals to check the adequacy of the fitted fractional polynomial regression model. If the residuals display the behavior of following a standard normal distribution, the model is a good fit. The four plots are:

- top left: residuals against the fitted values of the mean parameter.
- top right: residuals against the specified covariate.
- bottom left: a kernel density estimate of the residuals.
- bottom right: a QQ-normal plot of the residuals.



The residuals behave well if the top two plots show a random scatter around the horizontal line at 0, and the kernel density estimate of the residuals is approximately normal and the normal QQ-plot is approximately linear.

8.2 Estimate the centiles at given gestational weeks

We estimate the 2.5th, 50th, and 97.5th centiles at 14, 20, 28, 32, 36 gestational weeks using predictions from the FPR curves. Also compare the predicted hemoglobin value with empirical percentile at each time point.

Table 11. FPR predicted centiles & Empirical percentile - Zambia

GA	FPR-2.5	Empir-2.5	FPR-50	Empir-50	FPR-97.5	Empir-97.5
12	8.978	11.431	11.818	12.2	14.658	13.977
14	8.894	8.885	11.61	11.515	14.325	13.03
20	8.667	8.829	11.057	10.895	13.448	12.782
28	8.46	8.555	10.649	10.705	12.837	13.194
32	8.405		10.645		12.885	
36	8.358	8.295	10.802	10.95	13.246	13.611

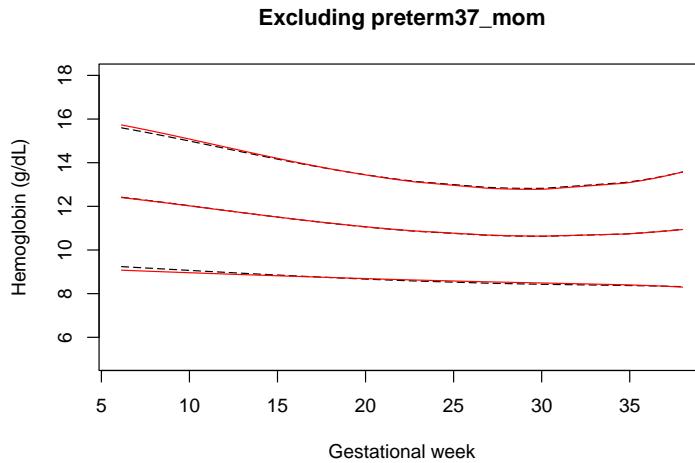
Note: No 32 gestational week data for Zambia. ANC-32 visit happened at or after 33 gestational week.

8.3 Sensitivity analysis

8.3.1 Sensitivity analysis by excluding the subgroup of subjects with adverse outcomes.

Compare sample under each subsample (red line) with all enrolled (black line).

- Sensitivity analysis by excluding the subgroup of subjects with preterm delivery



Check whether the centile plots are sensitive to removing the group with preterm delivery.

9 Fractional polynomial regression - India-CMC

Assuming we have normally distributed data and the heterogeneity of CBC-hemoglobin across sites cannot be ignored, we will do site specific analysis. We will use India-CMC data in this section and estimate the 2.5th, 5th, 10th, 50th, 95th and 97.5th centiles of hemoglobin at given gestational weeks during pregnancy and 6-week postpartum.

The FPR model for μ and σ are

$$\mu = 11.65454379 - 20.27017277 + 21.5406280(GA/10)^{-0.5} + 7.012179523\log(GA/10)$$

