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Original Article
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Association of haemoglobin levels in the first trimester and at 26–30 weeks with fetal and neonatal outcomes: a secondary analysis of the Global Network for Women's and Children's Health's ASPIRIN Trial

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Objective Limited data are available from low- and middle-income countries (LMICs) on the relationship of haemoglobin levels to adverse outcomes at different times during pregnancy. We evaluated the association of haemoglobin levels in nulliparous women at two times in pregnancy with pregnancy outcomes.

Design ASPIRIN Trial data were used to study the association between haemoglobin levels measured at 6^{+0}–13^{+6} weeks and 26^{+0}–30^{+0} weeks of gestation with fetal and neonatal outcomes.

Setting Obstetric care facilities in Pakistan, India, Kenya, Zambia, The Democratic Republic of the Congo and Guatemala.

Population A total of 11 976 pregnant women.

Methods Generalised linear models were used to obtain adjusted relative risks and 95% CI for adverse outcomes.

Main outcome measures Preterm birth, stillbirth, neonatal death, small for gestational age (SGA) and birthweight <2500 g.

Results The mean haemoglobin levels at 6^{+0}–13^{+6} weeks and at 26–30 weeks of gestation were 116 g/l (SD 17) and 107 g/l (SD 15), respectively. In general, pregnancy outcomes were better with increasing haemoglobin. At 6^{+0}–13^{+6} weeks of gestation, stillbirth, SGA and birthweight <2500 g, were significantly associated with haemoglobin of 70–89 g/l compared with haemoglobin of 110–129 g/l The relationships of adverse pregnancy outcomes with various haemoglobin levels were more marked at 26–30 weeks of gestation.

Conclusions Both lower and some higher haemoglobin concentrations are associated with adverse fetal and neonatal outcomes at 6^{+0}–13^{+6} weeks and at 26–30 weeks of gestation, although the relationship with low haemoglobin levels appears more consistent and generally stronger.

Keywords Haemoglobin levels, low- and middle-income countries, pregnancy outcomes.

Tweetable abstract Both lower and some higher haemoglobin concentrations were associated with adverse fetal and neonatal outcomes at 6–13 weeks and 26–30 weeks of gestation.

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Introduction

Anaemia in pregnancy is a common and important health concern around the world. Globally, anaemia, usually defined as haemoglobin <110 g/l, affects about 56 million pregnant women each year with a worldwide prevalence of about 40% (95% CI 36.4–44.6%)[1] ranging from about 24% in high-income countries to 43% in low-income countries.[2] Anaemia is extremely common in south Asia, where the prevalence approximates 88% and nearly half of pregnant women are moderately to severely anaemic, generally defined as having a haemoglobin <90 g/l.[3] Maternal anaemia is one of the leading risk factors for adverse pregnancy outcomes including stillbirth, preterm birth, low birthweight, pre-eclampsia, eclampsia, neurodevelopmental delay and maternal death.[4,5] However, a causal relationship of anaemia with adverse pregnancy outcomes remains inconclusive and some studies have failed to show any association.[6–8] Studies have also investigated whether when anaemia appears in the pregnancy is associated with adverse outcomes. For example, some studies found a significant association of first-trimester anaemia with adverse pregnancy outcomes,[9] whereas others found significant associations only in the second or third trimesters.[8,10] There is reasonable agreement that the severity of anaemia, especially when moderate or severe, is most strongly related to adverse outcomes.

Increased haemoglobin concentrations (>130 g/l) during pregnancy have also been associated with preterm and small-for-gestational age (SGA) births,[11,12] showing a U-shaped relationship with adverse outcomes at the extremes of haemoglobin levels.[12–14] Lower and higher haemoglobin appear to have different associations with pregnancy outcomes. For example, increased risk of preterm birth has been observed in anaemic women whereas SGA has been described in women with higher haemoglobin levels.[11]

Limited data are available from low- and middle-income countries (LMICs) about the association of poor pregnancy outcomes with different levels of haemoglobin and the duration of gestation. In this study, we sought to evaluate the association of various levels of haemoglobin measured from 6^{+0} to 13^{+6} weeks and 26^{+0} to 30^{+0} weeks of gestation in nulliparous women with pregnancy outcomes occurring at ≥20 weeks and at ≥26 weeks of gestation, respectively, in LMIC settings. We felt it important to evaluate haemoglobin levels at two times during pregnancy and to relate them to outcomes as there is evidence that these relationships are not consistent over the course of pregnancy. Also, because hypertension is known to influence many of the outcomes under consideration, we felt it important to control for blood pressure while assessing the relationship between haemoglobin levels and the pregnancy outcomes.

