MATERNAL ANEMIA AND ADVERSE PREGNANCY OUTCOMES

by

QIAOYI ZHANG

A dissertation submitted to the

School of Public Health

University of Medicine and Dentistry of New Jersey and

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Doctor of Philosophy

UMDNJ - School of Public Health

Written under the direction of

Professor Cande V. Ananth, PhD, MPH

And approved by

_____

_____

_____

_____

New Brunswick, New Jersey

January, 2008

ABSTRACT OF THE DISSERTATION

MATERNAL ANEMIA AND ADVERSE PREGNANCY OUTCOMES

By QIAOYI ZHANG

Dissertation Director:

Professor Cande V. Ananth, PhD, MPH

Background: Maternal anemia is a ubiquitous pregnancy complication, and has been associated with an array of adverse perinatal and reproductive outcomes. Despite scores studies, the association between anemia and perinatal outcomes remains poorly understood. The objectives of our study were to describe the epidemiology of maternal anemia, and to examine the associations of maternal anemia with perinatal mortality and preterm birth.

Method: A prospective cohort study was conducted, using existing data from a population-based pregnancy-monitoring system in 13 counties in East China (1993-96). Women who delivered singleton infants at 20 to 44 weeks with at least one hemoglobin assessment during pregnancy were included (n=164,667). The prevalence of anemia (hemoglobin <10 g/dL) during pregnancy and rates of stillbirth, neonatal death, and preterm birth were estimated. Multivariable log-Binomial regression models were used

to evaluate risk factors associated with anemia. Associations between anemia and adverse outcomes were examined using multiple Cox proportional hazards regression models after adjusting for a variety of confounding factors.

Results: The overall prevalence of anemia was 32.6%, with substantial variations across trimesters (11%, 20%, and 26% in the 1st, 2nd and 3rd trimesters, respectively). Older maternal age, lower education, farm occupation, delayed prenatal care, pregnancy-induced hypertension and preeclampsia were associated with increased risk for anemia, whereas peri-conceptional folic acid use was associated with reduced risk for 1st trimester anemia. Anemia in the first half of pregnancy was associated with increased risk of stillbirth (adjusted hazard ratio (HR) 1.7, 95% confidence internal (CI) 1.1, 2.7), but not neonatal deaths. Anemia in the 1st trimester was associated with increased risk for preterm premature rupture of membranes (PROM). Women with hemoglobin ≤5 g/dL were at highest risk (HR 3.3, 95% CI 1.4, 7.7) with progressively declining risk with increasing hemoglobin levels. In contrast, anemia in the 3rd trimester was associated with reduced risk for all preterm birth and spontaneous preterm labor, potentially due to hemo-dilution. Anemia was not associated with medically indicated preterm birth.

Conclusion: Anemia in early pregnancy was associated with increased risk for stillbirth and preterm PROM. These findings underscore that early identification and treatment to alleviate anemia may help improve adverse pregnancy outcomes and related complications.

ACKNOWLEDGEMENT

I would like to acknowledge my advisor, Dr Cande V. Ananth, and my dissertation committee members Drs Zhu Li, George Rhoads, and John C. Smulian. Thank you for all your time, patience and efforts. I really appreciate how you challenged me to learn and grow as a researcher and all the guidance and support you gave me throughout my dissertation.

To Drs. R.J. Berry and Jacqueline Gindler at Centers for Disease Control and Prevention, thank you for preparing for the data and providing thoughtful recommendations.

To my current colleagues at Merck & Co., Inc., Drs. Larry Ma, Panagiotis Mavros, Larry Radican, Tom Burke, and Weili He, thank you for all your technical and emotional support.

To my previous colleagues at Beijing Medical University, Drs. Rongwei Ye, Junchi Zheng, and Ms. Zhaoxia Xiong, thank you for all your timely help.

To Dr. Xu Xiong, thank you for sharing with me your previous work on this topic.

To the thousands of health professionals and village doctors in the 13 project counties of Taicong, Jiaxing, Haiyan, Kunshan, Wujiang, Wuxian, Wuxi, Jiaxing Suburban, Haining, Pinghu, Tongxiang, Jiashang, and Cixi in East China, thank you for your dedication and effort that made my dissertation research possible.

DEDICATION

This dissertation is dedicated to my great parents, Zhang Fushu and Lin Qingzhen, my wonderful husband, Hua Pan, and two sweet children, Erik and Jonathan.

TABLE OF CONTENTS

Title page ii

Abstract ii

Acknowledgement iv

Dedication. iv

Introduction 1

Chapter 1 37

Prevalence and Risk Factors of Anemia in Pregnant Women: A Population-based Prospective Cohort Study in China

Chapter 2 67

The Impact of Maternal Anemia on Perinatal Mortality: A Prospective, Population-based Cohort Study in China

Chapter 3 97

Maternal Anemia on Preterm Birth Clinical Subtypes:

A Prospective Cohort Study

Summary 128

Curriculum Vita 133

LIST OF TABLES

Table I – 1 25
Reported prevalence rates of and risk factors for maternal anemia from studies conducted in developing countries

Table I – 2 28
Evidence and strength of association linking major risk factors with anemia based on published studies

Table I – 3 29
Chinese Studies related to the association between maternal anemia and perinatal mortality

Table I – 4 30
Chinese studies related to the associations between maternal anemia and preterm birth

Table I – 5 31
Previous studies on maternal anemia and preterm birth clinical subtypes

Table I – 6 37
Definitions of outcomes in this dissertation

Table 1 – 1 56
Characteristics of maternal hemoglobin measures in 164, 667 women with at least 1 hemoglobin measure during pregnancy

Table 1 – 2 57
Distribution of maternal characteristics in relation to anemia during pregnancy

Table 1 – 3 60
Associations between maternal risk factors and anemia during pregnancy (n=164,677)

Table 1 – 4 62
Sensitivity analysis: adjusted prevalence rate ratio for maternal anemia using the WHO cut-off (hemoglobin <11 g/dL)

Table 2 – 1 88
Maternal and fetal characteristics and perinatal mortality

Table 2 – 2 90
The rates of stillbirth and neonatal mortality, by maternal anemia status

Table 2 – 3 92
Anemia status across trimesters and perinatal mortality

Table 3 – 1 118
Maternal and neonatal characteristics and preterm birth (<37 weeks) rates by clinical subtypes

Table 3 – 2 120
Maternal anemia and preterm birth (<37 weeks) rates by severity and clinical subtypes

Table 3 – 3 121
Adjusted hazard ratio for preterm birth (<37 weeks) and its clinical subtypes in relation to maternal anemia

LIST OF ILLUSTRATIONS

Figure I – 1 35
Suggested biologic mechanisms linking maternal anemia and preterm birth and perinatal morality

Figure I – 2 36
Study cohort selection flowchart

Figure 1 – 1 63
Prevalence of maternal anemia during pregnancy by severity

Figure 1 – 2 64
Average maternal hemoglobin level during pregnancy, by folic acid use status

Figure 1 – 3 65
Associations between maternal age and anemia during pregnancy

Figure 1 – 4 66
Prevalence of maternal anemia during pregnancy, according to Chinese (<10 g/dL) and the WHO (<11 g/dL) cut-offs

Figure 2 – 1 93
Birthweight distribution and perinatal mortality rates by anemia status

Figure 2 – 2 94
Gestational age specific-perinatal mortality rates by anemia status

Figure 2 – 3 95
Adjusted hazard ratio of stillbirth and 95% confidence interval for hemoglobin level in each trimester

Figure 2 – 4 96
Adjusted hazard ratio of early neonatal mortality and 95% confidence interval for hemoglobin level in each trimester

Figure 3 – 1 122
Distribution of gestational age by anemia status

Figure 3 – 2 123
Proportion of clinical subtypes among preterm births by preterm severity and maternal anemia status

Figure 3 – 3 124
Adjusted hazard ratio of preterm birth (< 37 weeks) and 95% confidence interval for hemoglobin level in each trimester

Figure 3 – 4 125
Adjusted hazard ratio of preterm premature rupture of membranes (< 37 weeks) and 95% confidence interval for hemoglobin level in each trimester

Figure 3 – 5 126
Adjusted hazard ratio of spontaneous preterm labor (<37 weeks) and 95% confidence interval for hemoglobin level in each trimester

Figure 3 – 6 127
Hemoglobin change and preterm birth (<37 weeks)

Introduction

Background

Maternal anemia is one of the most commonly prevalent pregnancy complications, which has been associated with an array of adverse pregnancy outcomes, including preterm birth, restricted fetal growth, and perinatal death (1). In addition, anemia is associated with increased risks of maternal deaths in developing countries (2) and a host of other maternal complications, including infection, pregnancy-induced hypertension, preeclampsia, eclampsia, premature rupture of fetal membranes (3), and postpartum depression (4). Normal pregnancy outcomes occur 30-45% less often in anemic mothers (5). Over half of women worldwide experience anemia during their pregnancy, with prevalence rates tipping over two-thirds to up to three-fourths of women in developing countries (6). Because of its high prevalence, the impact of maternal anemia on fetal development remains an important public health concern.

Despite numerous reports on associations between maternal anemia and adverse pregnancy outcomes, the findings remain largely inconclusive. To some extent, this is mitigated by inadequacies in study designs, e.g., failing to adjust for important confounders, ignoring the timing difference on anemia assessment, and aggregating heterogeneous exposures and outcomes.

Epidemiology of maternal anemia

The WHO recommendations for (any) anemia include hemoglobin <12 g/dL for non-pregnant women and <11 g/dL for pregnant women; severe anemia in pregnancy as hemoglobin <7 g/dL, and very severe anemia as hemoglobin <4 g/dL (5). In the United

States, maternal anemia is defined as hemoglobin below the fifth percentile of a trimester-specific hemoglobin reference level in iron-supplemented women during their pregnancy. According to the CDC recommendations, anemia in pregnant women is defined as hemoglobin <11 g/dL in the 1st and 3rd trimesters, and hemoglobin <10.5 g/dL in the 2nd trimester (7).

The WHO (2001) estimated the prevalence of anemia (hemoglobin <11 g/dL) among pregnant women (1990-95) based on blood hemoglobin concentration were 52% in non-industrialized and 23% in industrialized countries. The prevalence of anemia in low-income pregnant women enrolled in public health programs in the United States in 1993 was 9%, 14%, and 37% in the 1st, 2nd, and 3rd trimesters, respectively. Comparable data for the U. S. population of all pregnant women are unavailable. Levy et al. (8) reported a maternal anemia (hemoglobin <10 g/dL) prevalence of 8.6% during the 1st trimester in an Israeli population-based study in 1988-2002.

In our study maternal anemia was defined as hemoglobin <10 g/dL regardless of gestational age, which was pre-specified in the original protocol for a cohort of Chinese pregnant women without routine prenatal iron-supplementation. This is the commonly used clinical definition for maternal anemia in China. Severe anemia is defined as hemoglobin <8 g/dL (9). Although this Chinese cutoff was lower than cutoffs recommended both by the WHO and the CDC, it reflected how anemia was managed in this population during the time of the data collection.

In China, anemia is the most commonly reported complication during pregnancy. A national survey on the prevalence of anemia among child-bearing-aged (15-49 years)

women in China (1998), reported the prevalence of anemia was 35.6% and 42.1% for non-pregnant and pregnant women, respectively (10). Another Chinese nationwide epidemiologic survey conducted in 2000 focused on iron deficiency and iron deficiency anemia in women aged 20-29 years, and reported that 61.7% pregnant women had iron deficiency or iron deficiency anemia (11). The prevalence of iron deficiency anemia was 15.1%, 9.6%, 19.8%, and 33.8% in non-pregnant women and in the 1st, 2nd, and 3rd trimesters, respectively (11). Both surveys used the WHO recommended cutoffs of hemoglobin <12 g/dL for non-pregnant women and hemoglobin <11 g/dL for pregnant women to define anemia. When anemia was defined as hemoglobin <10 g/dL, Xiong et al.(9) found the prevalence of maternal anemia in Suzhou, China (1989-1990) was 10.3% in the 1st trimester, 18.9% in the 3rd trimester, and 26.2% during pregnancy. The prevalence of severe anemia (hemoglobin <8 g/dL) was 1.1%, 3%, and 3.4%, respectively, in the 1st, 2nd, and 3rd trimesters. Previously reported prevalence of maternal anemia in China and other developing countries, between late 80's and first 5 years of 21st century, are summarized in Table I – 1.

The etiology of pregnancy-associated anemia remains obscure, besides the known physiological hemo-dilution in normal pregnancy (12). Most studies in iron-supplemented women showed an increase in total blood volume of approximately 45%, with an increase in red blood cell mass of about 35% and a disproportionate increase in plasma volume of 50% (13). In normal pregnancy, hemoglobin levels decline throughout the 1st and 2nd trimesters and reach a nadir near the end of the 2nd and early of the 3rd trimesters, and then rise again nearer to term (14). The increased prevalence of anemia

from the 1st to 3rd trimester is partially caused by hemo-dilution. Therefore, more women tested positive for anemia in early pregnancy are true positives than those diagnosed in later pregnancy (15). Among pregnant women, expansion of blood volume and growth of fetus, placenta, and other maternal tissues greatly increase the demand for iron three-fold in the second and third trimesters to approximately 5.0 mg iron/day (7). Most pregnant women who do not take iron supplements to meet the increased demand cannot maintain adequate iron stores (16).