and

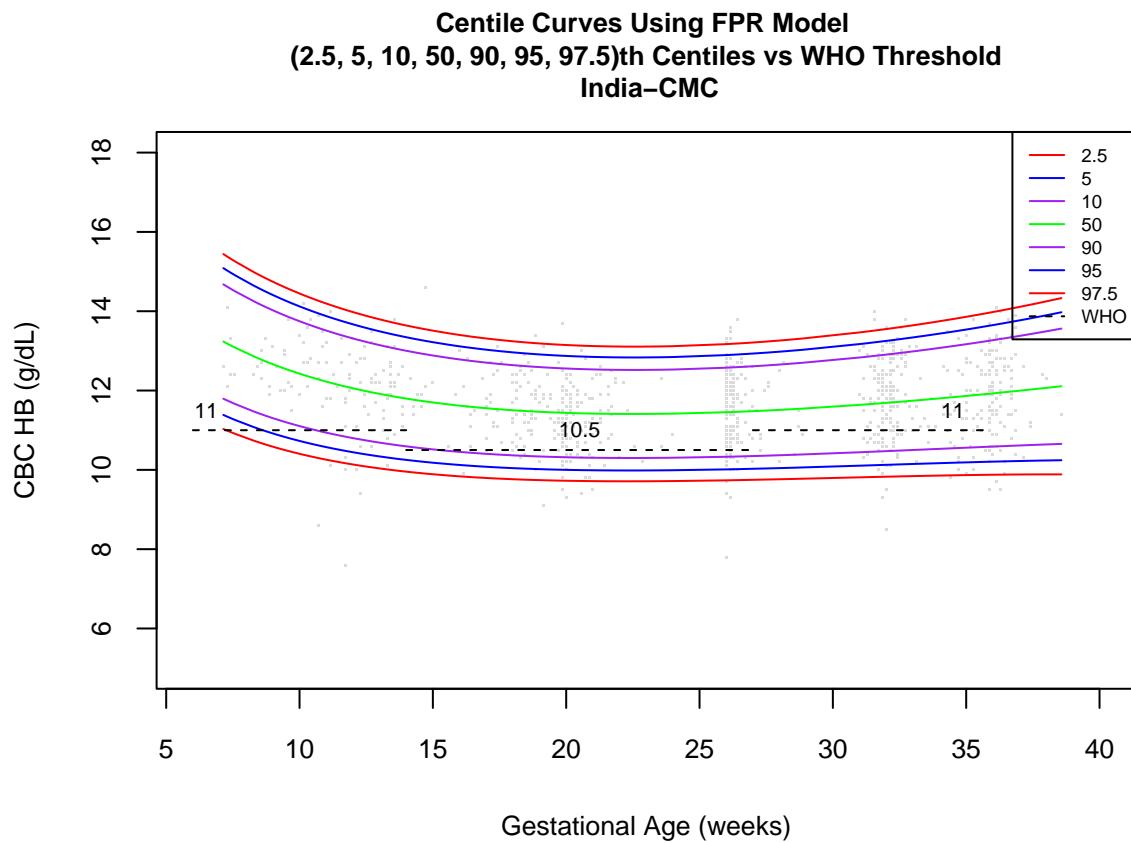
$$\log(\sigma) = -0.05134491 - 0.03422043 - 0.2371503^{-2} + 0.004719108(GA/10)^3$$

respectively.

9.1 Centile plots of hemoglobin against gestational weeks

Given the fitted FPR model, we can plot the centile curves of hemoglobin against the gestational weeks. Also add the WHO thresholds to compare with.

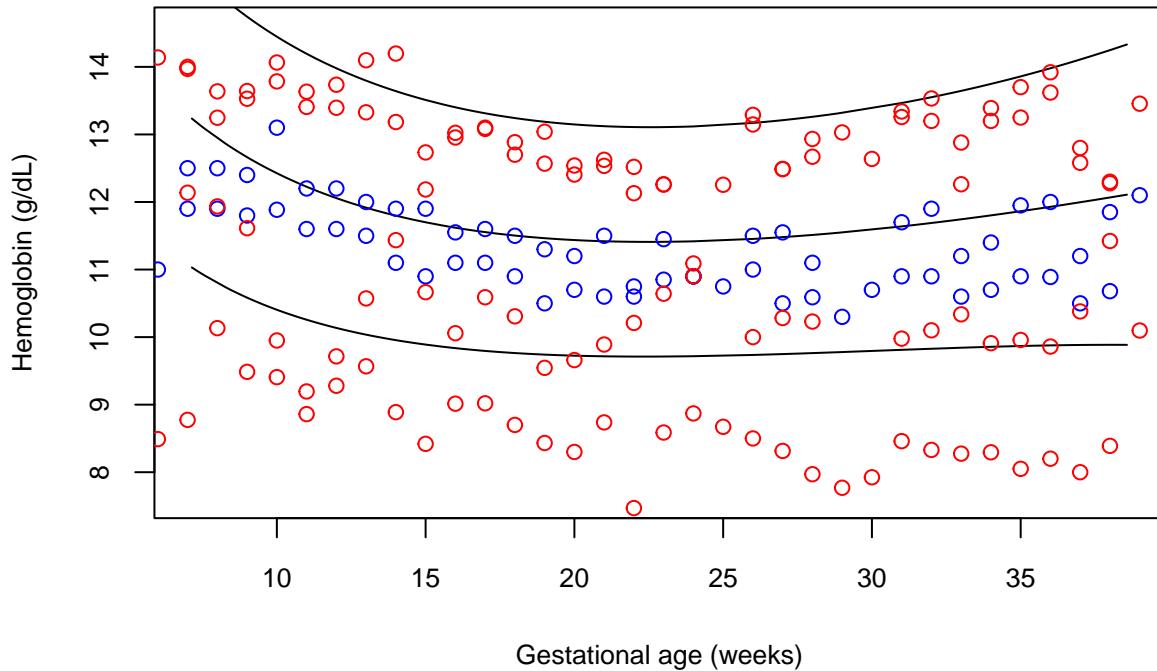
9.1.1 Centile curves using FPR model (2.5, 5, 10, 50, 90, 95, 97.5)th centiles vs WHO threshold



9.1.2 Diagnostic plot

We use diagnostic plots to examine if the FPR model is a good fit to the hemoglobin centile curves.