Methods

Study design and population

We used the data from the ASPIRIN Trial[15,16] to study the association between various levels of haemoglobin measured at two points in pregnancy and pregnancy outcomes. The ASPIRIN Trial was a multi-country community-based individually randomised placebo-controlled trial aimed at assessing the efficacy of low-dose aspirin in the reduction of preterm birth. The trial was conducted in seven sites in six countries (Pakistan, India, Zambia, Kenya, Democratic Republic of Congo and Guatemala) from 23 March 2016 to 11 April 2019. All women in the study were nulliparous with no more than two previous first-trimester pregnancy losses. Women were recruited between 6^{+0} weeks and 13^{+6} weeks, and had a haemoglobin level measured and an ultrasound examination at the time of enrolment. Of a total of 11 976 women enrolled in the ASPIRIN trial, 11 558 (aspirin: $n = 5787$, placebo: $n = 5771$) had pregnancy outcomes at ≥20 weeks of gestation and 10 751 (aspirin: $n = 5377$, placebo: $n = 5374$) women had pregnancy outcomes determined at ≥26 weeks of gestation, respectively (Figure 1). A second haemoglobin measurement was made between 26 and 30 weeks of gestation.

Ethics approval

The study was approved by the relevant ethics committees of each country and institutional review boards of collaborators in the USA and Research Triangle Institute International. All women provided informed consent for participation in the trial.

Haemoglobin concentration and other measurements

The main exposure variable for this study was a maternal haemoglobin level at randomisation between 6^{+0} and 13^{+6} weeks of gestation and again at follow up from 26 to 30 weeks of gestation. Haemoglobin concentration was measured in a standardised manner using calibrated HemoCue devices across all study sites. Women with a haemoglobin level of <70 g/l at screening were excluded from the

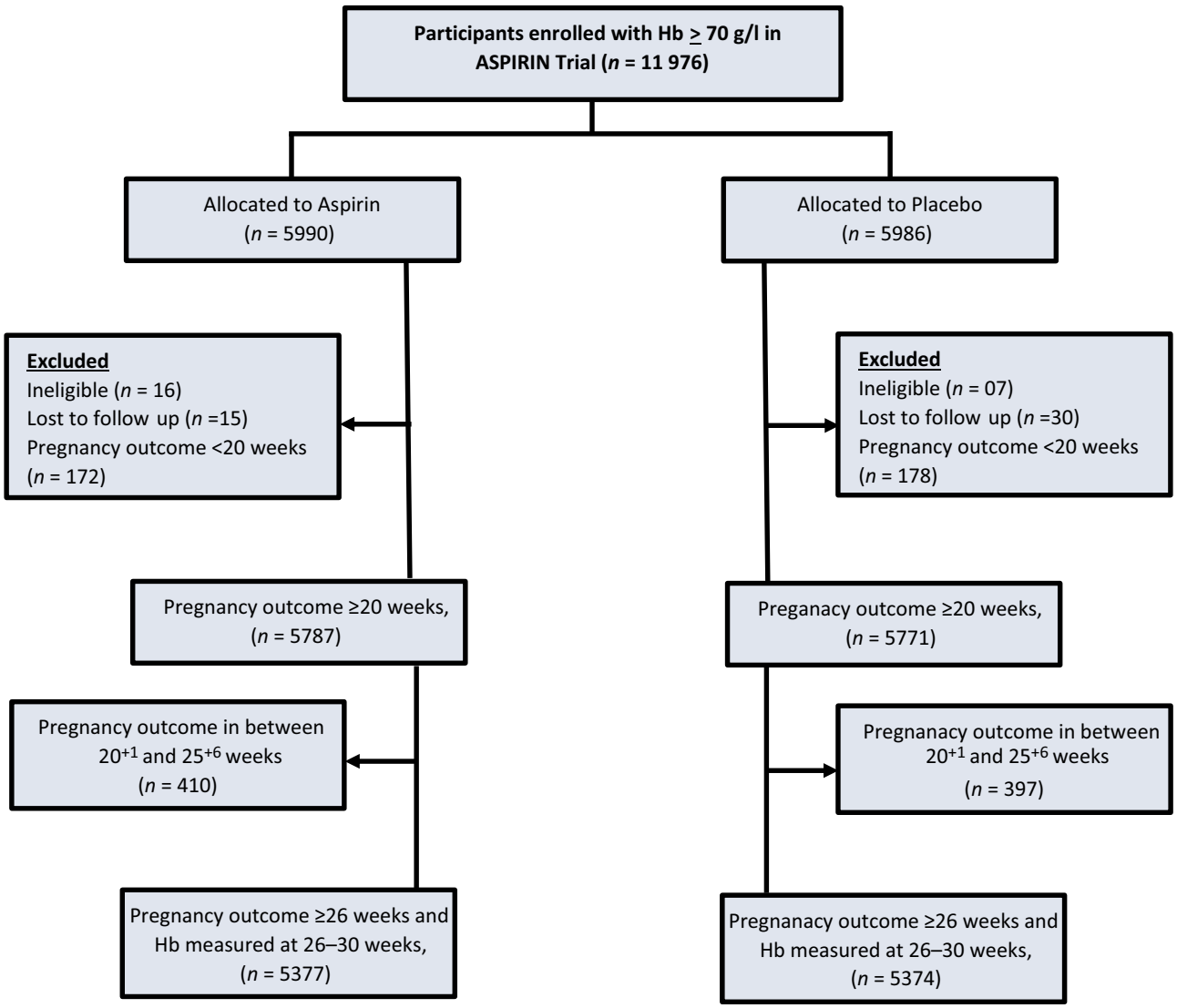

Figure 1. Study CONSORT diagram.