Although iron-deficiency is the most common cause of maternal anemia (17-20), other reported risk factors for maternal anemia include deficiencies in folic acid (18;21;22), vitamin B_{12} (22) and vitamin A (23;24), underweight (body mass index <18.5) (19;25;26), young (<25 years) (20;25;27) and older (≥30 years) maternal age (9), poorly educated (19;27), multiparity (18;19;26;28;29), twin pregnancy (28;30), short interpregnancy interval (<5 months) (28;30), malaria (6;17;19;24;28) and hookworm (23;24) infections, and pica in pregnancy (6). As shown in Table I – 1, the importance of these risk factors, and the strength of associations with anemia vary substantially from population to population. Table I – 2 summarizes the reported strength of association between major risk factors and maternal anemia.

Among all risk factors, iron deficiency is arguably the most prevalent cause, affecting nearly two thirds of Chinese women during their pregnancy (11). Other risk factors that were reported in previous Chinese studies include rural residence (10;11), deficiencies of folic acid and vitamin B_{12} (22), maternal age ≥30 years, low BMI (<20), late onset of prenatal care (after the 1st trimester), and ≤14 prenatal visits (9).

Due to the physiological hemo-dilution phenomenon coupled with medical interventions to treat women diagnosed with anemia, it is likely that the risk profile of women with anemia may present some heterogeneity across trimesters. However, previous epidemiologic studies on maternal anemia have paid little attention to assessing this inherent heterogeneity.

Maternal anemia and perinatal mortality

Given the aforementioned high prevalence of anemia in pregnant women, if maternal anemia is indeed associated with increased risk for perinatal mortality, this observation will have a significant public health implication. Unfortunately, data from existing studies on the association between anemia and perinatal mortality remains fairly inconclusive.

Allen (3) proposed 3 potential biologic mechanisms through which maternal anemia may be associated with increased risks of adverse pregnancy outcomes (Figure I – 1), including perinatal mortality and its major determinants such as restricted fetal growth and preterm birth (31-33). Low hemoglobin concentration can cause a state of hypoxia, which is presumably exacerbated in pregnancy when oxygen demands are particularly high because of the metabolism of the mother and the fetus. Hypoxia activates a stress response in the mother and fetus through elevations in corticotrophin-releasing hormone or cortisol resulting in restricted fetal growth (34) and preterm delivery (35;36). Iron deficiency may also increase oxidative stress resulting in oxidative damage to erythrocytes and the feto-placental unit. Oxidative stress is one mechanism

thought to cause pregnancy-induced hypertension, preeclampsia, and gestational diabetes (37;38), which are known risk factors of preterm birth and perinatal death. Furthermore, iron deficiency may increase the risk of maternal infections. The products of the activated immune system of mothers with infection may have crossed the placenta and activated the fetal hypothalamic-pituitary-adrenal axis (39). Thus, in turn, triggers the onset of spontaneous preterm labor. Maternal infection is also particularly associated with preterm premature rupture of membranes (40).

There are equivocal findings in regarding maternal anemia and perinatal mortality. Among a few retrospective cohort studies from developing countries, some reported increased risks of stillbirth and perinatal mortality in relation to maternal anemia (41-43); but not others (9;44-47), including 3 studies conducted in China (9;45;46). The few studies examining the association between anemia and stillbirth (48-50) or perinatal death (8;51) in industrialized countries also provide inconsistent results.

The lack of consistency in findings across studies may be due to variations in the timing of hemoglobin measurement. In mid- and late pregnancy, it may difficult to distinguish hemo-dilution and true iron deficiency anemia (52), therefore, separate assessment corresponding to the stage of pregnancy may reveal important insights of the associations. A Chinese study (9) examined associations between anemia at the first prenatal visit or in the 3rd trimester and risk of perinatal mortality, and found a modest association in early pregnancy, but not the 3rd trimester. In contrast, 2 other studies (45;46) studies failed to distinguish the timing of anemia exposure and reported an absence of association between anemia and stillbirth or perinatal mortality (Table I – 3).

Studies that stratified these associations by trimester reported significant findings (50;51). Murphy et al. (51) reported higher perinatal mortality rates in relation to anemia (hemoglobin <10.4 g/dL) during 13-24 weeks of gestation based on their analysis of Cardiff Births Survey (1970-1982) data. A secondary analysis of the data from the United States National Maternal and Infant Health Survey (1988) data reported that mild anemia in the 1st or 2nd trimester was not associated with stillbirth, whereas moderate anemia was significantly associated with increased risk among non-black women (adjusted hazards ratio 4.4, 95% CI 1.02, 19.01) (50). However, both studies were conducted in industrialized countries and the prevalence rates of maternal anemia in their study populations were fairly low. Therefore the etiology of anemia may differ from developing countries, including China, and the generalizability of their findings could be limited.

To our knowledge, all previous studies categorized hemoglobin values using one or multiple cutoffs, instead of keep it as a continuous variable. For instance, in all 3 previous Chinese studies (9;45;46), pregnant women were classified as either anemic or non-anemic using hemoglobin cut-off of ≤10 g/dL. This kind of approach implicitly assumes that risks are homogeneous within category, and are different at the cut-point (53). The arbitrarily selected cutoff points, based on such assumption might be problematic in the relationship between maternal hemoglobin levels and perinatal mortality.

Maternal anemia and preterm birth clinical subtypes

Preterm birth remains one of the strongest determinants of perinatal mortality and morbidity (31-33) in developed countries. As described earlier (Figure I – 1), maternal infection, hypoxia, and oxidative stress have been the 3 major postulated biological mechanisms by which anemia and iron deficiency could cause preterm birth (3). Despite numerous studies, the role of maternal anemia in preterm birth remains poorly defined. Anemia has been found to be associated with increased risks for preterm birth in some (8;54-58), but not other (9;46;59;60) studies. There was also a "U"-shaped relationship being widely reported (51;61-64). Similar to the studies on maternal anemia and perinatal mortality, many studies on the associations between anemia and preterm birth have failed to examine if the timing of anemia exposure during pregnancy exert independent effects on preterm birth. Therefore, the reported associations between anemia and preterm birth could have been influenced by the physiological hemo-dilution during pregnancy (15;60).

The findings from Chinese studies (9;46;54;58;64) on the relationship between anemia and preterm birth were also inconsistent. Four studies (9;54;58;64) were conducted in East China. In those studies, anemia was assessed by the stage of pregnancy, and preterm birth, however, was examined as an aggregated outcome (Table I – 4). While some reported anemia in the 1st trimester being associated with increased risk of preterm birth (54;58), others did not (9). The last study reported that only the 3rd trimester hemoglobin <7 g/dL (severe anemia) was associated with a marginally increased risk for preterm birth (64). The common and biggest limitation of preterm

birth studies is the failure to examine associations between anemia and within "risk sets" of preterm birth.

Although still controversial (65), considerable etiologic heterogeneity data have been reported, among preterm birth clinical subtypes, namely, spontaneous preterm birth (spontaneous onset of labor or following preterm premature rupture of membranes) and medically indicted preterm birth (31;66-69). However, too little has been done further to evaluate if the associations between anemia and preterm birth vary among its different clinical presentations (70). If maternal anemia is indeed associated with one preterm birth subtype, and not others, the association with preterm birth as an entity may be attenuated (71).

The associations between maternal anemia and preterm birth clinical subtypes in 5 studies (59;70;72-74) are summarized in Table I -5. Two of them examined anemia at first prenatal visit as one of the risk factors for different clinical subtypes and found not significant (72;74); one study in a iron- and folate-supplemented cohort found high hematocrit (>40%), instead of anemia, during 31-34 weeks was associated with increased risks for both spontaneous and medically indicated preterm births (59); two remaining studies focused on spontaneous preterm births, including preterm premature rupture of membranes, one reported a moderately increased risk in relation to 2nd trimester anemia, but not the 3rd trimester anemia (73), and the another one found an increased risk in relation to 3rd trimester anemia (70). All five studies were conducted in either the United States or the United Kingdom, where their study populations have lower maternal anemia prevalence rates but higher preterm birth incidence rates than those in East

China (9;54;58;64). Furthermore, all these earlier studies either dichotomized pregnant women as anemic or non-anemic using one cut-off point (9;72;73), or categorized them into multiple groups using several cut-off points (54;58;64;70;72;74). Categorization of a skewed-distributed exposure like anemia, assumes an implicit within-category homogeneity, degrades continuous exposure information and tends to be less accurate than other approaches that permit more flexible and robust analysis, such as restricted cubic spline analysis (53). Studies that stratify the timing of hemoglobin assessment by the stage of gestation, examine hemoglobin as a continuous measure, and separate preterm birth by its clinical subtypes, may likely reveal interesting clues to etiology and biologic mechanisms.

Research Questions

The goal of this dissertation is to understand the anemia during pregnancy and its impact on adverse perinatal outcomes. The dissertation is organized to address 3 specific research objectives, using data from a large, prospective, population-based Chinese cohort of pregnant women:

1. To describe the epidemiologic characteristics and risk factors associated with maternal anemia during pregnancy
2. To examine the association between maternal anemia and perinatal mortality, including stillbirth and early neonatal death.
3. To evaluate the association between maternal anemia and preterm birth and its clinical subtypes

Methods

Pregnancy Monitoring System, China (1993-96)

This is a prospective cohort study using the existing data from a population-based pregnancy-monitoring system established through a community interventional trial to prevent neural-tube defects in 21 counties in China. Every woman who resided in the project area and became pregnant was registered at their marital registration or first prenatal visit. At the time of entry to this project, a campaign program of peri-conceptional folic acid supplementation of 400 μg daily until the end of the 1st trimester was offered to women before pregnancy or during the 1st trimester. The project was approved by the institutional review boards of both the Centers for Disease Control and Prevention, and the Chinese Ministry of Health. Women who were pregnant and delivered between October 1993, and December 1996 were identified and monitored through the 42nd day following delivery. The data of the project included all women whose fetus or infant could be confirmed as either having or not having a neural tube defect (75-77).

The Perinatal Health Care Booklet, specifically designed for this project, served as the primary data collection tool. Every registered woman in the project area was issued the booklet at the entry to the project and was assigned a unique identification number. The data contained parental demographics and family history, maternal medical history and medical and obstetric conditions during the pregnancy, perinatal health outcomes, and perinatal health care utilization. Hemoglobin data were collected during a pre-marital physical exam before the marriage registration if applicable, and

repeatedly measured at the 1st, 2nd, and 3rd trimesters during pregnancy. The blood samples were collected by local health care professionals during the pre-marital exam or prenatal visits. Hemoglobin concentration was determined using the usual clinical methods, mostly by spectrophotometer. For women that had more than 1 hemoglobin assessment performed in the 2nd and 3rd trimesters, only the lowest value of that trimester was recorded. All data recorded in the individual booklets were computerized by trained staff at the county's maternal and child health care department. A standardized data entry application with built-in data checking filters was developed for the project and used by each county.

Cohort composition

Our study was restricted to a total of 172,414 women who delivered a singleton live birth or stillbirth between 20 and 44 completed weeks of gestation in the 13 counties of Zhejiang and Jiangsu provinces of East China. Among them, 164,667 women with at least 1 hemoglobin measure during pregnancy were included in the epidemiologic study of maternal anemia and the analysis for stillbirth; 153,952 women who delivered a live-born infant and were followed up to 28th days were included for the neonatal mortality analysis; 160,700 women who delivered live-born infants who had plausible gestational age and birthweight combination assessed by Alexander's algorithm (78) were included in the study for preterm birth (Figure I – 2).

This population in these 13 counties offers a unique opportunity to examine these research questions, because the hemoglobin levels were repeatedly measured and

recorded at each trimester before the pregnancy outcomes were determined. In addition, the population is relatively homogeneous with respect to maternal ethnicity, smoking, alcohol, and geographic altitude. Hookworm infection, malaria, and thalassemia are rare (54). During the time of this project, women had not been recommended routinely taking prenatal iron supplements in general practice. Instead, iron supplements were prescribed only when anemia was diagnosed (9;54).

Definition of outcomes and risk factors for maternal anemia

The definitions of outcomes that were examined in our study are provided in Table I – 6. Risk factors that were considered potentially associated with anemia during pregnancy included maternal age at delivery, parity (nulliparity and multiparity), maternal education (categorized as completion of high school or above, junior high school, and elementary school or less), maternal occupation (farmer, factory worker, and other), folic acid supplementation (400 μg daily) any time before pregnancy or during the 1st trimester, pre-pregnancy body mass index (kg/m^2, and categorized as <18.5, 18.5 to 24, and ≥25, corresponding to underweight, normal and overweight/obese (79), respectively), timing of entry to the project (either before or during pregnancy), timing of first prenatal visit (in the 1st, 2nd, or 3rd trimesters), vaginal bleeding in the 1st or 2nd trimesters, and pregnancy-induced hypertension (none, mild, and moderate or preeclampsia or eclampsia). Mild pregnancy-induced hypertension was defined as blood pressure ≥130/90 mmHg or an increase of ≥30/15 mmHg from baseline, and moderate as blood pressure ≥140/100 mmHg. Except for vaginal bleeding and

pregnancy-induced hypertension, other factors were also considered as potential confounding factors for the studies of anemia and adverse pregnancy outcomes. We found vaginal bleeding in the 1st or 2nd trimesters was not associated with maternal anemia in our cohort. We did not adjust for pregnancy-induced hypertension, due to the concern that it is likely on the causal pathway of the exposure-disease relationship.

