**Maternal haemoglobin drop in multiple pregnancy is associated with increased gestational age at delivery and birthweight: A retrospective study**

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**Short title:** Maternal haemoglobin in twins

**Abstract**

**Objective:** To investigate the hypothesis that maternal haemoglobin (Hb) levels in twin pregnancy fall between the first and second trimesters, and that the size of the fall is associated with gestational age at birth and birthweight (BW).

**Design:** Retrospective study.

**Setting:** Inner London Maternity Unit.

**Population:** Pregnant womenwith twin pregnancies delivering two live, phenotypically normal neonates, after 24+0 weeks of gestation, between October 2009 and September 2021.

**Methods:** Measurementof Hb, at ≤14+0 weeks of gestation, (Hb1) and again at 20+0-30+0 weeks gestation (Hb2). Hb drop was defined as Hb1-Hb2. Small for gestational age was defined as BW <10th percentile for gestation. The association of Hb drop with gestational age at birth, BW, SGA and intertwin BW discrepancy of ˃25%, was evaluated.

**Main outcome Measures:** Gestational age at birth**,** incidence of SGA neonates and/or intertwin BW discrepancy >25%.

**Results:** 925 women with twin pregnancies. Maternal Hb1 did not correlate with gestational age or SGA or twins with BW discrepancy >25%. However, a larger Hb drop was associated with a higher gestational age at birth (p<0.001), a larger BW of both twin 1 and 2 (p<0.001) and a trend towards reduction in the incidence of delivering one or two SGA neonates (p=0.005 and p=0.003, respectively) or twins with BW discrepancy of >25% (p=0.005).

**Conclusions:** The study has shown that a larger maternal Hb drop from the first to the second trimester is associated with a higher gestational age at birth, a larger BW and smaller BW discrepancy in twin pregnancies.

**FUNDING:** None

**KEYWORDS:** twin, pregnancy, haemoglobin, birthweight

1. **INTRODUCTION**

In the United Kingdom, the rate of twin pregnancies has risen from 9.8/1000 in 1980 to 13.7/1000 in 2021. 1 This increase is due to both the use of assisted reproductive techniques (ART) and rising maternal age (pregnant women aged over 30 have a 2.7 higher chance of spontaneous multiple pregnancy).2 Twin pregnancy is associated with increased maternal and perinatal morbidity and mortality,3 mainly due to preterm delivery (31-63%) 4 and delivery of small for gestational age (SGA) neonate(s) (25-35%).3-5 Small babies are at increased risk of hypoxia, neurological complications,6 hypoglycaemia, metabolic and haematological disturbances, and disrupted thermoregulation at birth.7 Preterm and SGA infants have increased long-term morbidity including sudden infant death,6 neurological sequelae and motor and cognitive delay.7

The measurement of maternal full blood count (FBC) including haemoglobin (Hb) level is routinely performed at least twice during pregnancy, initially at the first antenatal visit and again at around 28 weeks of gestation.8 In normal pregnancy, plasma volume increases progressively, producing a ‘physiological anaemia’ due to haemodilution9 and maternal Hb levels are reported to fall on average by 14g/L over the course of a normal uncomplicated pregnancy.10 Maternal Hb levels have a non-linear association with obstetric outcomes in singleton pregnancies. Several studies have reported a U-shaped association with low birthweight (LBW) neonates (both pre-term and SGA).11,12 A recent meta-analysis13 concluded that maternal anaemia, with definitions ranging from Hb <100 g/L to Hb <115 g/L, was associated with an increased risk of LBW and preterm birth. Less widely appreciated is that both high Hb14–18 levels and lower Hb “drop” during pregnancy17,19 have also been associated with LBW in singleton pregnancy, with or without pre-eclampsia (PE).16 Both high Hb levels (>140g/L) and lower Hb drop (<10g/L) are likely to reflect failure of the plasma expansion process.20,21

We have been able to identify only one previous study investigating the correlation between maternal Hb and pregnancy outcomes in twin pregnancies (n=247).22 It reported that second trimester maternal anaemia (Hb<100 g/L) was not associated with the development of preterm delivery, PE, gestational diabetes, Caesarean delivery or post-partum haemorrhage.22 The hypothesis that we tested in our study was that in twin pregnancy, the fall in Hb levels between the first and second half trimesters of pregnancy would be associated with the gestation at birth and BW for gestational age.

**2. METHODS**

**2.1 Study Population**

Pregnant women with twin pregnancies, who had had first trimester combined screening for major chromosomal abnormalities at 11-14 weeks of gestation and delivered at our maternity unit in an inner London Hospital in the UK between 1st October 2009 and 30th September 2021 were identified using the fetal medicine reporting system (ViewPoint 5 and 6TM, Health Net Connections, UK). Chorionicity was determined in the first trimester23 and the pregnancy was dated by the crown-rump length of the largest twin or by the date of embryo transfer if ART was used. Only women who gave birth to two live, phenotypically normal neonates, at or after 24+0 weeks of gestation, were included in the analysis.