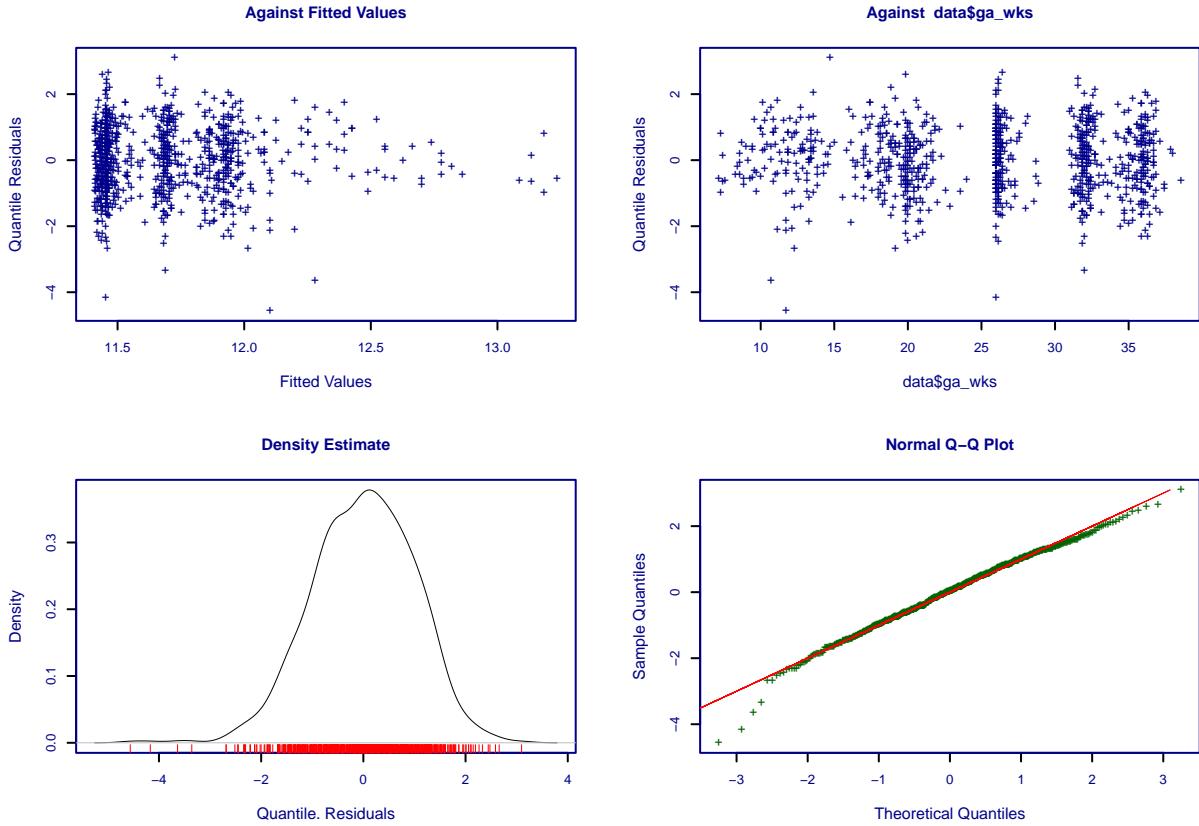
**FPR centiles VS Empirical centiles
2.5th, 50th, 97.5th centiles**



In the $\hat{}$ FPR centiles VS Empirical centiles $\hat{}$ plot, we will observe if the FPR curves are consistent with empirical curves.

In addition, we use four plots of normalized quantile residuals to check the adequacy of the fitted fractional polynomial regression model. If the residuals display the behavior of following a standard normal distribution, the model is a good fit. The four plots are:

- top left: residuals against the fitted values of the mean parameter.
- top right: residuals against the specified covariate.
- bottom left: a kernel density estimate of the residuals.
- bottom right: a QQ-normal plot of the residuals.



The residuals behave well if the top two plots show a random scatter around the horizontal line at 0, and the kernel density estimate of the residuals is approximately normal and the normal QQ-plot is approximately linear.

9.2 Estimate the centiles at given gestational weeks

We estimate the 2.5th, 50th, and 97.5th centiles at 14, 20, 28, 32, 36 gestational weeks using predictions from the FPR curves. Also compare the predicted hemoglobin value with empirical percentile at each time point.

Table 12. FPR predicted centiles & Empirical percentile - India-CMC

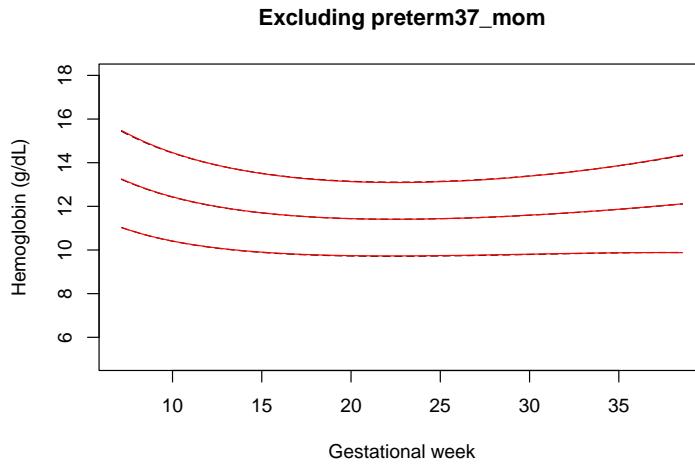
GA	FPR-2.5	Empir-2.5	FPR-50	Empir-50	FPR-97.5	Empir-97.5
12	10.136	9.715	12.058	12.2	13.979	13.395
14	9.955	11.435	11.798	11.9	13.641	14.198
20	9.725	9.66	11.437	11.2	13.148	12.54
28	9.763	10.23	11.512	11.1	13.261	12.67
32	9.827	10.1	11.689	11.9	13.551	13.532
36	9.877	9.86	11.928	12	13.98	13.92