It is likely that the present study may be affected by residual confounding, such as iron supplement use, smoking, and alcohol consumption. Smoking and alcohol consumption were rare in this population, the estimated exposure rates were less than 1% based on a pilot survey in the project area (personal communication, Zhu Li). However potential passive smoking could be much more prevalent than active smoking. Prenatal iron supplements were only prescribed to women with anemia as a treatment at the time of this project. If this intervention was effective, it might have attenuated the association between anemia and the adverse pregnancy outcomes.

Statistical analysis

In the unadjusted analysis, χ^2 test was used for rate comparisons, and student's t test and ANOVA were used for means. We used the fetuses-at-risk approach (80) to estimate the gestational age-specific perinatal mortality rate. Associations between the risk factors and anemia were based on prevalence rate ratios, both unadjusted and adjusted, derived from fitting log-Binomial regression models. Factors that were found to be associated with anemia ($P<0.05$) in the unadjusted analysis were included in the multiple regression models. It included maternal age at delivery, education, occupation, parity,

folic acid supplementation, pre-pregnancy BMI, pregnancy-induced hypertension, timing of entry to project, and timing of fist prenatal visit. Cox proportional hazards models were used to estimate the unadjusted and adjusted hazard ratios for perinatal mortality and preterm birth where the outcomes were associated with the weeks of gestation at delivery or days after birth. The above factors, except for pregnancy-induced hypertension, and fetal gender were adjusted for in the multivariable regression models.

Anemia status within and across trimesters was categorized using the hemoglobin <10g/dL as the cut-off point. Furthermore, after determining a non-linear association, we keep hemoglobin value in each trimester as a continuous variable, and modeled it with restricted cubic spline transformations (81;82) of 4 knots at approximately the 5^{th}, 35^{th}, 65^{th}, and 95^{th} percentile. The number of knots was determined by comparing the Akaike's information criterion (81) of nested multivariable Cox proportional hazards regression models. The model with 4 knots had the smallest Akaike's information criterion. We also applied spline transformation on maternal age to deal with its non-linear relationship with outcomes.

As a sensitivity analysis, we explored the multiple imputation method (83;84) to replace the missing hemoglobin value among women with the other 2 of 3 trimesters hemoglobin measurement during pregnancy. Other sensitivity analyses were conducted using a different anemia cut-off value, or using underlining subgroups of pregnant women. In our analysis, we cautiously retained heterogeneous exposures and outcomes as separate entities and also used the conventional approaches that aggregated them to

provide a broader picture of the associations between maternal anemia and adverse pregnancy outcomes. We also used the change of hemoglobin across trimester as the proxy of hemo-dilution, and assessed the effects of hemo-dilution on adverse outcomes.

References

(1) Rasmussen K. Is there a causal relationship between iron deficiency or iron-deficiency anemia and weight at birth, length of gestation and perinatal mortality? J Nutr 2001; 131(2S-2):5905-603s.

(2) Brabin BJ, Hakimi M, Pelletier D. An analysis of anemia and pregnancy-related maternal mortality. J Nutr 2001; 131(2S-2):604s-615s.

(3) Allen LH. Biological mechanisms that might underlie iron's effects on fetal growth and preterm birth. J Nutr 2001; 131(2S-2):581s-589s.

(4) Corwin EJ, Murray-Kolb LE, Beard JL. Low hemoglobin level is a risk factor for postpartum depression. J Nutr 2003; 133(12):4139-4142.

(5) World Health Organization. Iron deficiency anaemia assessment, prevention, and control A guide for programme managers. WHO/NHD/01 3 2001;1-132.

(6) Adam I, Khamis AH, Elbashir MI. Prevalence and risk factors for anaemia in pregnant women of eastern Sudan. Trans R Soc Trop Med Hyg 2005; 99(10):739-743.

(7) CDC. Recommendations to prevent and control iron deficiency in the United States. Morbidity and Mortality Weekly Report 1998; 47(No. RR-3):1-30.

(8) Levy A, Fraser D, Katz M, Mazor M, Sheiner E. Maternal anemia during pregnancy is an independent risk factor for low birthweight and preterm delivery. Eur J Obstet Gynecol Reprod Biol 2005; 122(2):182-186.

(9) Xiong X, Buekens P, Fraser WD, Guo Z. Anemia during pregnancy in a Chinese population. Int J Gynaecol Obstet 2003; 83(2):159-164.

(10) Capital Institute of Pediatrics C. [Survey of women anaemia status in child-bearing age in China in 1998]. Chinese Journal of Reproductive Health 2002; 13(3):102-107.

(11) Liao Qk. [Prevalence of iron deficiency in pregnant and premenopausal women in China: a nationwide epidemiological survey]. Zhonghua Xue Ye Xue Za Zhi 2004; 25(11):653-657.

(12) Hytten F. Blood volume changes in normal pregnancy. Clin Haematol 1985; 14(3):601-612.

(13) Bothwell TH. Iron requirements in pregnancy and strategies to meet them. Am J Clin Nutr 2000; 72(1 Suppl):257S-264S.

(14) Whittaker PG, Macphail S, Lind T. Serial hematologic changes and pregnancy outcome. Obstet Gynecol 1996; 88(1):33-39.

(15) Scholl TO, Hediger ML. Anemia and iron-deficiency anemia: compilation of data on pregnancy outcome. Am J Clin Nutr 1994; 59(2 Suppl):S492-S501.

(16) Thomsen JK, Prien-Larsen JC, Devantier A, Fogh-Andersen N. Low dose iron supplementation does not cover the need for iron during pregnancy. Acta Obstet Gynecol Scand 1993; 72(2):93-98.

(17) Hinderaker SG, Olsen BE, Lie RT et al. Anemia in pregnancy in rural Tanzania: associations with micronutrients status and infections. Eur J Clin Nutr 2002; 56(3):192-199.

(18) Marti-Carvajal A, Pena-Marti G, Comunian GC, Munoz S. Prevalence of anemia during pregnancy: Results of Valencia (Venezuela) anemia during pregnancy study. Arch Latinoam Nutr 2002; 52(1):5-11.

(19) Verhoeff FH, Brabin BJ, Chimsuku L, Kazembe P, Broadhead RL. An analysis of the determinants of anaemia in pregnant women in rural Malawi – a basis for action. Ann Trop Med Parasitol 1999; 93(2):119-133.

(20) Bergmann RL, Gravens-Muller L, Hertwig K et al. Iron deficiency is prevalent in a sample of pregnant women at delivery in Germany. Eur J Obstet Gynecol Reprod Biol 2002; 102(2):155-160.

(21) Rasmussen K. Is there a causal relationship between iron deficiency or iron-deficiency anemia and weight at birth, length of gestation and perinatal mortality? J Nutr 2001; 131(2S-2):5905-603s.

(22) Li S, Tang Y, Wu S. [Analysis of anemia causes during pregnancy and its effects on mothers and fetus]. Journal of Clinical Hematology 1994; 7(2):54-56.

(23) Bondevik GT, Eskeland B, Ulvik RJ et al. Anaemia in pregnancy: possible causes and risk factors in Nepali women. Eur J Clin Nutr 2000; 54(1):3-8.

(24) Dreyfuss ML, Stoltzfus RJ, Shrestha JB et al. Hookworms, malaria and vitamin A deficiency contribute to anemia and iron deficiency among pregnant women in the plains of Nepal. J Nutr 2000; 130(10):2527-2536.

(25) Bondevik GT, Ulstein M, Lie RT, Rana G, Kvale G. The prevalence of anemia in pregnant Nepali women – a study in Kathmandu. Acta Obstet Gynecol Scand 2000; 79(5):341-349.

(26) Robinson S, Godfrey K, Denne J, Cox V. The determinants of iron status in early pregnancy. Br J Nutr 1998; 79(3):249-255.

(27) Agarwal KN, Agarwal DK, Sharma A et al. Prevalence of anaemia in pregnant & lactating women in India. Indian J Med Res 2006; 124(2):173-184.

(28) Selo-Ojeme DO. Anemia in pregnancy: case control study of risk factors. Int J Gynaecol Obstet 1997; 59(1):53-54.

(29) Conde-Agudelo A, Belizan JM, Lindmark G. Maternal morbidity and mortality associated with multiple gestations. Obstet Gynecol 2000; 95(6 Pt 1):899-904.

(30) Conde-Agudelo A, Belizan JM. Maternal morbidity and mortality associated with interpregnancy interval: cross sectional study. BMJ 2000; 321(7271):1255-1259.

(31) Ananth CV, Vintzileos AM. Epidemiology of preterm birth and its clinical subtypes. J Matern Fetal Neonatal Med 2006; 19(12):773-782.

(32) Kramer MS, Demissie K, Yang H, Platt RW, Sauve R, Liston R. The contribution of mild and moderate preterm birth to infant mortality. Fetal and Infant Health Study Group of the Canadian Perinatal Surveillance System. JAMA 2000; 284(7):843-849.

(33) Slattery MM, Morrison JJ. Preterm delivery. Lancet 2002; 360(9344):1489-1497.

(34) Smith R. Alterations in the hypothalamic pituitary adrenal axis during pregnancy and the placental clock that determines the length of parturition. J Reprod Immunol 1998; 39:215-220.

(35) Sandman CA, Wadhwa PD, Chicz-DeMet A, Dunkel-Schetter C, Porto M. Maternal stress, HPA activity, and fetal/infant outcome. Ann N Y Acad Sci 1997; 814:266-275.

(36) McLean M, Bisits A, Davies J, Woods R, Lowry P, Smith R. A placental clock controlling the length of human pregnancy. Nat Med 1995; 1(5):460-463.

(37) Cester N, Staffolani R, Rabini RA et al. Pregnancy induced hypertension: a role for peroxidation in microvillus plasma membranes. Mol Cell Biochem 1994; 131(2):151-155.

(38) Poranen AK, Ekblad U, Uotila P, Ahotupa M. Lipid peroxidation and antioxidants in normal and pre-eclamptic pregnancies. Placenta 1996; 17(7):401-405.

(39) Falkenberg ER, Davis RO, DuBard M, Parker CRJ. Effects of maternal infections on fetal adrenal steroid production. Endocr Res 1999; 25(3-4):239-249.

(40) Romero R, Gomez R, Chaiworapongsa T, Conoscenti G, Kim JC, Kim YM. The role of infection in preterm labour and delivery. Paediatr Perinat Epidemiol 2001; 15 Suppl 2:41-56.

(41) Conde-Agudelo A, Belizan JM, az-Rossello JL. Epidemiology of fetal death in Latin America. Acta Obstet Gynecol Scand 2000; 79(5):371-378.

(42) Geelhoed D, Agadzi F, Visser L et al. Maternal and fetal outcome after severe anemia in pregnancy in rural Ghana. Acta Obstet Gynecol Scand 2006; 85(1):49-55.

(43) Watson-Jones D, Weiss HA, Changalucha JM et al. Adverse birth outcomes in United Republic of Tanzania – impact and prevention of maternal risk factors. Bull World Health Organ 2007; 85(1):9-18.

(44) Aimakhu CO, Olayemi O. Maternal haematocrit and pregnancy outcome in Nigerian women. West Afr J Med 2003; 22(1).

(45) Zhang J, Cai WW. Risk factors associated with antepartum fetal death. Early Hum Dev 1992; 28(3):193-200.

(46) Lao TT, Pun T-C. Anaemia in pregnancy – is the current definition meaningful? Eur J Obstet Gynecol Reprod Biol 1996; 68:53-58.

(47) Lone FW, Qureshi RN, Emanuel F. Maternal anaemia and its impact on perinatal outcome. Trop Med Int Health 2004; 9(4):486-490.

(48) Garn SM, Ridella SA, Petzold AS, Falkner F. Maternal hematologic levels and pregnancy outcomes. Semin Perinatol 1981; 5(2):155-162.

(49) Stephansson O, Dickman PW, Johansson A, Cnattingius S. Maternal hemoglobin concentration during pregnancy and risk of stillbirth. JAMA 2000; 284(20):2611-2617.

(50) Tomashek KM, Ananth CV, Cogswell ME. Risk of stillbirth in relation to maternal haemoglobin concentration during pregnancy. Matern Child Nutr 2006; 2:19-28.

(51) Murphy JF, O'Riordan J, Newcombe RG, Coles EC, Pearson JF. Relation of haemoglobin levels in first and second trimesters to outcome of pregnancy. Lancet 1986; 1(8488):992-995.

(52) Scholl TO. Iron status during pregnancy: setting the stage for mother and infant. Am J Clin Nutr 2005; 81(5).

(53) Greenland S. Problems in the average-risk interpretation of categorical dose-response analyses. Epidemiology 1995; 6(5):563-565.

(54) Ren A, Wang J, Ye RW, Li S, Liu JM, Li Z. Low first-trimester hemoglobin and low birth weight, preterm birth and small for gestational age newborns. Int J Gynaecol Obstet 2007; 98(2):124-128.

(55) Scanlon KS, Yip R, Schieve LA, Cogswell ME. High and low hemoglobin levels during pregnancy: differential risks for preterm birth and small for gestational age. Obstet Gynecol 2000; 96(5 Pt 1):741-748.

(56) Scholl TO, Hediger ML, Fischer RL, Shearer JW. Anemia vs iron deficiency: increased risk of preterm delivery in a prospective study. Am J Clin Nutr 1992; 55(5):985-988.