trial. For Guatemala, haemoglobin concentrations were adjusted with a correction factor for altitude above sea level.[17] Four Guatemalan women with haemoglobin measurements ≥70 g/l at screening had haemoglobin values adjusted to values <70 g/l. These women were included in the 70–89 g/l category. For this study, haemoglobin levels were classified into four categories (70–89, 90–109, 110–129 and ≥130 g/l) at the time of enrolment and into five categories (<70, 70–89, 90–109, 110–129 and ≥130 g/l) including those women who had haemoglobin levels <70 g/l at their follow-up assessment.

Gestational age was the best estimate based on an algorithm including maternal last menstrual period and the sonogram at the time of screening between 6^{+0} and 13^{+6} weeks of gestation, using the American College of Obstetrics and Gynecology standard for gestational age determination.[18] Every woman in the study had a first-trimester ultrasound examination. The body mass index (BMI) in kg/m^2 was measured and calculated using maternal height and weight at the time of screening. Blood pressures were recorded as the mean of three systolic and diastolic blood pressures measured using automated blood pressure equipment. For this study, the blood pressure values evaluated were those obtained at the study screening visit and the last obtained during prenatal care. Birthweight was measured in all sites using a calibrated weighing scale as soon as possible after birth. Values obtained after 4 days were not included in birthweight or SGA statistics. A categorical variable 'region' (Africa, India, Pakistan and Guatemala) was created to assess regional

differences in the association of haemoglobin levels with pregnancy outcomes.

Study outcomes

The study outcomes included preterm birth, stillbirth, neonatal death, SGA and low birthweight (<2500 g). Preterm birth was defined as the delivery at $\geq 20^{+0}$ weeks and before 37^{+0} weeks of gestation. Stillbirth was defined as any fetal loss at $\geq 20^{+0}$ weeks. SGA was defined as having a birthweight less than the Intergrowth 10th centile for a given gender and gestational age. Neonatal death was defined as the death of a live-born baby at <28 days. All study outcomes were collected through the Global Network's Maternal and Newborn Health Registry, an ongoing geographically based registry that collects pregnancy outcome data for all births in defined geographic areas.[19,20]

Statistical analyses

Data analyses were performed using SAS software (version 9.4; SAS Institute, Cary, NC, USA). First, haemoglobin levels were compared across both study arms, i.e. the aspirin and placebo groups, to assess the difference between the two groups. The descriptive statistics of various sociodemographic, obstetric and clinical characteristics were compared with haemoglobin levels at screening. Moreover, the study outcomes were analysed by baseline haemoglobin levels at 6^{+0}–13^{+6} weeks of gestation and at follow up between 26 and 30 weeks of gestation, respectively. In addition, Loess plots of haemoglobin at screening and at 26–30 weeks of gestation were also plotted by study outcomes to graphically explore the association between haemoglobin and study outcomes.

For each of the fetal and neonatal outcomes, a preliminary generalised linear model with a binomial or Poisson distribution and a log link was run including the following potential confounders as co-variates: haemoglobin at screening, site, treatment, gestational age at randomisation (to account for potential bias regarding late enrolment), gravidity, maternal age, BMI, blood pressure at screening and the interaction of the trial treatment arm and haemoglobin at screening (to detect differences by study arm). Then, models were run including the same variables except that the site was replaced with a variable for region and the interaction of region and haemoglobin level at screening to obtain the relative risks for each outcome by region. The relative risks of each outcome and the 95% CI were obtained for the haemoglobin concentration at screening using 110–129 g/l as the reference category for the overall and regional models. The same process was repeated using haemoglobin levels obtained between 26 and 30 weeks of gestation and the last blood pressures before pregnancy outcome. The interaction terms for haemoglobin levels with study intervention as well as systolic and diastolic blood pressure with gestational age at which the measures were obtained were also included in the models.

Funding

This trial was funded by grants from the Eunice Kennedy Shriver Institute of Child Health and Human Development to study sites. The grants were competitively awarded to study investigators following external review of scientific quality. Representatives of the funder (MKT and MM) participated in oversight of the study implementation; however, they did not influence the study manuscript.

Results

The mean haemoglobin levels at 6^{+0}–13^{+6} weeks of gestation and at 26–30 weeks of gestation were 116 g/l [standard deviation (SD) 17] and 107 g/l (SD 15), respectively. The distribution and mean haemoglobin levels were virtually identical in the aspirin and control groups at both time-points, strongly suggesting that aspirin did not influence haemoglobin concentrations. The mean haemoglobin concentrations at enrolment were 116 g/l (SD 17) for the women in the aspirin group and 116 g/l (SD 17) for the women in the placebo group, whereas at 26–30 weeks the mean haemoglobin concentrations were 107 g/l (SD 15) for the women in the aspirin group and 107 g/l (SD 16) for the women in the placebo group. Hence, the entire population was combined for further analyses.