9.3 Sensitivity analysis

9.3.1 Sensitivity analysis by excluding the subgroup of subjects with adverse outcomes.

Compare sample under each subsample (red line) with all enrolled (black line).

- Sensitivity analysis by excluding the subgroup of subjects with preterm delivery



Check whether the centile plots are sensitive to removing the group with preterm delivery.

10 Fractional polynomial regression - India-SAS

Assuming we have normally distributed data and the heterogeneity of CBC-hemoglobin across sites cannot be ignored, we will do site specific analysis. We will use India-SAS data in this section and estimate the 2.5th, 5th, 10th, 50th, 95th and 97.5th centiles of hemoglobin at given gestational weeks during pregnancy and 6-week postpartum.

The FPR model for μ and σ are

$$\mu = 11.0906273 - 1.7733238 + 2.4330598(GA/10)^{-2} + 5.5855978(GA/10)^{-2}\log(GA/10)$$

and

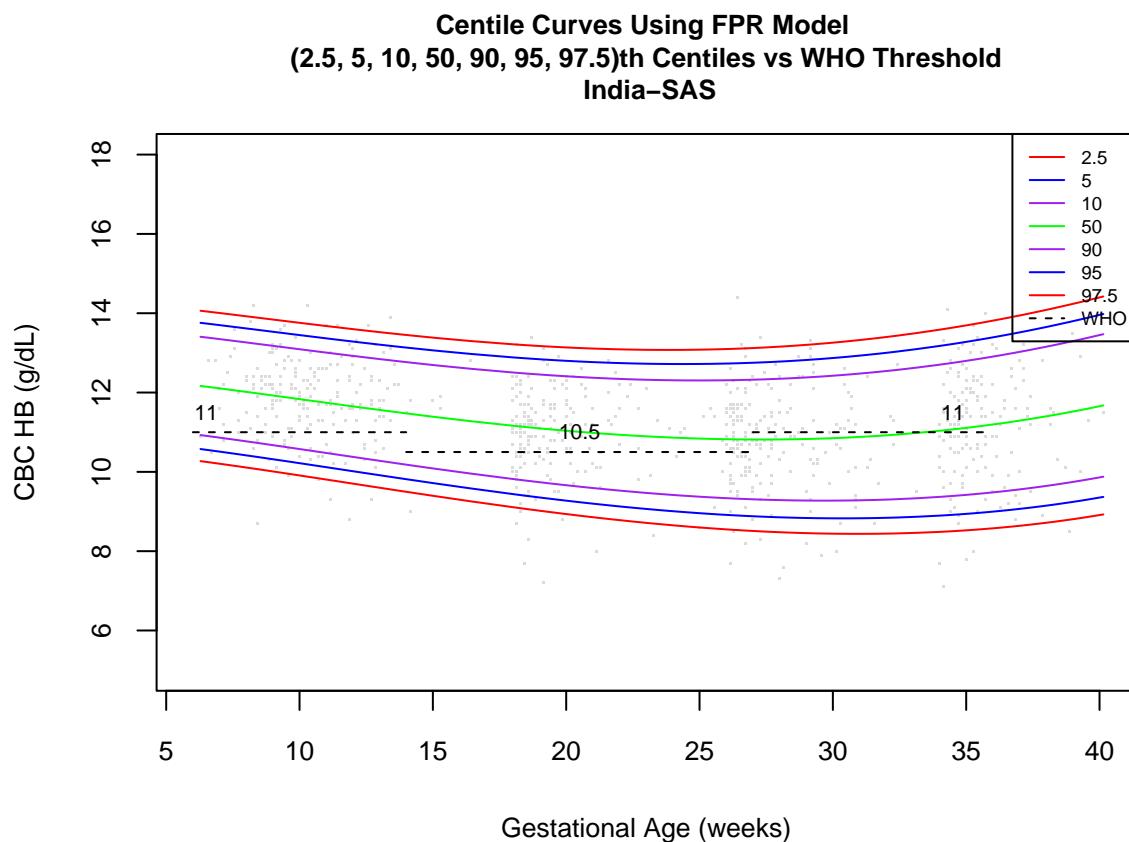
$$\log(\sigma) = 0.1552803 - 0.2291292 + 0.1377563(GA/10)^3 - 0.1276461(GA/10)^3\log(GA/10)$$

respectively.