(57) Lieberman E, Ryan KJ, Monson RR, Schoenbaum SC. Association of maternal hematocrit with premature labor. Am J Obstet Gynecol 1988; 159(1):107-114.

(58) Zhou LM, Yang WW, Hua JZ, Deng CQ, Tao XG, Stoltzfus RJ. Relation of hemoglobin measured at different times in pregnancy to preterm birth and low birth weight in Shanghai, China. Am J Epidemiol 1998; 148(10):998-1006.

(59) Lu ZM, Goldenberg RL, Cliver SP, Cutter G, Blankson M. The relationship between maternal hematocrit and pregnancy outcome. Obstet Gynecol 1991; 77(2):190-194.

(60) Klebanoff MA, Shiono PH, Berendes HW, Rhoads GG. Facts and artifacts about anemia and preterm delivery. JAMA 1989; 262(4):511-515.

(61) Chang SC, O'Brien KO, Nathanson MS, Mancini J, Witter FR. Hemoglobin concentrations influence birth outcomes in pregnant African-American adolescents. J Nutr 2003; 133(7):2348-2355.

(62) Knottnerus JA, Delgado LR, Knipschild PG, Essed GG, Smits F. Haematologic parameters and pregnancy outcome. A prospective cohort study in the third trimester. J Clin Epidemiol 1990; 43(5):461-466.

(63) Steer P, Alam MA, Wadsworth J, Welch A. Relation between maternal haemoglobin concentration and birth weight in different ethnic groups. BMJ 1995; 310(6978):489-491.

(64) Wang J, Ren Ag, Ye Rw et al. [Study on the third trimester hemoglobin concentrations and the risk of low birth weight and preterm delivery]. Zhonghua Liu Xing Bing Xue Za Zhi 2007; 28(1):15-18.

(65) Klebanoff MA, Shiono PH. Top-down, bottom up and inside-out – reflections on preterm birth. Paediatr Perinat Epidemiol 1995; 9(2):125-129.

(66) Berkowitz GS, Blackmore-Prince C, Lapinski RH, Savitz DA. Risk factors for preterm birth subtypes. Epidemiology 1998; 9(3):279-285.

(67) Pickett KE, Abrams B, Selvin S. Defining preterm delivery – the epidemiology of clinical presentation. Paediatr Perinat Epidemiol 2000; 14(4):305-308.

(68) Zhang J, Savitz DA. Preterm birth subtypes among blacks and whites. Epidemiology 1992; 3(5):428-433.

(69) Tucker JM, Goldenberg RL, Davis RO, Copper RL, Winkler CL, Hauth JC. Etiologies of preterm birth in an indigent population: is prevention a logical expectation? Obstet Gynecol 1991; 77(3):343-347.

(70) Siega-Riz AM, Adair LS, Hobel CJ. Maternal hematologic changes during pregnancy and the effect of iron status on preterm delivery in a West Los Angeles population. Am J Perinatol 1998; 15(9):515-522.

(71) Savitz DA, Blackmore CA, Thorp JM. Epidemiologic characteristics of preterm delivery: etiologic heterogeneity. Am J Obstet Gynecol 1991; 164(2):467-471.

(72) Adams MM, Sarno AP, Harlass FE, Rawlings JS, Read JA. Risk factors for preterm delivery in a healthy cohort. Epidemiology 1995; 6(5):525-532.

(73) Klebanoff MA, Shiono PH, Selby JV, Trachtenberg AI, Graubard BI. Anemia and spontaneous preterm birth. Am J Obstet Gynecol 1991; 164(1 Pt 1):59-63.

(74) Meis PJ, Michielutte R, Peters TJ et al. Factors associated with preterm birth in Cardiff, Wales. II. Indicated and spontaneous preterm birth. Am J Obstet Gynecol 1995; 173(2):597-602.

(75) Berry RJ, Li Z, Erickson JD et al. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. N Engl J Med 1999; 341(20):1485-1490.

(76) Li S, Moore CA, Li Z et al. A population-based birth defects surveillance system in the People's Republic of China. Paediatr Perinat Epidemiol 2003; 17(3):289-293.

(77) Li Z, Berry RJ, Li S. [Preventing neural tube defects with periconceptional folic acid supplementation: a population-based intervention program in the China]. Zhonghua Yi Xue Za Zhi 2000; 80(7):493-498.

(78) Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. Obstet Gynecol 1996; 87(2):163-168.

(79) World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO Consultation. 894. 2000. Geneva, World Health Organization. WHO Technical Report Series.
Ref Type: Report

(80) Joseph KS. Incidence-based measures of birth, growth restriction, and death can free perinatal epidemiology from erroneous concepts of risk. J Clin Epidemiol 2004; 57(9):889-897.

(81) Harrell FE. Regression modeling strategies. New York: Springer-Verlag, 2001.

(82) Durrleman S, Simon R. Flexible regression models with cubic splines. Stat Med 1989; 8(5):551-561.

(83) Raghunathan TE. What do we do with missing data? Some options for analysis of incomplete data. Annu Rev Public Health 2004; 25:99-117.

(84) Schafer JL. Multiple imputation: a primer. Stat Methods Med Res 1999; 8:3-15.

(85) van den Broek NR, Rogerson SJ, Mhango CG, Kambala B, White SA, Molyneux ME. Anaemia in pregnancy in southern Malawi: prevalence and risk factors. Br J Obstet Gynaecol 2000; 107(4):445-451.

(86) Hinderaker SG, Olsen BE, Bergsjo P, Lie RT, Gasheka P, Kvale G. Anemia in pregnancy in the highlands of Tanzania. Acta Obstet Gynecol Scand 2001; 80:18-26.

Table I - 1

Reported prevalence rates of and risk factors for maternal anemia from studies conducted in developing countries

Study site, Authors, (Reference)	Sample size, Study setting, Years of study	Timing of anemia assessment	Anemia definition or cut-offs	Anemia prevalence rates	Risk factors for anemia* Odds Ratio (95% confidence interval)
China Capital Institute of Pediatrics, 2002 (10)	n=19,185 National survey on a random sample of women aged 15 – 49 years 1998	Cross-sectional	Hemoglobin <12 g/dL for non-pregnant and <11 g/dL for pregnant women	35.6% for non-pregnant and 42.1% for pregnant women	Resided in rural or remote area
China Liao, *et al* 2004 (11)	n=7,312 National survey on a random sample of women aged 20 – 29 years 2000	Cross-sectional	Hemoglobin <12 g/dL for non-pregnant and <11 g/dL for pregnant women, and serum ferritin <20 μg/L	15.1% for non-pregnant and 9.6%, 19.8%, and 33.8% for the 1st , 2nd, and 3rd trimesters, respectively	Resided in urban
Suzhou, China Xiong, *et al* 2003 (9)	n=16,936 A retrospective cohort study 1989 – 1990	First prenatal visit and the 3rd trimester	Hemoglobin <10 g/dL	Overall 26.2%; 10.3% in the first trimester, and 18.9% in the 3rd trimester	Maternal age ≥30 years; initiate prenatal care >12 weeks; BMI <20; high school or higher education; prenatal visits ≤ 14 times.

Beijing, China Li, *et al* 1994 (22)	n=227 Hospital-based random sample of pregnant women aged 23-36 years 1988 – 1989	Each trimester, depended on the stage of pregnancy at entry	Hemoglobin <11 g/dL	Overall 30.4%; 0%, 5.2%, and 56.4% for the 1st , 2nd, and 3rd trimesters, respectively	Deficiencies of iron, folic acid, and vitamin B_{12}
Eastern Sudan Adam, *et al* 2005 (6)	n=744 Hospital-based pregnant women 2003 – 2004	Each trimester, depended on availability	Hemoglobin <11 g/dL	Overall 62.6%; 44.4%, 56.2%, and 67.7% for the 1st , 2nd, and 3rd trimesters, respectively	Malaria: 4.5 (2.5, 8.1); Pica: 1.7 (1.1, 2.6)
Kathmandu, Nepal Bondevik, *et al* 2000 (25)	n=2,280 Hospital-based pregnant women 1994 – 1995	At first prenatal visit	Hematocrit ≤ 34% for the 1st and 3rd trimesters and ≤33% for the 2nd trimester	Overall 62.2%; 53.1%, 65.4%, and 73.0% for the 1st , 2nd, and 3rd trimesters, respectively	Maternal height: 0.7 (0.5, 0.9) per 5 cm increase; BMI: 0.8 (0.6, 0.96) per 2 kg/m^2 increase
Seven states, India Agarwal, *et al* 2006 (11)	n=1,751 A random sample of pregnant or lactating women 2001 – 2003	Cross-sectional	Hemoglobin <11 g/dL for pregnant women	Overall 84%; ranged 57.8% to 97% by state	Illiteracy; younger age at marriage; working as laborer; lower height; multiparity; no routine iron and folate supplement; and unaware of anemia

Southern Malawi, van den Broek, *et al* 2000 (85)	n=7,436 Pregnant women attending a semi-urban hospital and a rural prenatal clinic 1997 – 1999	At first prenatal visit	Hemoglobin <11 g/dL	72% in rural area and 57% in urban area	Primigravida: 1.9 (1.5, 2.4) in urban area; 2.3 (1.7, 3.3) in rural area
Valencia, Venezuela Marti-Carvajal, *et al* 2002 (18)	n=630 Cross-sectional study of a hospital-based cohort, 1996	At labor in the 3rd trimester	Hemoglobin <11 g/dL	Overall 34.4%	Multiparity: 2.0 (1.3, 3.0)
Highlands of Tanzania, Hinderaker, *et al* 2001 (86)	n=3,836 Cross-sectional study of a hospital-based cohort 1995 – 1996	Cross-sectional	Hemoglobin <11 g/dL	Overall 23%	Higher maternal age: 1.2 per 5 years increase, p<0.05; Rainy season: 6..0, p <0.001; Malaria: 1.8, p<0.01
Dhaka, Bangladesh Ahmed, *et al* 2003 (11)	n=383 Pregnant women aged 20 – 30 years attending 1 clinic 2000	Cross-sectional	Hemoglobin <11 g/dL	Overall 40%	Vitamin A deficiency: 1.8, p<0.05

* If odds ratios were not provided, risk factors were reported by the authors based on unadjusted prevalence rates.

Table I - 2

Evidence and strength of association linking major risk factors with anemia based on published studies

Risk factors	Range of relative risk or odds ratio	Strength of association
Age <25 years	1.4-2.9	+
Multiparity	0.7 - 2.0	+/-
Primigravida	1.9 - 2.3	+
Twin	1.8 - 4.8	++
Interpregnancy interval <5 months	1.3	+
No iron supplementation	1.8 - 3.3	++
Vitamin A deficiency	1.6- 5.3	++
Malaria	1.7- 4.5	++
Hookworm	5.4-7.5	+++
Pica in pregnancy	1.7	+

Table I - 3

Chinese Studies related to the association between maternal anemia and perinatal mortality

Study city, Authors, (Reference)	Sample size, Study setting, Years of study	Timing of anemia assessment	Anemia definition or cut-offs	Perinatal mortality rate (per 1,000 births)	Odds Ratio (95% confidence interval) for perinatal morality in relation to anemia
Shanghai Zhang, *et al* 1992 (45)	n=856 A case-control study in 29 hospitals 1986 - 1987	Anytime during pregnancy	Hemoglobin ≤10 g/dL	-	For antepartum stillbirth: 1.3 (0.8, 2.1)
Hong Kong Lao, *et al* 1996 (46)	n=10,942 Hospital-based retrospective cohort with routine multivitamin and iron supplement 1990 – 1992	Lowest hemoglobin value during pregnancy	Hemoglobin <10 g/dL	anemic vs. non-anemic: 10 vs. 12	-
Suzhou Xiong, *et al* 2003 (9)	n=16,936 A retrospective cohort study 1989 – 1990	First prenatal visit and the 3rd trimester	Hemoglobin <10 g/dL	At first visit anemic vs. non-anemic: 14 vs. 9; In the 3rd trimester, 12 vs. 10, respectively	Anemia at first visit: 1.5 (0.9, 2.5); Anemia in the 3rd trimester: 1.2 (0.8, 1.8)

Table I - 4

Chinese studies related to the associations between maternal anemia and preterm birth

Study city, Authors, (Reference)	Sample size, Study setting, Years of study	Timing of anemia assessment	Anemia definition or cut-offs	Preterm birth rate	Odds Ratio (95% confidence interval) for preterm birth in relation to anemia
Shanghai Zhou, *et al* 1998 (58)	n=829 Random sample cohort study 1991 - 1992	The 1st trimester; the 5th and 8th month	Hemoglobin <11 g/dL	6.9%	Anemia in the 1st trimester: 2.1 (1.0, 4.1)
Suzhou Xiong, *et al* 2003 (9)	n=16,936 A retrospective cohort study 1989 – 1990	First prenatal visit and the 3rd trimester	Hemoglobin <10 g/dL	4.5%	Anemia at first visit: 1.0 (0.8, 1.4); Anemia in the 3rd trimester: 0.7 (0.6, 0.9)
4 cities/county, East China Ren, *et al* 2007 (54)	n=88,149 Population-based, prospective cohort study 1995 – 2000	The 1st trimester	Hemoglobin <11 g/dL	4.5%	Overall: 1.2 (1.1, 1.3); Hemoglobin <8 g/dL: 1.9 (0.9, 3.8); Hemoglobin 8-9.9 g/dL: 1.3 (1.2, 1.6) Hemoglobin 10-11.9 =ref
4 cities/county, East China Wang, *et al* 2007 (64)	n=95,102 Population-based, prospective cohort study 1995 – 2000	The 3rd trimester	Hemoglobin <11 g/dL	4.5%	Hemoglobin <7 g/dL: 1.8 (1.0, 3.3) Hemoglobin 9-9 .9=ref