Table 1 describes sociodemographic and clinical characteristics of pregnant women by haemoglobin categories at enrolment (6^{+0}–13^{+6} weeks of gestation). There was wide variation in haemoglobin concentrations across the participating sites. Among the three African sites, the Democratic Republic of Congo had 5.2 and 35.8% women with haemoglobin levels 70–89 and 90–109 g/l, respectively; Kenya had 3.2 and 18.0% women with haemoglobin levels of 70–89 and 90–109 g/l, respectively and Zambia had 1.2 and 17.1% women with haemoglobin levels 70–89 and 90–109 g/l, respectively. In addition, Kenya had the highest proportion (52.0%) of women with haemoglobin levels 110–129 g/l and Zambia had the highest proportion (33.9%) of women with a haemoglobin level ≥130 g/l compared with the other two African sites.

Of the two Indian sites, Nagpur had the highest proportion of women with haemoglobin levels of 70–89 (5.4%) and 90–109 g/l (52.8%), respectively. Nearly 37% of women in Nagpur had a haemoglobin level of 110–129 g/l and 4.9% had a haemoglobin level of ≥130 g/l. Belagavi had 4.6 and 27.2% women with haemoglobin levels of 70–89 and 90–109 g/l, respectively. Furthermore, about 54 and 14% of women in Belagavi had haemoglobin levels of 110–129 and

Table 1. Baseline sociodemographic and clinical characteristics of pregnant women in ASPIRIN Trial by haemoglobin levels at enrolment (6^{+0}–13^{+6} weeks of gestation)

	Total	Haemoglobin at enrolment (g/l)			
		70–89	90–109	110–129	≥130
Pregnancies ≥20 weeks of gestation, *n* (%)	11 558	732 (6.3)	3525 (30.5)	5277 (45.7)	2024 (17.5)
Democratic Republic of Congo	1320	68 (5.2)	473 (35.8)	594 (45.0)	185 (14.0)
Zambia	1010	12 (1.2)	173 (17.1)	483 (47.8)	342 (33.9)
Kenya	1328	42 (3.2)	239 (18.0)	691 (52.0)	356 (26.8)
Belagavi, India	2650	123 (4.6)	722 (27.2)	1427 (53.8)	378 (14.3)
Nagpur, India	2046	111 (5.4)	1080 (52.8)	754 (36.9)	101 (4.9)
Pakistan	1533	353 (23.0)	686 (44.7)	445 (29.0)	49 (3.2)
Guatemala	1671	23 (1.4)	152 (9.1)	883 (52.8)	613 (36.7)
Maternal age (years), *n* (%)	11 558	732	3525	5277	2024
<20	4506 (39.0)	234 (32.0)	1203 (34.1)	2182 (41.3)	887 (43.8)
20–29	6801 (58.8)	481 (65.7)	2263 (64.2)	2964 (56.2)	1093 (54.0)
30–40	251 (2.2)	17 (2.3)	59 (1.7)	131 (2.5)	44 (2.2)
Mean (SD)	21 (3)	21 (3)	21 (3)	21 (3)	21 (3)
Gestational age at enrolment, *n*	11 558	732	3525	5277	2024
Median (P25, P75)	10.1 (8.6, 12.0)	10.1 (8.4, 12.1)	10.4 (8.9, 12.1)	10.1 (8.6, 12.0)	10.0 (8.6, 11.7)
Maternal education, *n* (%)	11 556	732	3525	5275	2024
No formal schooling	1658 (14.3)	312 (42.6)	673 (19.1)	559 (10.6)	114 (5.6)
1–6 years of schooling	1713 (14.8)	80 (10.9)	449 (12.7)	801 (15.2)	383 (18.9)
7–12 years of schooling	6924 (59.9)	292 (39.9)	2015 (57.2)	3293 (62.4)	1324 (65.4)
≥13 years of schooling	1261 (10.9)	48 (6.6)	388 (11.0)	622 (11.8)	203 (10.0)
Maternal BMI (kg/m^2), *n* (%)	11 551	732	3524	5275	2020
<18.5	2986 (25.9)	264 (36.1)	1232 (35.0)	1204 (22.8)	286 (14.2)
18.5–24.9	7039 (60.9)	427 (58.3)	2023 (57.4)	3311 (62.8)	1278 (63.3)
25.0–29.9	1262 (10.9)	38 (5.2)	230 (6.5)	632 (12.0)	362 (17.9)
≥30	264 (2.3)	3 (0.4)	39 (1.1)	128 (2.4)	94 (4.7)
Mean (SD)	21.0 (3.7)	19.6 (2.8)	20.0 (3.3)	21.3 (3.6)	22.5 (4.0)
Blood pressure measured at screening (mmHg), *n*	11 524	724	3513	5267	2020
Systolic blood pressure, mean (SD)	106.8 (10.7)	105.3 (10.6)	105.6 (10.3)	106.8 (10.8)	109.7 (10.6)
Diastolic blood pressure, mean (SD)	65.6 (8.5)	65.1 (8.5)	65.5 (8.5)	65.3 (8.5)	66.8 (8.2)

Haemoglobin measurements have been adjusted for altitude in Guatemala according to the formula in the linked paper: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-3156.2008.02143.x

Four haemoglobin measurements at screening which were adjusted to values <70 g/l are included in the 70–89 g/l category.