10.1 Centile plots of hemoglobin against gestational weeks

Given the fitted FPR model, we can plot the centile curves of hemoglobin against the gestational weeks. Also add the WHO thresholds to compare with.

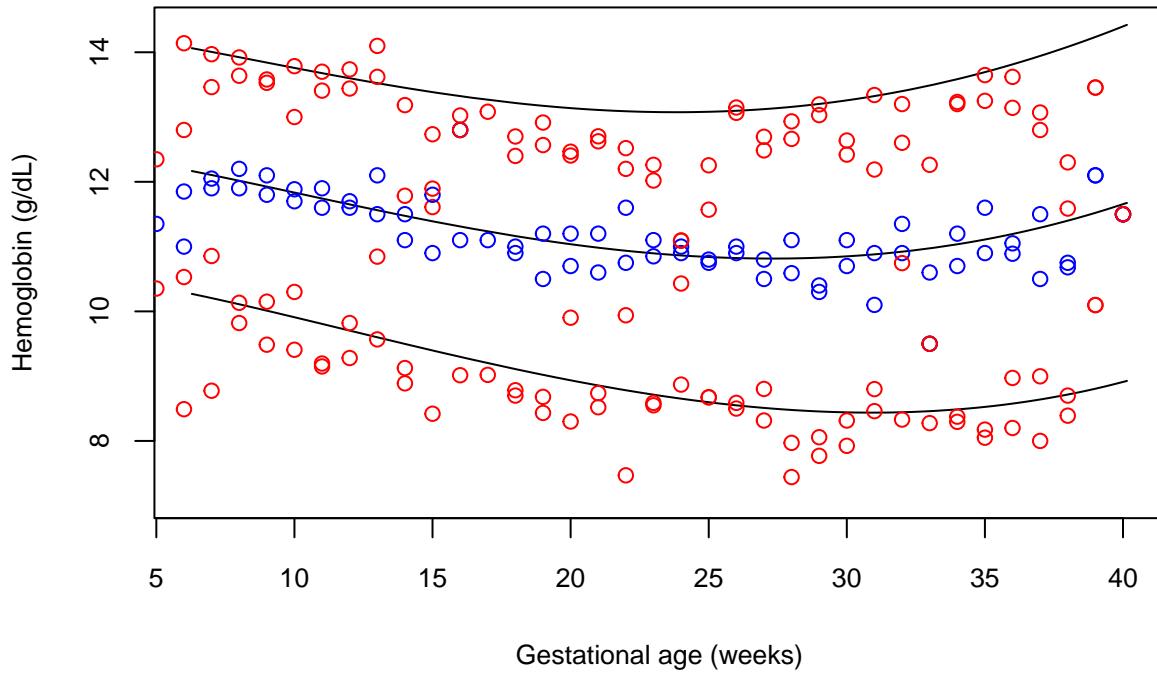
10.1.1 Centile curves using FPR model (2.5, 5, 10, 50, 90, 95, 97.5)th centiles vs WHO threshold



10.1.2 Diagnostic plot

We use diagnostic plots to examine if the FPR model is a good fit to the hemoglobin centile curves.

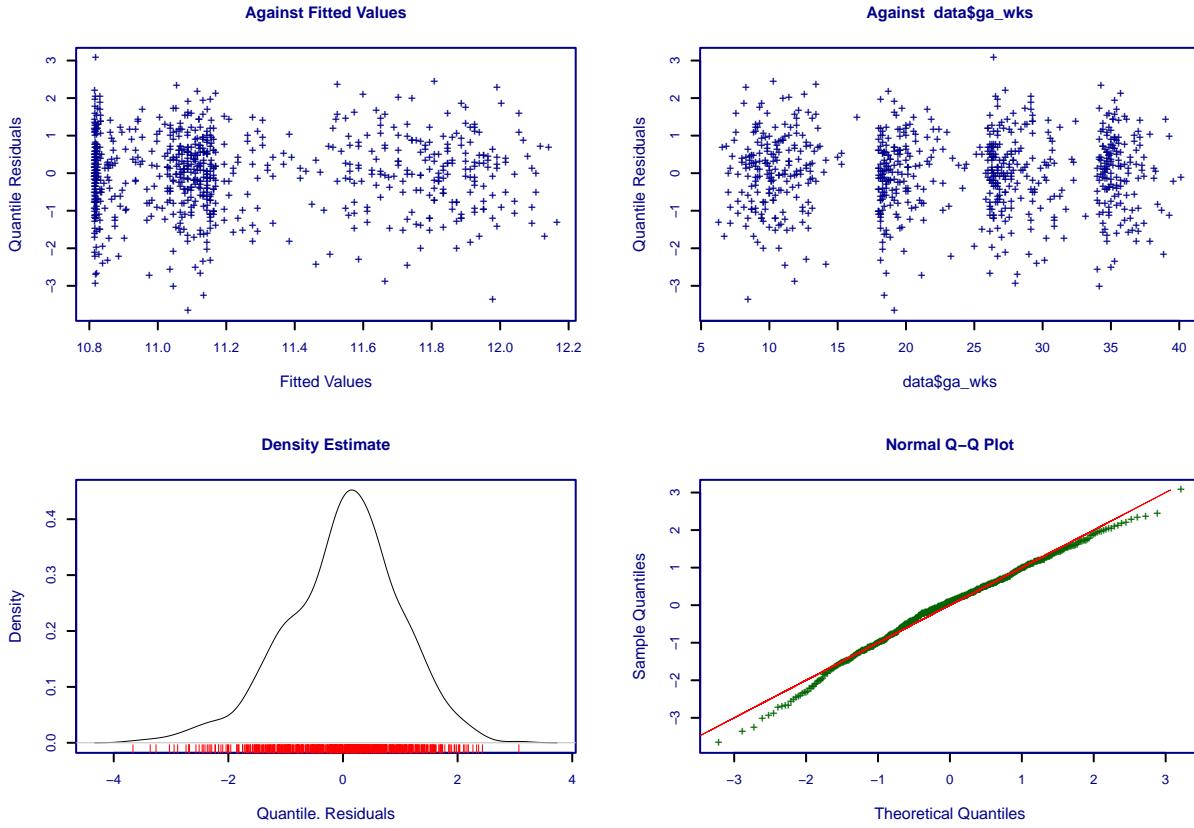
**FPR centiles VS Empirical centiles
2.5th, 50th, 97.5th centiles**