Table I - 5

Previous studies on maternal anemia and preterm birth clinical subtypes

Authors, year, (Reference)	Sample size, Study setting, Years of study	Timing of anemia assessment	Anemia definition or cut-offs	Preterm birth clinical subtypes (rate)	Results in relation to anemia Odds Ratio (95% confidence interval)
Klebanoff *et al* 1991 (73)	n=1,706 Two-stage case-control design in a multiethnic cohort from California Kaiser Permanente Birth Defects Study 1974-77	13-36 weeks, repeated every 2 weeks	<10th percentile for specific ethnic group in each 2-week interval	Spontaneous preterm birth (not applicable)	4.3 (1.2, 1.5) at 17-18 weeks 3.5 (1.1, 11.3) at 25-26 weeks Not significant at other intervals
Lu *et al* 1991 (59)	n=17,149 Medical record system at Birmingham, AL black and white iron- and folate-supplemented 1983-88	At first prenatal visit and repeated every 4-8 weeks	Hematocrit <30%; 30-32%; 33-36%; 37-39%; 40-42%; ≥43%	All preterm birth (12.6%) Spontaneous preterm birth and Medically indicated	No associations with anemia

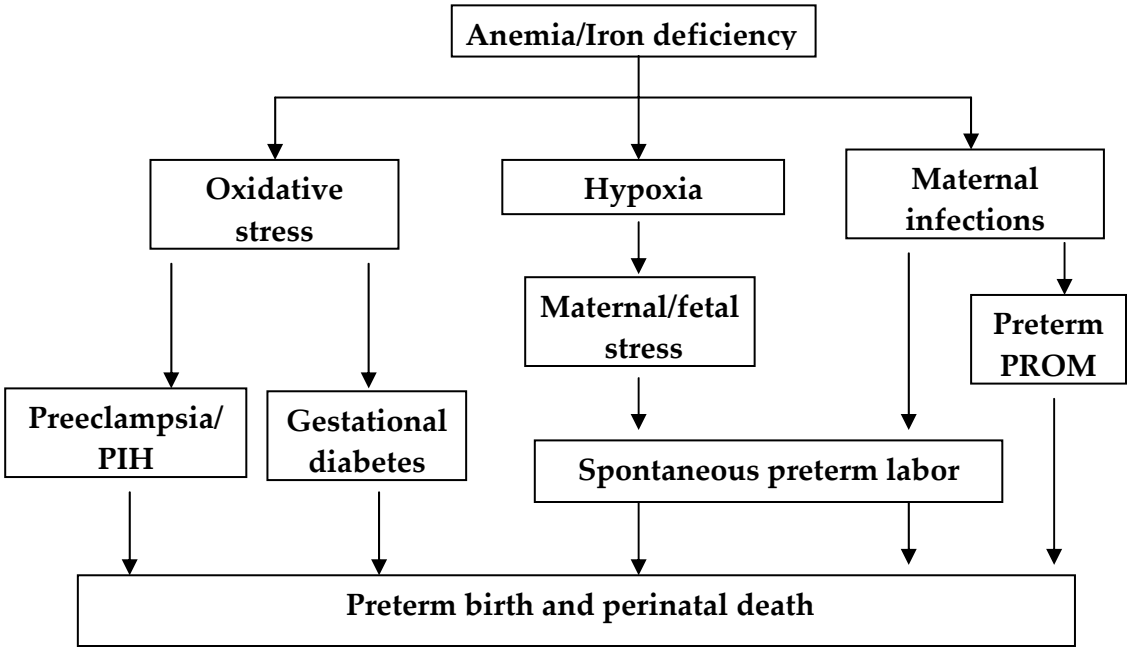
Adams *et al.* 1995 (72)	n=1,825 Servicewoman delivered at 4 US army medical centers black and white 1987 – 1990	At first prenatal visit	Hemoglobin <11 g/dL for 1st and 3rd trimester ; <10.5 g/dL for 2nd trimester	Spontaneous preterm birth (7.1%) and Medically indicated (3%)	1.2 (0.8, 2.0) for spontaneous preterm birth 1.0 (0.5, 2.0) for medically indicated preterm birth
Meis *et al.* 1995 (74)	n=39,449 Singleton births to residents of Cardiff, Wales 1970 – 1979	At first prenatal visit	Hemoglobin <10.4 g/dL	Spontaneous preterm birth (2.6%) and Medically indicated (1.7%)	1.3 (0.9, 2.0) for spontaneous preterm birth 1.0 (0.5, 1.7) for indicated preterm birth
Siega-Riz *et al.* 1998 (70)	n=6,873 Predominantly Hispanic population in served by public health clinics in West Los Angeles Iron-supplemented 1983 – 1986	At first prenatal visit and repeated at 28-32 weeks	Hemoglobin <11 g/dL for 1st and 3rd trimesters; <10.5 g/dL for 2nd trimester	Spontaneous preterm labor (4%) and Preterm PROM (1%)	No association at initial evaluation At 28-32 weeks: 1.8 (1.2, 2.8) for spontaneous preterm birth 1.8 (1.0, 3.0) for spontaneous preterm labor 2.3 (0.9, 5.5) for preterm PROM

Table I - 6

Definitions of outcomes in this dissertation

Outcomes	Definitions
Maternal anemia during pregnancy	Hemoglobin <10 g/dL, regardless of stage of pregnancy
Mild to moderate anemia	Hemoglobin 8 to <10 g/dL
Severe anemia	Hemoglobin <8 g/dL
Live birth	A baby who shows any sign of life after delivery, such as breathing, heart beat, pulsation of umbilical cord regardless of whether the umbilical cord or placenta were intact
Stillbirth	Intrauterine death of fetus at ≥ 20 weeks of gestation
Antepartum stillbirth	Fetal death before the onset of labor
Intrapartum stillbirth	Fetal death during labor
Neonatal death	Death of a live-born infant within the first 27 days (≤ 27)
Early neonatal death	Death of a live-born infant at ≤6 days
Late neonatal death	Death of a live-born infant at 7-27 days
Perinatal mortality	Any stillbirth at ≥20 weeks of gestation and neonatal death during 0 to 6 days (≤ 6) after birth

Preterm birth	Delivery at <37 weeks, resulting in live-born infants
Preterm premature rupture of membrane	Spontaneous rupture of chorioamniotic membranes prior to the onset of labor and delivery at <37 weeks
Medically indicated preterm birth	Preterm birth following labor induction and/or cesarean performed before onset of labor, presumably performed for impending in utero fetal compromise
Spontaneous preterm labor	Preterm birth following spontaneous onset of labor
Very preterm birth	Preterm birth at <32 weeks of gestation
Moderate preterm birth	Preterm birth at 32-33 weeks of gestation
Mild preterm birth	Preterm birth at 34-36 weeks of gestation



PIH, Pregnancy-induced hypertension; PROM, premature rupture of membranes

Figure I - 1.
Suggested biologic mechanisms linking maternal anemia and preterm birth and perinatal morality

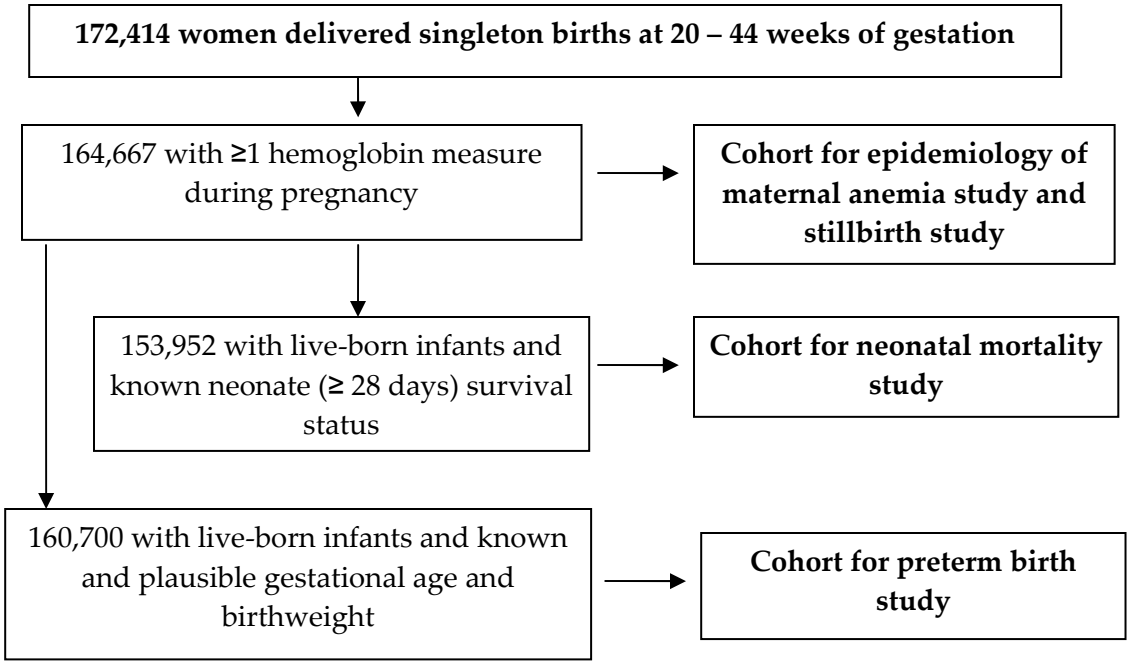


Figure I - 2
Study cohort selection flowchart

Chapter 1

Prevalence and Risk Factors of Anemia in Pregnant Women: A Population-based Prospective Cohort Study in China

Abstract

Objective: Maternal anemia is the most common pregnancy complication in developing countries; however, its epidemiology remains largely unexplored in China. Our study was designed to explore epidemiologic characteristics and risk factors of maternal anemia during pregnancy.

Methods: A prospective cohort study was conducted, using existing data from a population-based pregnancy-monitoring system in 13 counties in East China (1993-96). Women who delivered singleton infants at 20 to 44 weeks with at least one hemoglobin assessment during pregnancy were included (n=164,667). The prevalence of anemia (hemoglobin <10 g/dL) during pregnancy as well as in each trimester were estimated. Multivariable log-Binomial regression models were used to evaluate risk factors associated with anemia.

Results: The overall prevalence of anemia was 32.6%, with substantial variations across trimesters (11.2%, 20.1%, and 26.2% in the 1st, 2nd and 3rd trimesters, respectively). Risk factors for anemia included older maternal age, education below junior high schooling (relative risk (RR) 1.10, 95% confidence interval (CI) 1.08, 1.12), farmer (1.05, 95% CI 1.03, 1.06), and pregnancy-induced hypertension (RR 1.09, 95% CI 1.05, 1.13 for mild and RR 1.13, 95% CI 1.06, 1.19 for moderate/preeclampsia). Peri-conception folic acid use was associated with a reduced risk for anemia in the 1st trimester (RR 0.75, 95% CI 0.72, 0.78). Initiating prenatal care after the 1st trimester was associated with increased risk of anemia in the 2nd and 3rd trimesters.

Conclusion: Anemia during pregnancy is highly prevalent in this indigent Chinese population. The risk increases with the severity of hypertensive disorders. Folic acid supplementation during the peri-conception period is associated with reduced risk of 1st trimester anemia. Early prenatal care may reduce the risk of anemia later in pregnancy.

Introduction

Maternal anemia is a ubiquitous pregnancy complication in developing countries. The WHO estimated prevalence (1990-95) of anemia (hemoglobin <11 g/dL) based on hemoglobin concentration is approximately 23% in industrialized countries, with the prevalence in non-industrialized countries being at least twice as high (1). The prevalence of anemia in low-income pregnant women enrolled in public health programs in the United States in 1993 was 9%, 14%, and 37% in the 1st, 2nd, and 3rd trimesters, respectively (2). Anemia has been associated with a number of adverse pregnancy outcomes, including preterm birth, restricted fetal growth, and perinatal mortality (3). The reported associations are largely confined to studies in industrialized countries, and comparable studies in developing countries in general and China in particular, are lacking. In fact, little is known about anemia in pregnancies from China.

The etiology of pregnancy-associated anemia remains obscure, besides the known physiological hemo-dilution in normal pregnancy (4). The 3-fold increased demand for iron during pregnancy (2), and the disproportionate increase in plasma volume by 50% with a only 35% increase in red blood cell mass (5) can contribute to lower hemoglobin concentrations in pregnancy. Other reported risk factors for maternal anemia include deficiencies in iron (6-9), folic acid (3;7;10) and vitamin A (11;12), underweight (body mass index <18.5) (8;13;14), young maternal age (<25 years) (9;13;15), poorly educated (8;15), multiparity (7;8;14;16;17), twin pregnancy (16;18), short interpregnancy[cva1] interval (<5 months) (16;18), malaria (6;8;12;16;19) and hookworm (11;12) infections, and pica in pregnancy (19). Iron deficiency is the most prevalent cause,

affecting nearly two thirds of Chinese women during their pregnancy (20). Other risk factors reported in Chinese studies include deficiencies of folic acid and vitamin B_{12} (10), rural residence (20;21), maternal age ≥30 years, low BMI (<20), late onset of prenatal care (after first trimester), and ≤14 prenatal visits (22).