≥130 g/l, respectively. In Pakistan, 23.0 and 44.7% of women had haemoglobin levels of 70–89 and 90–109 g/l, respectively, whereas 29.0 and 3.2% of women had haemoglobin levels of 110–129 and ≥130 g/l, respectively. In Guatemala, 1.4 and 9.1% of women had haemoglobin levels of 70–89 and 90–109 g/l, respectively, whereas 52.8 and 36.7% of women had haemoglobin levels of 110–129 and ≥130 g/l, respectively.

Among all women, the mean maternal age was 21.0 (SD 3.0) years. At screening, the median gestational age was 10.1 weeks (P_{25}, P_{75}: 8.6, 12.0) and these did not differ across all levels of haemoglobin concentrations. About 43% of women with haemoglobin levels of 70–89 g/l had no formal schooling; this proportion decreased with increasing haemoglobin concentration levels. Similarly, about 36% women with a haemoglobin level of 70–89 g/l were underweight (BMI <18.5 kg/m^2) and this proportion decreased with increases in haemoglobin levels. The overall mean systolic blood pressure was 106.8 (SD 10.7) mmHg; however, it was higher in women with haemoglobin levels ≥130 g/l.

The overall pregnancy outcomes at ≥20 weeks of gestation and at ≥26 weeks of gestation including preterm birth, stillbirth, neonatal mortality <28 days, SGA and birthweight <1500 g and 1500–2499 g were evaluated by haemoglobin level categories at 6^{+0}–13^{+6} weeks and 26–30 weeks of gestation (Table 2). At 6^{+0}–13^{+6} weeks of gestation, in general, the pregnancy outcomes were better with increasing haemoglobin levels. However, for stillbirth, there was a U-shaped relationship showing a higher proportion of fetal deaths at both lower and higher concentrations of haemoglobin (Table 2 and Figure 2).

Table 2. Fetal and neonatal outcomes by haemoglobin levels at enrolment (6^{+0}–13^{+6} weeks of gestation) for pregnancies ≥20 weeks of gestation and at 26–30 weeks of gestation for pregnancies ≥26 weeks of gestation

	Total	Haemoglobin levels (g/l)				
		<70	70–89	90–109	110–129	≥130
Pregnancies ≥20 weeks of gestation, *n* (%)	11 558	–	732 (6.3)	3525 (30.5)	5277 (45.7)	2024 (17.5)
Preterm delivery, *n/N* (%)	1422/11 544 (12.3)	–	135/732 (18.4)	457/3520 (13.0)	610/5269 (11.6)	220/2023 (10.9)
Stillbirth, *n/N* (rate/1000)	307/11 544 (26.6)	–	38/732 (51.9)	100/3520 (28.4)	116/5269 (22.0)	53/2023 (26.2)
Neonatal mortality <28 days, *n/N* (rate/1000)	327/11 235 (29.1)	–	30/694 (43.2)	114/3419 (33.3)	137/5152 (26.6)	46/1970 (23.4)
SGA, *n/N* (%)	3070/10 959 (28.0)	–	229/684 (33.5)	1075/3375 (31.9)	1334/5003 (26.7)	432/1897 (22.8)
Birthweight*, *n* (%)	11 342	–	725	3493	5161	1963
<1500 g	215 (1.9)	–	17 (2.3)	70 (2.0)	97 (1.9)	31 (1.6)
1500–2499 g	2064 (18.2)	–	181 (25.0)	713 (20.4)	860 (16.7)	310 (15.8)
≥2500 g	9063 (79.9)	–	527 (72.7)	2710 (77.6)	4204 (81.5)	1622 (82.6)
Mean (SD)	2787 (512)	–	2664 (512)	2721 (508)	2816 (505)	2872 (517)
Pregnancies ≥26 weeks of gestation, *n* (%)	10 751	147 (1.4)	1182 (11.0)	4909 (45.7)	3959 (36.8)	554 (5.2)
Preterm delivery, *n/N* (%)	1205/10 746 (11.2)	30/147 (20.4)	190/1182 (16.1)	546/4908 (11.1)	380/3956 (9.6)	59/553 (10.7)
Stillbirth, *n/N* (rate/1000)	201/10 746 (18.7)	10/147 (68.0)	29/1182 (24.5)	86/4908 (17.5)	63/3956 (15.9)	13/553 (23.5)
Neonatal mortality <28 days, *n/N* (rate/1000)	269/10 544 (25.5)	9/137 (65.7)	47/1152 (40.8)	121/4822 (25.1)	83/3893 (21.3)	9/540 (16.7)
SGA, *n/N* (%)	2982/10 315 (28.9)	34/133 (25.6)	328/1131 (29.0)	1453/4728 (30.7)	1055/3793 (27.8)	112/530 (21.1)
Birthweight*, *n* (%)	10 569	144	1162	4834	3880	549
<1500 g	109 (1.0)	4 (2.8)	18 (1.5)	45 (0.9)	34 (0.9)	8 (1.5)
1500–2499 g	1968 (18.6)	36 (25.0)	243 (20.9)	942 (19.5)	673 (17.3)	74 (13.5)
≥2500 g	8492 (80.3)	104 (72.2)	901 (77.5)	3847 (79.6)	3173 (81.8)	467 (85.1)
Mean (SD)	2790 (482)	2660 (503)	2713 (466)	2764 (469)	2832 (488)	2915 (520)