In the $\hat{}$ FPR centiles VS Empirical centiles $\hat{}$ plot, we will observe if the FPR curves are consistent with empirical curves.

In addition, we use four plots of normalized quantile residuals to check the adequacy of the fitted fractional polynomial regression model. If the residuals display the behavior of following a standard normal distribution, the model is a good fit. The four plots are:

- top left: residuals against the fitted values of the mean parameter.
- top right: residuals against the specified covariate.
- bottom left: a kernel density estimate of the residuals.
- bottom right: a QQ-normal plot of the residuals.



The residuals behave well if the top two plots show a random scatter around the horizontal line at 0, and the kernel density estimate of the residuals is approximately normal and the normal QQ-plot is approximately linear.

10.2 Estimate the centiles at given gestational weeks

We estimate the 2.5th, 50th, and 97.5th centiles at 14, 20, 28, 32, 36 gestational weeks using predictions from the FPR curves. Also compare the predicted hemoglobin value with empirical percentile at each time point.

Table 13. FPR predicted centiles & Empirical percentile - India-SAS

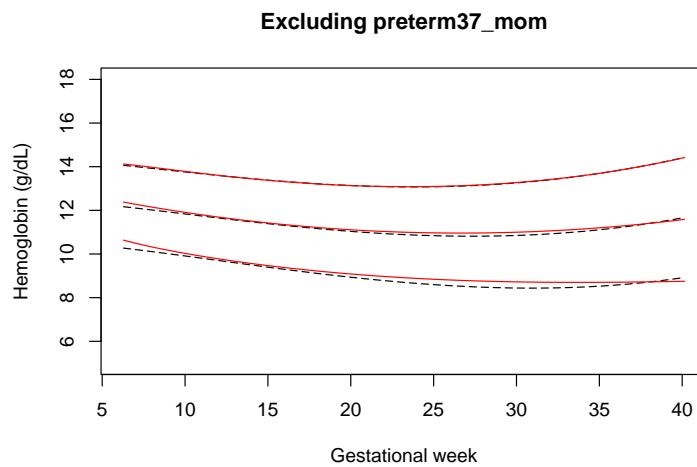
GA	FPR-2.5	Empir-2.5	FPR-50	Empir-50	FPR-97.5	Empir-97.5
12	9.703	9.82	11.651	11.7	13.599	13.44
14	9.498	9.125	11.475	11.5	13.452	11.785
20	8.936	9.902	11.037	11.2	13.138	12.465
28	8.478	7.442	10.818	11.1	13.157	12.662
32	8.443	10.745	10.921	11.35	13.4	12.602
36	8.576	8.972	11.195	11.05	13.813	13.142

10.3 Sensitivity analysis

10.3.1 Sensitivity analysis by excluding the subgroup of subjects with adverse outcomes.

Compare sample under each subsample (red line) with all enrolled (black line).