The first trimester combined screening for the major chromosomal abnormalities included measurement of nuchal translucency and maternal free beta human chorionic gonadotrophin and pregnancy associated plasma protein-A.8 Information on maternal demographic characteristics including weight, ethnic/racial group, smoking and method of conception was recorded prospectively at the screening assessment. Other maternal characteristics, including maternal height, parity, past medical and obstetric history, as well as pregnancy and delivery outcomes including development of hypertension or PE, gestation and mode of birth, sex and BW of the neonates were recorded in the Hospital’s perinatal database (Ciconia Maternity Information System; CMiS). Maternal booking body mass index (BMI) was calculated as weight (kg) / height (m)2. Preterm delivery was defined as delivery <37+0 completed weeks of gestation. Pre-eclampsia was defined as maternal hypertension of ≥140/90 mmHg, four hours apart, and proteinuria of ≥300mg/24hours or protein/creatinine ratio ≥30 on a random urine sample, after 20 weeks of gestation, in a previously normotensive woman.24 If PE was documented in the perinatal database, individual patient’s medical records were reviewed to confirm the diagnosis. Small for gestational age (SGA) was defined as BW<10th percentile for gestational age in either twin, as assessed by comparison with singleton charts.25 An intertwin BW discrepancy of ˃25%, calculated as [((BW larger twin-BW smaller twin)/ BW of the larger twin) X100], was also considered as an outcome.

Women were included in the study if they had a full blood count (FBC) estimation and electrophoresis at ≤14+0 weeks of gestation, and a further FBC at 20+0 to 30+0 weeks gestation (in accordance with national guidance).8 The values of maternal Hb (g/L) were obtained from the electronic patient records. The study was approved by the local Research Ethics Committee (REC reference: 13/NI/0070). There was no patient involvement in our study.

**2.2 Statistical analysis**

The Kolmogoroff–Smirnoff test was used to assess normality of the data distribution. Data were expressed as number (%) and median (interquartile range). Groups were compared using the unpaired Student t-test/Mann-Whitney or chi-square (χ2) for numerical and categorical data respectively. Univariate regression analysis was used to investigate the relationship between maternal Hb level and gestational age at sampling, and adjustments were performed to standardise the values to a particular gestational age where necessary to correct for the effect of variation in gestation at sampling. Pearson correlation coefficient and linear regressions were used to assess the relationship between maternal Hb and other maternal characteristics. Haemoglobin values were not adjusted for maternal demographics as it was hypothesised that any link between the demographics and gestational age and BW would be due at least in part to plasma volume (and resultant Hb) changes and thus correction would obtund the relationships we were investigating. Maternal Hb levels at the first (Hb1) and second visit (Hb2), adjusted for gestational age at sampling where necessary, and Hb drop (Hb1-Hb2), were correlated with pregnancy outcomes, including gestational age at delivery, development of PE, delivery of at least one or both SGA neonates and inter-twin BW discrepancy of >25%, using multiple linear or logistic regression. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS, version 28.0, IBM Corp., Armonk, New York, USA). Because of multiple testing, significance has been taken as p<0.001.

**3. RESULTS**

The initial search for twin pregnancies booked and delivered in our maternity unit identified 1,287 twin pregnancies. We excluded 332 pregnancies for the reasons shown in Figure 1. The final study cohort included 925 twin pregnancies resulting in two live births of phenotypically normal neonates. No women were included twice.

The maternal characteristics, Hb values and pregnancy outcomes of the study participants are shown in Table 1. There was an inverse linear correlation between the initial Hb value (Hb1) and gestational age at sampling (Hb1 = 140.299 – (1.571 X gestational age at sampling; R=0.251, p<0.001) throughout the gestation range of 6-14 weeks (Figure 2), and therefore an adjusted value of Hb1 (adjHb1) value was calculated, standardised to the level expected at 6 weeks gestation. In contrast, the second Hb (Hb2) value, obtained at 20+0-30+0 weeks, inclusive, did not correlate significantly with the gestation at sampling and therefore no adjustment was required. The ‘Hb drop’ was then determined as the difference between the adjusted Hb1 and Hb2 (Hb drop= adjHb1-Hb2).