*Birthweight measured within 4 days of delivery or estimated.

The relationships of adverse pregnancy outcomes with various haemoglobin levels at 26–30 weeks of gestation were more marked, often appearing worse at the highest haemoglobin levels (≥130 g/l). Higher proportions of preterm birth and stillbirth were observed at both extremes of haemoglobin concentration (Table 2 and Figure 2). To better understand these relationships and account for the large differences in haemoglobin concentrations between regions as well as in maternal characteristics, generalised linear models were performed.

Both in the overall and regional models, of adjusted relative risks for fetal/neonatal outcomes for haemoglobin at screening, stillbirth, SGA and birthweight <2500 g, were significantly associated with haemoglobin levels of 70–89 g/l compared with haemoglobin levels of 110–129 g/l (see Supplementary material, Table S1). For Africa, all three outcomes had a significant association with haemoglobin levels of 70–89 g/l. Moreover, birthweight of <2500 g was also associated with haemoglobin levels of 90–109 g/l compared with 110–129 g/l. For Pakistan, birthweight of <2500 g showed a significant association with haemoglobin levels of 70–89 g/l compared with haemoglobin levels of 110–129 g/l. Because the Guatemalan site had very few women with low haemoglobin, the site was excluded from the regional models in which no outcomes were detected in any haemoglobin category.

Overall, preterm birth and neonatal mortality <28 days were not associated with maternal haemoglobin concentrations; however, in Pakistan, neonatal mortality <28 days had a significant association with maternal haemoglobin levels ≥130 g/l compared with haemoglobin levels of 110–129 g/l. The interaction between haemoglobin levels and study intervention remained insignificant in all models at 6^{+0}–13^{+6} weeks of gestation ($P > 0.05$).

We also assessed the relative risks for fetal/neonatal outcomes for haemoglobin at 26–30 weeks of gestation (see Supplementary material, Table S2). In the overall model, stillbirth was the only outcome significantly associated with haemoglobin <70 g/l compared with haemoglobin levels of 110–129 g/l).

For the regional models, in Pakistan, preterm birth was significantly associated with all lower (<70, 70–89 and