Given the gaps in the epidemiology of maternal anemia, and lack of data on its association with adverse outcomes in China, we assessed the rates of, and risk factors associated with, maternal anemia among pregnant women in a large prospective cohort in China of approximately 173,000 women. In particular, we evaluated if the profile of risk factors for anemia showed temporal heterogeneity across trimesters during pregnancy.

Material and Methods

Data source

This is a prospective cohort study using the existing data from a population-based pregnancy-monitoring system in China established through a community interventional trial to prevent neural-tube defects. The project was conducted in Hebei Province in North China with a relatively high incidence of neural tube defects and two provinces (Zhejiang and Jiangsu) in East China with lower rates, including an overall total of 21 counties. Every woman who resided in the project area and became pregnant was registered at their marital registration or first prenatal visit, and was routinely followed up by local health care professionals. At the time of entering this project, a program of peri-conception folic acid supplementation of 400 μg daily until the end of the 1st

trimester was offered to women before pregnancy or during the 1st trimester. The project was approved by the institutional review boards of both the Centers for Disease Control and Prevention, and the Chinese Ministry of Health. Women who were pregnant and delivered between October 1993, and December 1996 were identified and monitored through the 42nd day following delivery. Subjects included all women whose fetus or infant could be confirmed as either having or not having a neural tube defect (23-25).

The Perinatal Health Care Booklet, specifically designed for this project, served as the primary data collection tool. Every registered woman in the project area was issued the booklet and was assigned a unique identification number. The data contained parental demographics and family history, maternal medical history and medical conditions during the pregnancy, perinatal health outcomes, and perinatal health care utilization. Hemoglobin data were collected during a pre-marital physical exam before the marriage registration, and repeatedly at 1st, 2nd, and 3rd trimesters during pregnancy. The blood samples were collected by local health care professionals during the pre-marital exam or prenatal visits. Hemoglobin concentration was determined at each local laboratory using the usual clinical methods, mostly by spectrophotometer. For women that had more than 1 hemoglobin assessment performed in the 2nd and 3rd trimesters, only the lowest value of that trimester was recorded. All data recorded in the individual booklets were computerized by trained staff at the county's maternal and child health care department. A standardized data entry application with built-in data checking filters was developed for the project and used by each county.

Study cohort

Our study was restricted to a total of 172,414 women from the 13 counties of Zhejiang and Jiangsu provinces in East China that delivered a singleton live birth or stillbirth between 20 and 44 completed weeks. Among them, 164,667 women with at least 1 hemoglobin measure during pregnancy were included. Data from three (Fenghua, Yinxian, and Ninghai) of the 16 counties in East China were excluded due to concerns regarding external validity of hemoglobin measures (Personal communication, Zhu Li, 2007). Gestational age, in completed weeks, was estimated based on the last menstrual period.

The population in these 13 counties is relatively homogeneous with respect to maternal ethnicity, smoking, alcohol, and geographic altitude. The prevalence of smoking and alcohol use among Chinese women was very low (26;27), and pregnant women generally avoid these risky behaviors. Hookworm infection, malaria, and thalassemia are rare in this population (28). During the time the project was conducted (1993-96), the women in our cohort had not taken routine prenatal iron or multivitamin supplements. Iron was prescribed only for those who were diagnosed as anemic to be taken until the anemic status was corrected (22;28). However, the data on iron supplement use were not collected.

Definition of anemia

Anemia in each trimester was defined as hemoglobin level <10 g/dL, which was pre-specified in the original protocol and has been also a commonly used cut-off in China

(22). Severe anemia was defined as hemoglobin <8 g/dL. Any anemia was defined as the presence of anemia in any trimester during pregnancy. The WHO threshold recommendation of hemoglobin <11 g/dL (1) was used in a sensitivity analysis.

Risk factors for maternal anemia

Risk factors that were considered potentially associated with anemia during pregnancy and were examined included maternal age at delivery, maternal education (categorized as completion of high school or above, junior high school, and elementary school or less), occupation (farmer, factory worker, and other), parity (0, and 1 or more) , taking 400 μg daily folic acid supplement any time before pregnancy or during the 1st trimester, pre-pregnancy body mass index (kg/m^2, and categorized as <18.5, 18.5 to 24, and ≥25, corresponding to underweight, normal and overweight/obese, respectively), timing of entering to the project (either before or during pregnancy), timing of first prenatal visit (1st, 2nd, or 3rd trimesters), vaginal bleeding in the 1st or 2nd trimesters, and pregnancy-induced hypertension in the 3rd trimester (none, mild (blood pressure ≥130/90 mmHg or increased ≥30/15 mmHg from baseline), and preeclampsia, including moderate pregnancy-induced hypertension (blood pressure ≥140/100 mmHg), preeclampsia, and eclampsia).

Statistical analysis

The prevalence of maternal anemia overall and within trimesters was derived. Associations between the risk factors and anemia were based on prevalence rate ratios

(RR), both unadjusted and adjusted, derived from fitting log-Binomial regression models. Factors that were found to be associated with anemia ($P<0.05$) in the unadjusted analysis were included in the multiple regression models. We fitted log-Binomial regression models to facilitate direct estimation of prevalence rate ratios (29). We created an indicator variable for the missing value of each risk factor and included them in the models. After determining that the relationship between age and anemia was non-linear in the preliminary analysis, we modeled maternal age with restricted cubic spline transformations (30;31) of 4 knots at 21, 23, 25, and 31 years. The number of knots was determined by the values of Akaike's information criterion (30) of the multivariable log-Binomial regression models. The model with 4 knots had the smallest Akaike's information criterion.

We also tested interactions between maternal age and parity on risks of anemia. Since we did not observe any particular interaction on a multiplicative scale ($P>0.2$) effects, the interaction terms were dropped. Sensitivity analyses were conducted by applying the WHO cut-off for maternal anemia. All statistical analyses were carried out in SAS (version 9.1; SAS Institution, Cary, NC).

Results

Distributional characteristics of hemoglobin measures in each trimester are provided in Table 1 - 1. About 60% women had complete hemoglobin measures for all three trimesters during pregnancy. Of a total of 164,667 women, almost a third (33%) had anemia at some time during pregnancy, the prevalence increased substantially with

advancing gestation between the 1st to the 3rd trimesters. The prevalence of severe anemia (hemoglobin < 8g/dL) increased nearly 3- and 5-fold in the 2nd and the 3rd trimesters compared with 1st trimester (Figure 1 - 1). Over half of all women (53%) were on folic acid supplement before pregnancy and during the 1st trimester. The hemoglobin level in each trimester was, on average, higher among those with folic acid supplementation, compared with those without folic acid supplement (Figure 1 -2).

Table 1 - 2 shows the distribution of the characteristics of the pregnant women cohort and the corresponding anemia prevalence rates and unadjusted prevalence rate ratios. Table 1 - 3 shows the adjusted prevalence rate ratios for any maternal anemia as well as the associations stratified by trimester. The adjusted prevalence rate ratios for anemia in relation to maternal age are shown in Figure 1 – 3. Risk factors associated with anemia included older maternal age (≥26 years), lower education (elementary school or less), occupation being farmer, initiation of prenatal care after the 1st trimester, and pregnancy-induced hypertension/preeclampsia. Folic acid supplementation during the peri-conception period was associated with a 25% reduced risk for 1st trimester anemia and a slightly reduced risk for 2nd trimester anemia. Pre-pregnancy overweight was associated with a 12% reduced risk for anemia. In addition, women who registered for the project before pregnancy were associated with a reduced risk for 1st trimester anemia, but they also more likely to be detected with anemia in the 2nd trimester. Women who sought prenatal care for the first time in the 3rd trimester had missed the opportunity to be tested for anemia in the 1st and 2nd trimesters, but appeared to have increased risk for 3rd trimester anemia. Furthermore, the association between pregnancy-induced

hypertension and anemia was significant and stronger among those with moderate or severer case, preeclampsia, or eclampsia.

To examine if different hemoglobin cut-off values produced different patterns of associations, we conducted sensitivity analyses using the WHO recommendation for anemia (hemoglobin <11 g/dL). The prevalence of anemia was more than doubled, with approximately two-thirds (69%) of the women classified as being anemic during pregnancy (Figure 1 - 4). Table 1 – 4 shows the corresponding adjusted prevalence rate ratios for overall anemia. We found the increased cut-off did not substantially change risk patterns for most factors, but the magnitude of the effects was shifted more towards the null. Analyses restricted to women without pregnancy-induced hypertension (not shown) did not change the findings.

Discussion

Maternal anemia is the one of most prevalent pregnancy complications. Despite reported associations with adverse pregnancy outcomes, progress toward understanding of causal contribution to anemia and the role of its primary prevention during pregnancy has been disappointing. Research has not addressed some fundamental epidemiologic questions regarding anemia, most notably, the variations in risk across trimesters during pregnancy. Importantly, the epidemiology of anemia in pregnancy in China is largely unexplored.

The overall prevalence rates of maternal anemia were 32.6% in our cohort. The anemia prevalence was close to a retrospective cohort study in Suzhou, a city of East

China in 1989-1990, which reported a rate of 26.2% (22). However, the anemia prevalence of 69% in our cohort using the WHO recommendation, was higher than a Chinese national epidemiologic survey in 1998 that reported a rate of 42% (20). The differences in the prevalence rate of anemia is perhaps driven by the nature of the study designs (with the previous Chinese national survey being cross-sectional) and ascertainment methods (anemia was determined by only one hemoglobin measure during the survey, regardless of trimester of pregnancy and iron supplementation). Another issue that may have contributed to differences in prevalence rates is iron supplementation by physician intervention. The initial diagnoses of anemia in this project were made prior to receiving prescription of iron supplementation whereas women diagnosed as being anemic in the Chinese survey might have already been taking iron supplements at the time of anemia assessment. Comparing with other developing countries, the anemia prevalence in our study cohort was comparable to that reported in eastern Sudan (19) and Nepal (13); lower than that reported in India (15) and Malawi (32); and higher than that in Venezuela (7), Tanzania (33), Bangladesh and the WHO estimate of 52% for non-industrialized counties (1).

Our study suggests that older maternal age (≥26 years), elementary school or less education, and occupation being farmer are associated with an increased risk for anemia. These findings were consistent with earlier report (8;15) on education and occupation. Unlike other studies (9), we found maternal age to be inversely associated with anemia in this population, especially during the 2nd and 3rd trimesters. It is likely because our cohort contained very few teenagers (<20 years) who are more likely to have lower iron

stores and more socio-demographic risk factors (9). Our findings on pre-pregnancy body mass index also differ from previous studies that reported that underweight women were at increased risk of anemia (8;11;14). We found that underweight women were not associated with maternal anemia, whereas overweight women were associated with a increased risk for maternal anemia. It has been reported that the mean body mass index was much lower for Chinese than Western women (34) and the correlation between the maternal body mass and body fatness varied by sex, race, and age (35;36). Our findings may indicate that the commonly used body mass index threshold for underweight (<18.5) may not be an appropriate marker for anemia risk in Chinese child-bearing women. The observation that pregnancy-induced hypertension is significantly associated with increased risk of anemia, and the prevalence rate ratios increase with the severity of hypertension, is intriguing. Biologically, pregnancy-induced hypertension and maternal anemia could share some common pathways such as oxidative stress or placental hypoxia (37-39). More severe preeclampsia may be associated with hemolysis as well.

Over half of the women in our cohort received folic acid supplementation daily, which allowed us to examine the association between peri-conceptional folic acid use and anemia. Our results showed that folic acid use was significantly associated with a 25% reduction of the risk for 1st trimester anemia. Folic acid deficiency has been considered the next most common nutritional cause of maternal anemia after iron deficiency (3;10;15;37). Our study also revealed that folic acid supplementation until the end of the 1st trimester increased maternal hemoglobin confined to the 1st trimester and

effects gradually diminished in the later pregnancy. Initiating prenatal visit after the 1st trimester was associated with 8 - 10% increase of the risks for 2nd and 3rd trimester anemia, suggested starting prenatal care early might help reduce the risks. Women with pre-gestational insufficient iron stores are likely to present anemia at significantly earlier gestational ages (40), which can be treated by early iron supplementation.

The anemia prevalence rate is very sensitive to the hemoglobin cutoff value. When the threshold was increased from <10 g/dL (the Chinese standard) to <11 g/dL (the WHO recommendation), anemia prevalence increased from 33% to 69%. It may not be practical from clinical prospective to use the higher threshold for anemia among Chinese pregnant women. Hemoglobin cut-offs established for specific adverse outcomes would be more clinically relevant. However, that was not the focus for this study. Altering the hemoglobin thresholds in our study did not change the risk pattern for anemia.

Biases and limitations

Our findings are subject to potential limitations. Since hemoglobin levels were not the primary interest of the original project, it was measured in local laboratories using the usual clinical methods instead of a standardized method. However, spectrophotometer was commonly used at the time. Four percent of women were excluded because they had no hemoglobin measures during pregnancy. The cut-off for anemia in this project was lower than other recommended standards (1;2) which limits comparison of our study with those of others. However, using the WHO criteria gave a similar pattern of findings. Data on a few previously identified risk factors for anemia

including gestational diabetes, iron supplement use, interpregnancy interval, and malaria and hookworm infections (although uncommon) and pica were unavailable.