The correlations of Hb values and Hb drop with maternal characteristics were calculated, and further analysed using Pearson correlation coefficient (Table 2). It is notable that although maternal weight at booking, height, ethnic/racial group and method of conception correlated with Hb1 (adjHb1) and Hb 2, these demographics did not correlate significantly with the Hb drop. In contrast, significantly greater Hb drop was seen in parous women compared to nullipara (Hb1 was similar but Hb2 was lower in parous women) and, to some extent, in smokers than in non-smokers (Hb1 higher and Hb2 lower in smokers). Chorionicity was not significantly related to the haemoglobin drop (20.543 with dichorionic and 21.193 with monochorionic twins, p=0.237)

39.2% and 49.7% of twins 1 and 2, respectively, were classified as SGA on singleton charts25 and in 226/925 pregnancies (24.4%) both twins were SGA. In 91 pregnancies (9.83%), the inter-twin BW discrepancy was >25%. Initial maternal Hb levels (Hb1 and adjHb1) did not correlate with delivery of at least one SGA neonate or inter-twin BW discrepancy >25% (p=0.22, p=0.51, and p=0.26, p=0.34, respectively). However, both Hb2 and Hb drop correlated, but not significantly, with these two variables (p=0.019, p=0.005 and p=0.039, p=0.005, respectively). In particular, a larger Hb drop was associated with a higher gestational age at birth (R=0.157; p<0.001), a larger BW of both twin 1 (R=0.167; p<0.001) and twin 2 (R=0.208; p<0.001) and a trend towards a reduction in the incidence of delivering one twin SGA (R=-0.092; p=0.005), both twins SGA (R=-0.099; p=0.003) or twins with BW discrepancy of >25% (R=-0.093; p=0.005) (Figure 3). For each 10 g/L drop in Hb from Hb1 (adjusted) to Hb2, gestational age at birth increased by 0.33 of a week (R=0.157, gestational age = 35.256 + (Hbdrop x 0.033)), the BW of twin 1 increased by 79.74g (R=0.167, BW twin 1 = 2256.93 + (Hb drop x 7.974)), and the BW of twin 2 increased by 104.3g (R=0.208, BW twin 2 = 2133.37 + (Hb drop x 10.430)).

The value of Hb1 showed, as expected, a significant correlation with the Hb drop (Pearson correlation coefficient 0.530, p <0.001) because high Hbs were more likely to fall, an effect balanced by lower Hbs falling less (and a few actually rising, as shown in Figure 3). However, adjusting Hb drop for this effect made no substantial differences to the correlations between the change in Hb and gestation at delivery or BW (Supplementary Table 1). We further investigated the Hb drop as a percentage of Hb1 (((Hb1adj-Hb2)/Hb1adj) x 100) and again found now substantial changes to the significance of associations (Supplementary Table 1). The incidence of PE was 133/925 (14.4%) and there was no significant correlation between adjHb1, Hb2, Hb drop and the development of PE (Table 2).

We investigated the possibility that excluding women who delivered at or before the 30+0 weeks limit, because they had not yet had a Hb2 estimation, but including those who had already had a Hb2 measurement, might have affected the results, (Table 3.) The Pearson correlation coefficients between the maternal Hb values and gestational age at birth and BW for all cases compared with excluding the 27 women who delivered at 24+0-30+0 weeks showed no significant alteration in the values.

**4. DISCUSSION**

**4.1 Main Findings**

We found that a larger Hb drop throughout pregnancy was associated with a longer gestation, a greater BW of both twins and a smaller intertwin BW discrepancy. No significant relationship between Hb levels and PE was found. Our findings are in accordance with studies in singleton pregnancies.14–18,25

**4. 2 Interpretation**

The “physiological anaemia” of pregnancy begins early in the first trimester, and Hb levels are lowest at 24-34 weeks gestation.19,21 This fall in Hb correlates significantly with plasma expansion20,21,26 and the resulting physiological haemodilution.9 Duvekot et al.27 has suggested that plasma expansion is caused by a drop in maternal total vascular resistance (TVR), which in turn activates the renin-angiotensin-aldosterone system. This results in a proportionally greater rise in intravascular volume than in the erythropoietin stimulated increase in red blood cell mass, resulting in haemodilution.28 Reduced blood viscosity29 increased stroke volume and cardiac output (CO), as a result of the fall in vascular tone,27 further enhance placental blood flow and the delivery of nutrients to the developing utero-placental unit.28 Asmaller than average Hb drop has been reported in cases of low BW to placental ratio17 suggesting that placental insufficiency is associated with impaired plasma expansion, restricted fetal growth, and low BW. Impaired haemodilution results in a smaller fall in blood viscosity, increasing the risk of vascular occlusion and reducing oxygen delivery to the inter-villous space, thereby stunting placental development.30 Additionally, Hb has a direct role in the regulation of nitric oxide (NO) as free Hb molecules can inactivate NO through binding, preventing vasodilation and leading to endothelial vasoconstriction and hypertension.31 This could directly damage the placenta and the maternal endothelium, resulting in restricted fetal growth. Despite the association between maternal Hb and plasma expansion as well as NO,31 which are both known to be involved in the pathophysiology of PE,32,33 we did not find a correlation between maternal Hb levels and PE suggesting that other mechanisms may be involved in the pathogenesis of PE.