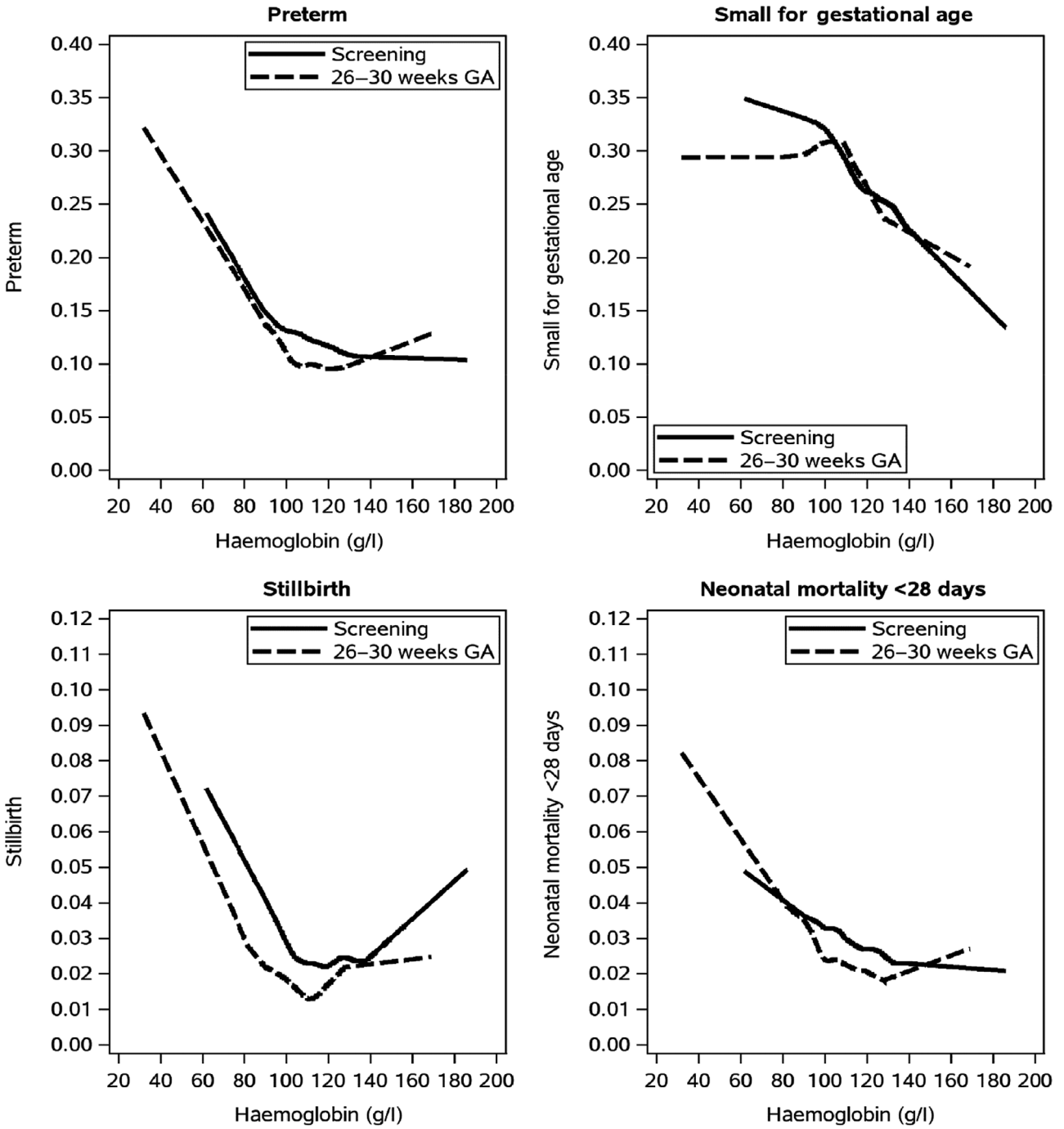

Figure 2. LOESS plots of haemoglobin at enrolment by fetal and neonatal outcomes. Note: Binomial variables are plotted at 0 and 1 and the current figures truncate values of 1 for better viewing of the LOESS curves.

90–109 g/l) and higher (≥130 g/l) haemoglobin levels. In Africa, stillbirth was significantly associated with haemoglobin levels <70 g/l compared with levels of 110–129 g/l. Moreover, in Africa and Pakistan, birthweight <2500 g was associated with haemoglobin levels <90 and <70 g/l compared with levels of 110–129 g/l. Because the Guatemalan site had very few women with low haemoglobin, the site was excluded from the regional models in which no outcomes were detected in one or more of the haemoglobin categories. In India and Guatemala, there were no significant associations of any adverse fetal or neonatal outcome with haemoglobin concentrations at 6–13 weeks or at 26–30 weeks of gestation.

Discussion

Main findings

The present study included a large multi-country cohort of nulliparous pregnant women who participated in the ASPIRIN trial and had haemoglobin levels measured at 6^{+0}–13^{+6} weeks of gestation; of them, 93% had haemoglobin levels measured at 26–30 weeks of gestation. The strength of this study is that we had two measurements at standardised times in pregnancy and linked them with carefully obtained outcomes in seven sites in six countries in Asia, Africa and Central America. We found that anaemia in pregnancy is common in LMICs and that both lower and higher haemoglobin concentrations were risk factors for several adverse pregnancy outcomes; however, there was wide variation in these relationships across regions.

This study, from three geographically distinct regions, confirms the global importance of anaemia in pregnancy, which is especially prevalent in our sites in south Asia, particularly in Pakistan, followed by our sites in Africa and Guatemala. However, the prevalence of anaemia reported in this study for our sites is less than that of global estimates and other studies from these regions.[2,3,21,22] One possible reason for this lower prevalence could be that the women were enrolled in pregnancy at 6^{+0}–13^{+6} weeks of gestation, earlier than many other studies. Haemoglobin levels tend to decrease as the pregnancy progresses, especially during the second trimester.

The study showed that haemoglobin levels of 70–89 g/l at 6^{+0}–13^{+6} weeks of gestation had a significant association with stillbirth, SGA and birthweight <2500 g in the overall population with wide regional differences. It was observed that in African women, stillbirth and SGA were associated with haemoglobin levels of 70–89 g/l and birthweight <2500 g was associated with haemoglobin levels <90 g/l, whereas only in Pakistani women, birthweight <2500 g was associated with haemoglobin levels of 70–89 g/l. Various studies, systematic reviews and meta-analyses across the world are consistent with the study findings.[4,23]

Many studies suggest that haemoglobin concentrations in pregnancy usually decrease from the first to the second trimester because of plasma volume expansion as a physiological consequence of pregnancy; however, pregnant women may have higher haemoglobin concentrations later in pregnancy as the result of insufficient plasma volume expansion leading to poor placental perfusion and fetal distress,[24] and so have more frequent adverse pregnancy outcomes at higher haemoglobin concentrations. A systematic review and meta-analyses and other studies have shown a U-shaped relationship of haemoglobin levels with various fetal and neonatal adverse outcomes including stillbirth, SGA, low birthweight and neonatal mortality.[25,26] The finding of neonatal mortality <28 days with higher haemoglobin concentration (≥130 g/l) at 6^{+0}–13^{+6} weeks of gestation in the Pakistan site is consistent with these studies.