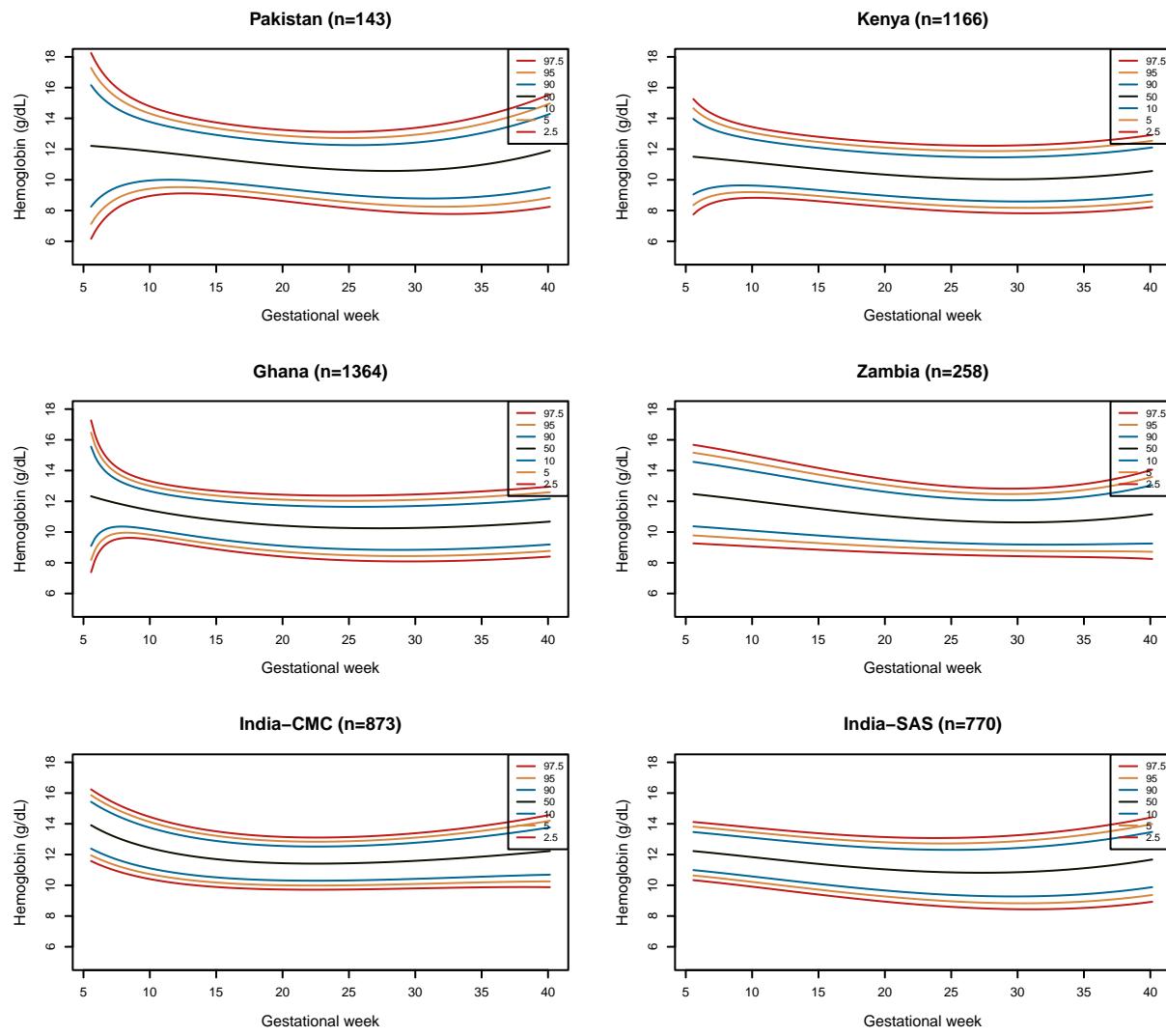
- Sensitivity analysis by excluding the subgroup of subjects with preterm delivery



Check whether the centile plots are sensitive to removing the group with preterm delivery.