Twin pregnancy puts a much greater burden on maternal physiology, including the haematological and cardiovascular systems, as evidenced by a higher CO and an even greater fall in TVR compared to singleton pregnancies.34 In our cohort, the maternal Hb dropped from 131 (adjusted) to 104 g/dL and this drop of 27g/dL is larger than that observed in singleton pregnancies (14g/dL).10,17 Our results suggest that this reflects a larger degree of plasma expansion in twins to cope with increased fetal demand. Campbell et al35 described greater plasma volume expansion in both nulliparous and parous women with twin pregnancies, compared with singleton pregnancy. Nevertheless, the larger Hb drop, demonstrating exaggerated plasma expansion in multifetal pregnancies, is not sufficient to allow growth of two fetuses to their full individual genetic potential, hence the higher incidence of SGA and perinatal mortality.3 The Hb1 level we report is slightly higher than that usually reported in singleton pregnancies because it is adjusted to 6 weeks of gestation (a unique feature of our study) when plasma volume expansion has not yet become established, whereas most reports of first trimester Hb level in singletons are related to convenience samples at gestational ages of 10-12 weeks, when some plasma volume expansion has already occurred. However, the Hb2 value in our cohort is lower than that usually reported for singleton pregnancies, because of the extra plasma volume expansion seen in twin pregnancies.10,14,16–18

According to NICE guidance,36 over half of women in our study would have been labelled anaemic in the second half of the pregnancy. However, our findings suggest that low Hb levels in the last half of pregnancy may more commonly indicate a “superior” maternal physiological adaptation than true ‘anaemia’. Shinar et al. noted that only 42% of twin pregnancies defined as “anaemic” had ferritin levels consistent with iron deficiency anaemia.37 Reduced iron intake has been found not to account for the lower BW observed and doubling prophylactic iron supplementation has no significant effect on maternal Hb levels.38 Furthermore, low neonatal iron status has not been associated with maternal anaemia.39 These findings add weight to the concept that plasma expansion is more likely than iron deficiency to account for the low mid-trimester Hb levels seen in multifetal pregnancy. Treatment of ‘anaemic’ women with iron supplementation is therefore usually unnecessary and can cause unpleasant side effects and should not be instituted without first establishing true iron deficiency.

Other maternal factors, including smoking and parity were associated with a larger Hb drop. In our study, smokers had a higher Hb1 and a lower Hb2, which accounts for the larger Hb drop seen in this group of women. Carbon monoxide affecting the erythrocytes of cigarette smokers is thought to trigger hypoxic peripheral stimulation, increasing Hb levels as a compensatory mechanism to maintain oxygen levels.40 While the higher Hb drop might be expected to be associated with increased BW, it may be that the negative effects on placental perfusion of regular nicotine inhalation are greater than the effects of any putative increase in plasma volume expansion. Parity is also known to be associated with larger neonates and reduced neonatal mortality than in singleton pregnancies.41 Our findings of a larger Hb drop in parous women with twins may be due to heightened plasma expansion and better adaptation to pregnancy in subsequent pregnancies,42 which may be immunological in nature.43 This may explain the lower incidence of SGA neonates born in parous women.44

**4.3 Strengths and Limitations**

The main strength of the study is the large number of twin pregnancies included over a 13 years’ period. Robust methodology was used, including the use of prospectively collected data on maternal characteristics, obtained as part of the first trimester combined screening for chromosomal abnormalities. As we included women who were looked after and delivered in our unit, we ensured homogeneity in the care received by all study participants. The main weakness of the study is that it is retrospective and we did not have information on the use of iron supplementation during pregnancy. Our study included predominantly women with a normal initial Hb, and our results may not be applicable to populations where maternal anaemia is more prevalent.

**5. CONCLUSION**

The study has shown that in twin pregnancies a larger maternal Hb drop from the first to the second half of pregnancy is associated with higher gestational age at birth, larger BW and a smaller inter-twin BW discrepancy. This Hb drop is more likely therefore to result from plasma volume expansion than iron deficiency anaemia, which could be examined in more detail by the direct measurement of plasma volume supplemented with iron studies.

**Disclosure of interests**

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

**Contribution of authorship**

MS conceived and planned the study. KT and TM collected the data. KT and PJS performed the analysis of the data. KT, MS and PJS contributed to the interpretation of the data. The manuscript was written by KT, MS and PJS. All authors contributed to editing the drafts and approved the final manuscript. All authors take responsibility for the final version.

**Details of Ethics Approval**

The study was approved by the Local REC (No: 13/NI/0070; 21 February 2022

**Funding**

None

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