Strengths

Despite these aforementioned limitations, existing data from this large, prospective, population-based cohort offer a unique opportunity to evaluate prevalence and risk factors for anemia in this Chinese population. The availability of serial hemoglobin measurements across trimesters enabled us to distinguish associations with anemia at different stages of pregnancy. Unlike many smaller and hospital-based studies that are affected by selection bias, the population-based nature of our study permits generalizability of findings to substantial areas of China. In addition, the population is relatively homogeneous by race, smoking, alcohol, and geographic altitude.

Conclusions

Anemia during pregnancy is highly prevalent in this Chinese population. The risk increases with the severity of pregnancy-induced hypertension. Folic acid supplementation during the peri-conceptional period is associated with reduced risk of anemia in the 1st trimester, but not later in pregnancy. Early prenatal care may reduce the risk of anemia during mid-late pregnancy. These findings suggest extending folic acid supplement to the entire pregnancy and initiating prenatal care in the 1st trimester may be able to prevent and early diagnose and treat anemia during pregnancy.

Reference

(1) World Health Organization. Iron deficiency anaemia assessment, prevention, and control a guide for programme managers. WHO/NHD/01 3 2001;1-132.

(2) CDC. Recommendations to prevent and control iron deficiency in the United States. Morbidity and Mortality Weekly Report 1998; 47(No. RR-3):1-30.

(3) Rasmussen K. Is there a causal relationship between iron deficiency or iron-deficiency anemia and weight at birth, length of gestation and perinatal mortality? J Nutr 2001; 131(2S-2):5905-603s.

(4) Hytten F. Blood volume changes in normal pregnancy. Clin Haematol 1985; 14(3):601-612.

(5) Bothwell TH. Iron requirements in pregnancy and strategies to meet them. Am J Clin Nutr 2000; 72(1 Suppl):257S-264S.

(6) Hinderaker SG, Olsen BE, Lie RT et al. Anemia in pregnancy in rural Tanzania: associations with micronutrients status and infections. Eur J Clin Nutr 2002; 56(3):192-199.

(7) Marti-Carvajal A, Pena-Marti G, Comunian GC, Munoz S. Prevalence of anemia during pregnancy: results of Valencia (Venezuela) anemia during pregnancy study. Arch Latinoam Nutr 2002; 52(1):5-11.

(8) Verhoeff FH, Brabin BJ, Chimsuku L, Kazembe P, Broadhead RL. An analysis of the determinants of anaemia in pregnant women in rural Malawi--a basis for action. Ann Trop Med Parasitol 1999; 93(2):119-133.

(9) Bergmann RL, Gravens-Muller L, Hertwig K et al. Iron deficiency is prevalent in a sample of pregnant women at delivery in Germany. Eur J Obstet Gynecol Reprod Biol 2002; 102(2):155-160.

(10) Li S, Tang Y, Wu S. [Analysis of anemia causes during pregnancy and its effects on mothers and fetus]. Journal of Clinical Hematology 1994; 7(2):54-56.

(11) Bondevik GT, Eskeland B, Ulvik RJ et al. Anaemia in pregnancy: possible causes and risk factors in Nepali women. Eur J Clin Nutr 2000; 54(1):3-8.

(12) Dreyfuss ML, Stoltzfus RJ, Shrestha JB et al. Hookworms, malaria and vitamin A deficiency contribute to anemia and iron deficiency among pregnant women in the plains of Nepal. J Nutr 2000; 130(10):2527-2536.

(13) Bondevik GT, Ulstein M, Lie RT, Rana G, Kvale G. The prevalence of anemia in pregnant Nepali women--a study in Katmandu. Acta Obstet Gynecol Scand 2000; 79(5):341-349.

(14) Robinson S, Godfrey K, Denne J, Cox V. The determinants of iron status in early pregnancy. Br J Nutr 1998; 79(3):249-255.

(15) Agarwal KN, Agarwal DK, Sharma A et al. Prevalence of anaemia in pregnant & lactating women in India. Indian J Med Res 2006; 124(2):173-184.

(16) Selo-Ojeme DO. Anemia in pregnancy: case control study of risk factors. Int J Gynaecol Obstet 1997; 59(1):53-54.

(17) Conde-Agudelo A, Belizan JM, Lindmark G. Maternal morbidity and mortality associated with multiple gestations. Obstet Gynecol 2000; 95(6 Pt 1):899-904.

(18) Conde-Agudelo A, Belizan JM. Maternal morbidity and mortality associated with interpregnancy interval: cross sectional study. BMJ 2000; 321(7271):1255-1259.

(19) Adam I, Khamis AH, Elbashir MI. Prevalence and risk factors for anaemia in pregnant women of eastern Sudan. Trans R Soc Trop Med Hyg 2005; 99(10):739-743.

(20) Liao Qk. [Prevalence of iron deficiency in pregnant and premenopausal women in China: a nationwide epidemiological survey]. Zhonghua Xue Ye Xue Za Zhi 2004; 25(11):653-657.

(21) Capital Institute of Pediatrics C. [Survey of women anaemia status in child-bearing age in China in 1998]. Chinese Journal of Reproductive Health 2002; 13(3):102-107.

(22) Xiong X, Buekens P, Fraser WD, Guo Z. Anemia during pregnancy in a Chinese population. Int J Gynaecol Obstet 2003; 83(2):159-164.

(23) Berry RJ, Li Z, Erickson JD et al. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. N Engl J Med 1999; 341(20):1485-1490.

(24) Li S, Moore CA, Li Z et al. A population-based birth defects surveillance system in the People's Republic of China. Paediatr Perinat Epidemiol 2003; 17(3):289-293.

(25) Li Z, Berry RJ, Li S. [Preventing neural tube defects with periconceptional folic acid supplementation: a population-based intervention program in the China]. Zhonghua Yi Xue Za Zhi 2000; 80(7):493-498.

(26) Cochrane J, Chen H, Conigrave KM, Hao W. Alcohol -use in China. Alcohol 2003; 38(6):537-542.

(27) Yang G, Fan L, Tan J et al. Smoking in China findings of 1996 national prevalence survey. JAMA 1999; 282(13):1247-1253.

(28) Ren A, Wang J, Ye RW, Li S, Liu JM, Li Z. Low first-trimester hemoglobin and low birth weight, preterm birth and small for gestational age newborns. Int J Gynaecol Obstet 2007; 98(2):124-128.

(29) Spiegelman D, Hertzmark E. Easy SAS calculation for risk or prevalence ratios and differences. Am J Epidemiol 2005; 162(3):199-200.

(30) Harrell FE. Regression modeling strategies. New York: Springer-Verlag, 2001.

(31) Durrleman S, Simon R. Flexible regression models with cubic splines. Stat Med 1989; 8(5):551-561.

(32) van den Broek NR, Rogerson SJ, Mhango CG, Kambala B, White SA, Molyneux ME. Anaemia in pregnancy in southern Malawi: prevalence and risk factors. Bjog 2000; 107(4):445-451.

(33) Hinderaker SG, Olsen BE, Bergsjo P, Lie RT, Gasheka P, Kvale G. Anemia in pregnancy in the highlands of Tanzania. Acta Obstet Gynecol Scand 2001; 80:18-26.

(34) Zhang Q, Du W, Hu X, Liu A, Pan H, Ma G. The relation between body mass index and percentage body fat among Chinese adolescent living in urban Beijing. Zhonghua Liu Xing Bing Xue Za Zhi 2004;(2):113-116.

(35) Gallagher D. How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? 1996.

(36) Prentice AM, Jebb SA. Beyond body mass index. Obesity Reviews 2001;(3):141-147.

(37) Allen LH. Biological mechanisms that might underlie iron's effects on fetal growth and preterm birth. J Nutr 2001; 131(2S-2):581s-589s.

(38) Cester N, Staffolani R, Rabini RA et al. Pregnancy induced hypertension: a role for peroxidation in microvillus plasma membranes. Mol Cell Biochem 1994; 131(2):151-155.

(39) Poranen AK, Ekblad U, Uotila P, Ahotupa M. Lipid peroxidation and antioxidants in normal and pre-eclamptic pregnancies. Placenta 1996; 17(7):401-405.

(40) Casanueva E, Pfeffer F, Drijanski A, Fernandez-Gaxiola AC, Gutierrez-Valenzuela V, Rothenberg SJ. Iron and folate status before pregnancy and anemia during pregnancy. Ann Nutr Metab 2003; 47(2):60-63.

Table 1 - 1

Characteristics of maternal hemoglobin measures in 164, 667 women with at least 1 hemoglobin measure during pregnancy

	Hemoglobin (g/dL)		
	1st trimester	**2nd trimester**	**3rd trimester**
Number (%)	125,364 (76.1%)	136,633 (83.0%)	146,872 (89.2%)
Mean (SD)	11.2 (1.3)	10.8 (1.3)	10.6 (1.3)
Median (IQR)	11.1 (10.5, 12.0)	10.8 (10.0, 11.5)	10.5 (9.9, 11.3)
Correlation coefficients*			
1st trimester	1.000	0.572	0.381
2nd trimester		1.000	0.519
3rd trimester			1.000

* P<0.05 for all the correlations between trimesters
SD, standard deviation; IQR, inter-quartile range

Table 1 - 2

Distribution of maternal characteristics in relation to anemia during pregnancy

Characteristics	Total births	Proportion with attribute (%)	Anemia prevalence rate (%)	Prevalence rate ratio (95% CI)*
All the women	164,667	100.0	32.6	—
Maternal age (years)				
<25	102,912	62.5	30.8	0.91 (0,89, 0.92)
25-29	42,650	25.9	33.9	1.00 (Reference)
30-34	17,965	10.9	39.2	1.16 (1.13, 1.18)
≥35	1,138	0.7	38.3	1.13 (1.05, 1.22)
Education				
High school/college	18,593	11.3	30.7	0.98 (0.96, 1.00)
Junior high school	98,362	59.9	31.3	1.00 (Reference)
Elementary school or less	47,300	28.8	36.0	1.15 (1.13, 1.17)
Occupation				
Farmer	90,525	55.0	33.8	1.09 (1.07, 1.10)
Factory worker	64,956	39.5	31.1	1.00 (Reference)
Other	8,987	5.5	30.3	0.97 (0.94, 1.01)
Ethnicity				
Han	163,555	99.4	32.4	0.95 (0.87, 1.04)
Other	917	0.6	34.4	1.00 (Reference)

Gravidity				
1	76,652	46.6	29.1	1.00 (Reference)
2	48,665	29.6	31.0	1.17 (1.15, 1.19)
≥ 3	39,233	23.8	37.5	1.29 (1.27, 1.31)
Parity				
0	138,713	84.2	31.8	1.00 (Reference)
≥1	25,954	15.8	36.9	1.16 (1.14, 1.18)
Folic acid use				
Yes	87,723	53.3	32.2	0.97 (0.96, 0.99)
No	76,944	46.7	33.0	1.00 (Reference)
BMI				
Underweight	23,601	16.7	31.7	0.97 (0.95, 0.99)
Normal weight	113,051	79.9	32.4	1.00 (Reference)
Overweight/Obese	4,869	3.4	29.5	0.90 (0.86, 0.94)
Registration				
Pre-pregnancy	66,679	40.5	32.8	1.01(0.99, 1.03)
During pregnancy	97,988	59.5	32.4	1.00 (Reference)
First prenatal visit				
<12 weeks	134,090	81.4	32.3	1.00 (Reference)
12-27 weeks	23,827	14.5	34.5	1.07 (1.05, 1.09)
≥28 weeks	6,750	4.1	30.2	0.93 (0.90, 0.97)
Early bleeding				
Yes	1,052	0.7	30.8	0.93 (0.85, 1.02)
No	150,032	99.3	33.0	1.00 (Reference)

Hypertension				
None	155,554	95.8	32.7	1.00 (Reference)
Mild	4,962	3.1	35.7	1.10 (1.06, 1.14)
Preeclampsia	1,874	1.1	37.0	1.14 (1.08, 1.21)

* Unadjusted prevalence rate ratio; CI: confidence interval

Table 1 - 3

Associations between maternal risk factors and anemia during pregnancy (n=164,677)

Maternal risk factors	Adjusted prevalence rate ratio (95% confidence interval) for anemia			
	Overall	Associations stratified by trimester in pregnancy		
		1st trimester	2nd trimester	3rd trimester
Maternal age		See Figure 1-3		
Education high school/college	0.97 (0.95, 0.99)	0.92 (0.88, 0.98)	0.96 (0.93, 1.00)	0.98 (0.95, 1.01)
Education elementary school or less	1.10 (1.08, 1.12)	1.13 (1.09, 1.17)	1.15 (1.12, 1.18)	1.10 (1.07, 1.12)
Farmer (vs factory worker)	1.05 (1.03, 1.06)	1.17 (1.13, 1.21)	1.08 (1.06, 1.11)	1.09 (1.07, 1.11)
Other occupation (vs factory worker)	0.97 (0.94, 1.01)	0.86 (0.79, 0.93)	0.92 (0.87, 0.97)	0.99 (0.94, 1.03)
Multiparity (vs primiparity)	1.02 (0.99, 1.04)	1.09 (1.03, 1.15)	1.03 (0.99, 1.07)	1.02 (0.99, 1.05)
Folic acid use (vs no use)	1.00 (0.98, 1.02)	0.75 (0.72, 0.78)	0.97 (0.94, 0.99)	1.01 (0.98, 1.03)
Pre-pregnancy BMI underweight	0.98 (0.96, 1.01)	0.99 (0.94, 1.03)	—	0.99 (0.97, 1.02)
Pre-pregnancy BMI overweight/obese	0.88 (0.84, 0.92)	0.88 (0.80, 0.96)	—	0.88 (0.83, 0.93)
Pre-pregnancy registration	1.05(1.03, 1.07)	0.94 (0.91, 0.98)	1.06 (1.04, 1.09)	1.02 (0.99, 1.04)
First prenatal visit at 12-27 wks	1.03 (1.01, 1.06)	—	1.08(1.05, 1.11)	1.10 (1.06, 1.13)
First prenatal visit at ≥28 wks	0.88 (0.85, 0.92)	—	—	1.09 (1.04, 1.14)