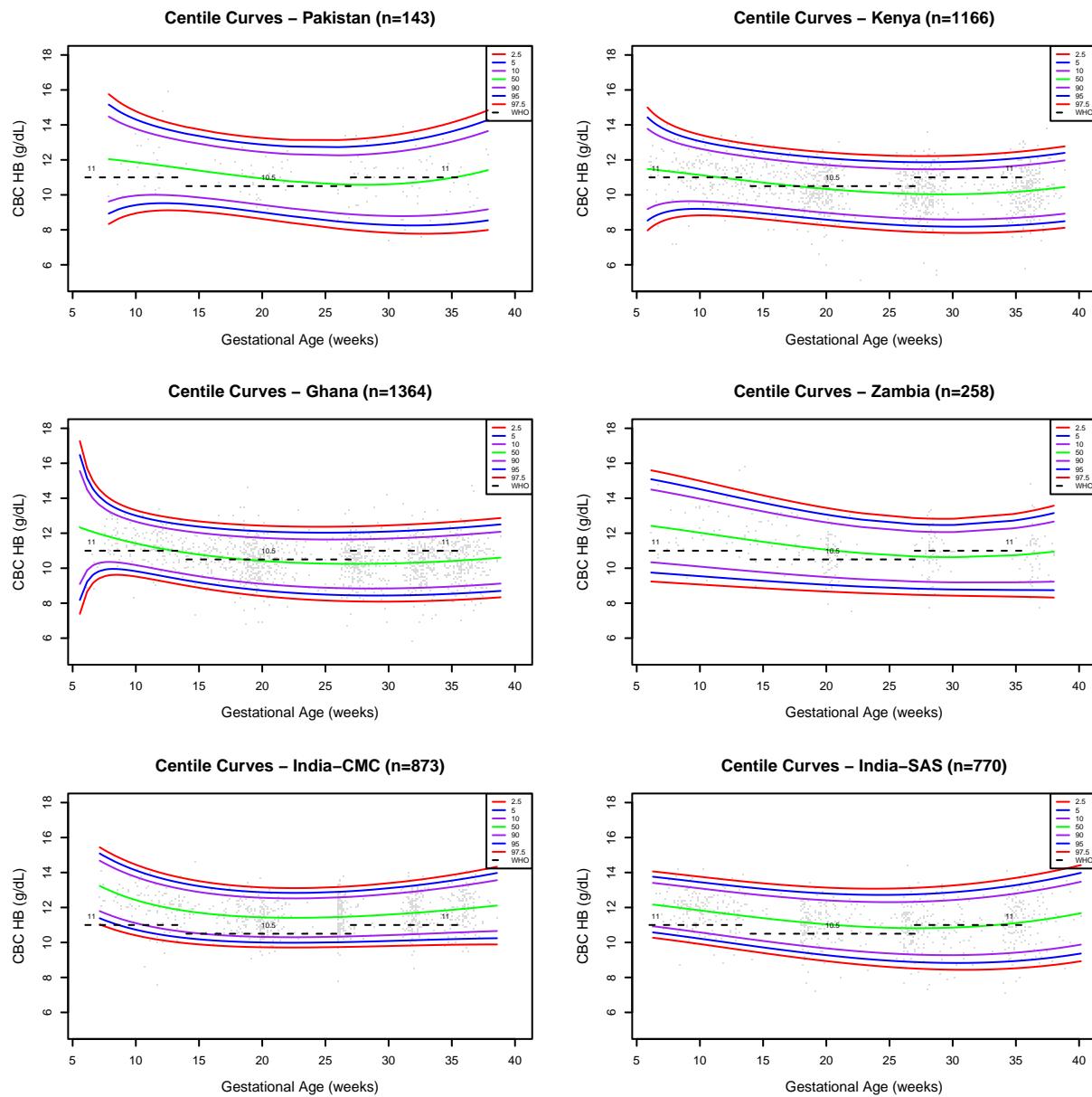
11 Predicted centile plots of hemoglobin against gestational weeks - site comparison

11.1 Site specific prediction of hemoglobin percentile



Note: n shown here is the sample size of data used to predict the centiles

12 Centile plots of hemoglobin against gestational weeks by site



13 Estimate centiles at given gestational weeks - site comparison

Table 14. FPR predicted centiles & Empirical percentile - Site Comparison

GA	FPR-2.5	Empir-2.5	FPR-50	Empir-50	FPR-97.5	Empir-97.5
Pakistan						
12	9.111	14.828	11.679	15.35	14.247	15.873
14	9.093	11.8	11.485	11.8	13.878	11.8
20	8.624	9.35	10.937	11.4	13.25	12.155
28	7.932	9.76	10.577	11	13.222	12.78
32	7.782	7.98	10.699	10.9	13.616	13.2
Kenya						
36	7.859	9.318	11.107	10.85	14.356	14.108
12	8.789	8.825	10.959	11.2	13.13	13.14
14	8.677	9.36	10.787	10.95	12.897	12.898
20	8.246	8.33	10.34	10.3	12.433	11.69
28	7.856	7.907	10.037	10.2	12.218	12.392
Ghana						
32	7.828	9.08	10.063	10.3	12.298	10.77
36	7.941	8.36	10.231	10.4	12.52	12.1
12	9.267	9.5	11.122	11.5	12.977	12.645
14	9.003	8.278	10.882	10.7	12.76	11.843
20	8.405	7.94	10.418	10.1	12.431	12
Zambia						
28	8.094	8.51	10.248	10.4	12.402	12.045
32	8.107	7.883	10.31	10.2	12.514	12.2
36	8.211	7.9	10.454	10.3	12.697	13.23
12	8.978	11.431	11.818	12.2	14.658	13.977
14	8.894	8.885	11.61	11.515	14.325	13.03
India-CMC						
20	8.667	8.829	11.057	10.895	13.448	12.782
28	8.46	8.555	10.649	10.705	12.837	13.194
32	8.405		10.645		12.885	
36	8.358	8.295	10.802	10.95	13.246	13.611
12	10.136	9.715	12.058	12.2	13.979	13.395
India-SAS						
14	9.955	11.435	11.798	11.9	13.641	14.198
20	9.725	9.66	11.437	11.2	13.148	12.54
28	9.763	10.23	11.512	11.1	13.261	12.67
32	9.827	10.1	11.689	11.9	13.551	13.532
36	9.877	9.86	11.928	12	13.98	13.92
12	9.703	9.82	11.651	11.7	13.599	13.44
14	9.498	9.125	11.475 51	11.5	13.452	11.785
20	8.936	9.902	11.037	11.2	13.138	12.465
28	8.478	7.442	10.818	11.1	13.157	12.662
32	8.443	10.745	10.921	11.35	13.4	12.602