With regards to the association of adverse fetal and neonatal outcomes with haemoglobin levels at 26–30 weeks of gestation, stillbirth was the only adverse outcome associated with haemoglobin <70 g/l at 26–30 weeks of gestation in the overall population, as well as in the African region. These findings are consistent with the studies assessing the relation between haemoglobin concentrations and pregnancy outcomes during the second or third trimester.[10,25]

We also found a significant association of birthweight <2500 g with haemoglobin levels <90 g/l in African women and <70 g/l in Pakistani women at 26–30 weeks of gestation. The findings are consistent with studies evaluating the association of low birthweight with anaemia in the third trimester[27] and regional differences in this relationship.[28]

There was an interaction between the last measured systolic blood pressure, haemoglobin levels and gestational age. Maternal blood pressure is a well-studied determinant of low birthweight and other adverse birth outcomes. Higher maternal blood pressure is associated with impaired fetal growth during the third trimester of pregnancy and increased risks of adverse birth outcomes.[29] Moreover, greater increases in blood pressures from 18 weeks of gestation onwards are related to reduced fetal growth and shorter gestation even in women whose blood pressure does not cross the threshold for hypertensive disorders of pregnancy.[30]

Our findings of no significant association of haemoglobin levels at 6^{+0}–13^{+6} weeks of gestation and at 26–30 of gestation with any of the adverse fetal and neonatal outcomes in India and Guatemala may be in part because the majority of these women had haemoglobin levels ≥90 and ≥110 g/l, respectively. Previous studies have found that moderate to severe anaemia is associated with poor pregnancy outcomes in LMICs.[3,31]

Strengths and limitations

The strengths of this study include the large sample size, prospective multi-country study, standardised haemoglobin measurements at 6^{+0}–13^{+6} weeks and at 26–30 weeks of gestation and assessment of study outcomes using the Global Network Maternal Newborn Health Registry. One limitation of the study was that we did not evaluate the presence of malaria in most of the study participants, which might have been a factor especially in the African sites. Another potential weakness is the exclusion of women with severe anaemia (<70 g/l) at early pregnancy in the study. However, it should not have a major impact on the study findings as there were only 26 women with haemoglobin concentrations <70 g/l who were excluded at the time of enrolment (0.2%). We also made multiple comparisons and it is possible that some of the reported associations occurred by chance.

Interpretation

This study confirms the association of low haemoglobin levels with various adverse pregnancy outcomes and confirms that the levels of haemoglobin differ widely by location. Similar haemoglobin levels drawn at different times in pregnancy have different associations with various adverse outcomes and these relationships differ by country. The study confirms that high haemoglobin levels may be associated with adverse outcomes.

Conclusions

Anaemia during pregnancy in any trimester is a major public health problem in LMICs with wide regional differences in prevalence and outcome. Both lower and higher haemoglobin concentrations are associated with adverse fetal and neonatal outcomes at 6^{+0}–13^{+6} weeks and 26–30 weeks of gestation, although the relationship with low haemoglobin levels appears more consistent and generally stronger.

Disclosures of interests

None declared. Completed disclosure of interests form available to view online as supporting information.

Contribution to authorship

SJ, SS and RLG drafted the manuscript with input from MKH, SSG, RJD, AG, NFK, AT, CLB, EC, WAC, AP, PLH, FE, SB, EAL, EMM, MKT and MM. MKH, SSG, RJD, AG, NFK, AT, CLB, EC, WAC, AP, PLH, FE, EAL, EMM, MKT and MM conceived the trial. SJ, SS, AG, LF, JO, AT, MMw, EC, PKD, AP and FE oversaw field implementation. MKH, SSG, RJD, AG, NFK, AT, CLB, EC, WAC, AP, PLH, FE, SB, EAL, EMM, JLM, TN, MKT, MMe and RLG provided oversight of the ongoing trial. JLM, TLN and EMM conducted the statistical analyses. All authors reviewed and approved the manuscript.

Details of ethics approval

The ethics review committee at each site reviewed and approved the study protocol before implementation.

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Clinical trial registration

This trial was registered at ClinicalTrials.gov (NCT ID 425252).

Data availability

The study data will be available through the NICHD data and specimen hub (https://dash.nichd.nih.gov).

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Association of haemoglobin levels at enrolment (6^{+0}–13^{+6} weeks of gestation) with fetal and neonatal outcomes.

Table S2. Association of haemoglobin levels at 26–30 weeks of gestation with fetal and neonatal outcomes for pregnancies ≥26 weeks. ■

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