Mild pregnancy-induced hypertension	1.09 (1.05, 1.13)	—	—	1.08 (1.03, 1.13)
Preeclampsia	1.13 (1.06, 1.19)	—	—	1.16 (1.08, 1.24)

Table 1 - 4

Sensitivity analysis: adjusted prevalence rate ratio for maternal anemia using the WHO cut-off (hemoglobin <11 g/dL)

Maternal risk factors*	Adjusted prevalence rate ratio (95% confidence interval)
Education high school/college	0.99 (0.98, 1.00)
Education elementary school or less	1.03 (1.02, 1.04)
Factory worker (vs farmer)	1.00 (0.99, 1.00)
Other occupation (vs farmer)	0.96 (0.94, 0.97)
Multiparity (vs primiparity)	0.99 (0.98, 0.99)
Folic acid supplement (vs no)	1.01 (1.00, 1.02)
Pre-pregnancy registration	1.00 (0.99, 1.00)
First prenatal visit at 12-27 wks	1.03 (1.02, 1.04)
First prenatal visit at ≥28 wks	0.92 (0.90, 0.93)
Mild pregnancy-induced hypertension	1.01 (0.99, 1.03)
Preeclampsia	1.01 (0.98 1.04)

* Spline transformed maternal age variables were also included in the models.

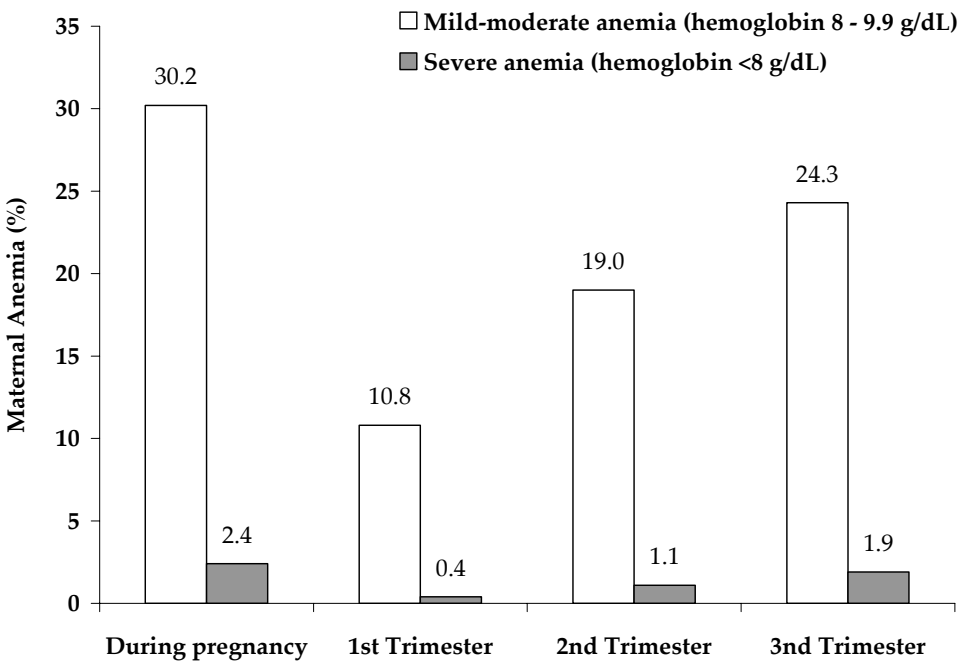


Figure 1 - 1
Prevalence of maternal anemia during pregnancy by severity

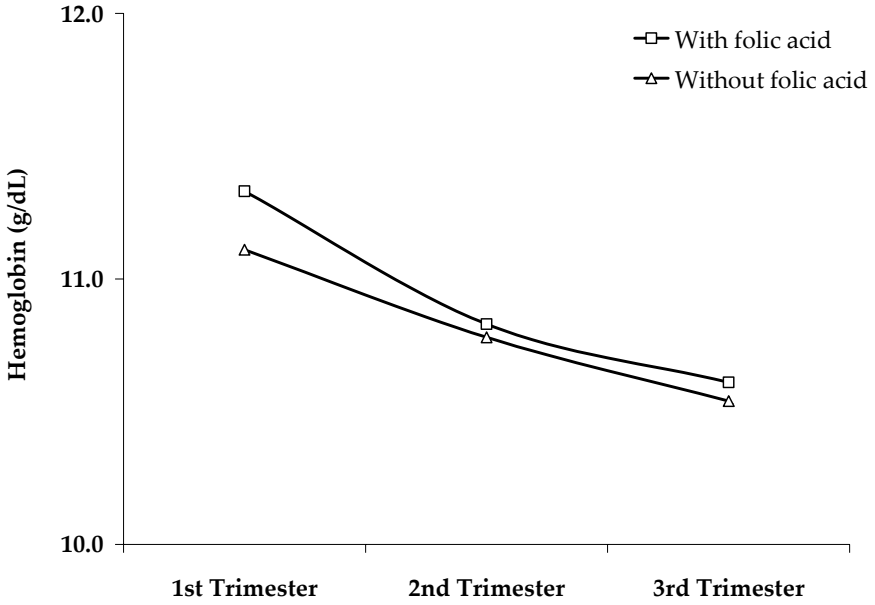


Figure 1 - 2
Average maternal hemoglobin level during pregnancy, by folic acid use status

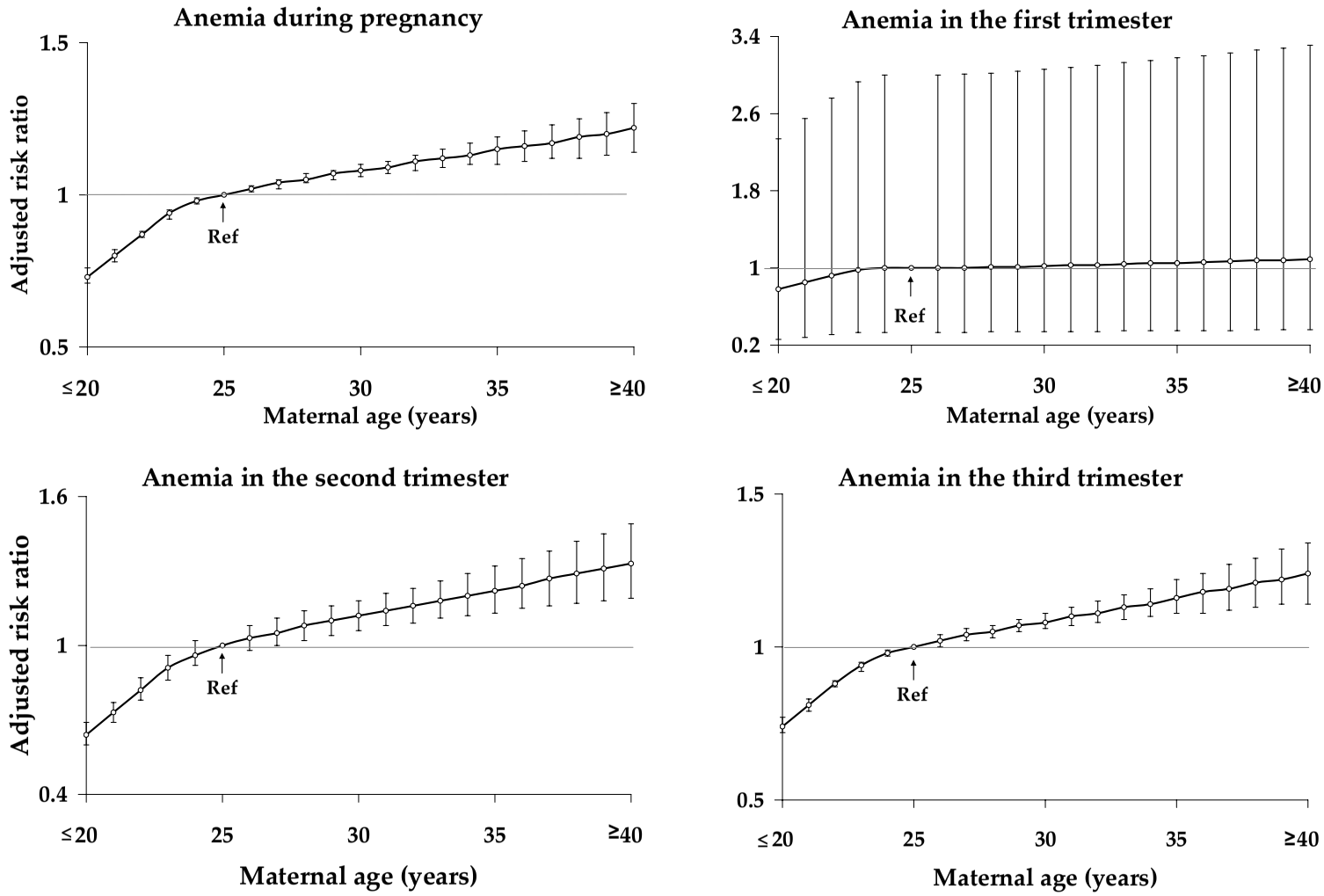


Figure 1 - 3 Associations between maternal age and anemia during pregnancy

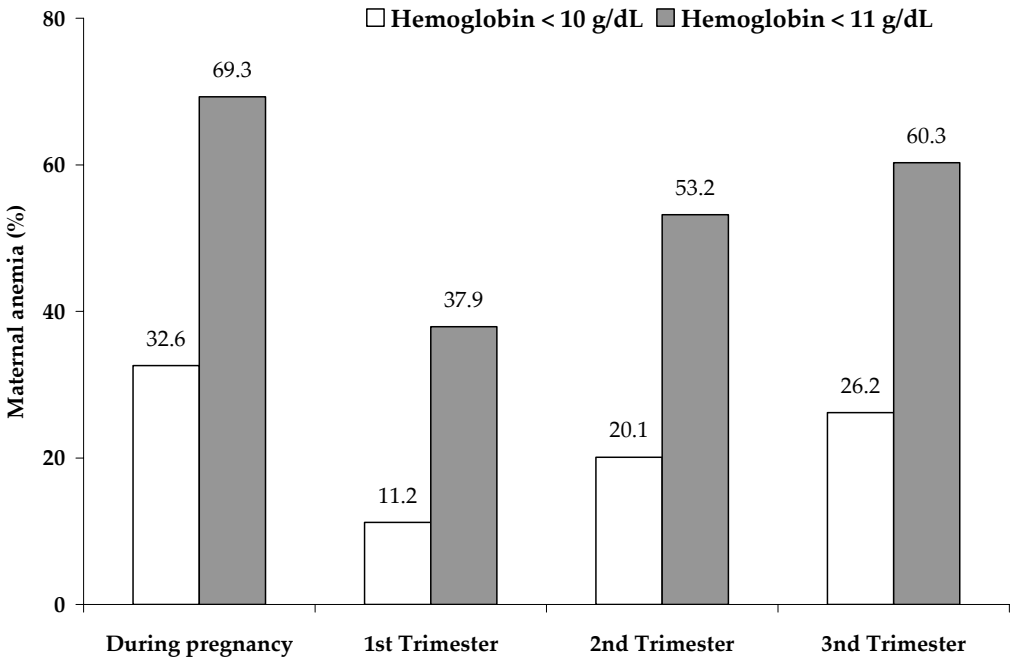


Figure 1 - 4
Prevalence of maternal anemia during pregnancy, according to Chinese (<10 g/dL) and the WHO (<11 g/dL) cut-offs

Chapter 2

The Impact of Maternal Anemia on Perinatal Mortality: A Prospective, Population-based Cohort Study in China

Abstract

Objective: Maternal anemia is a ubiquitous pregnancy complication in developing countries; however its impact on perinatal mortality remains speculative. We evaluated if maternal anemia during gestation is associated with increased risk of stillbirth and neonatal death.

Methods: A prospective cohort study, using existing data from a population-based pregnancy-monitoring system in 13 counties in East China (1993-96) was conducted. Singleton live births (n=163,313) and stillbirths (n=1,354) delivered at 20-44 weeks to women with at least 1 hemoglobin measure during pregnancy were included. Stillbirth and neonatal mortality rates by anemia status in each trimester were estimated. Multivariable Cox proportional hazards regression models were used to evaluate the association between mortality risk and hemoglobin levels both within and across trimester of exposure during pregnancy.

Results: The stillbirth rates were 6.2 and 9.2 per 1,000 births in women with and without anemia, respectively (P<0.05). Anemia (hemoglobin <10 g/dL) in both the 1st and 2nd trimesters was associated with increased risk for stillbirth (adjusted hazard ratio (HR) 1.7, 95% confidence interval (CI) 1.1, 2.7). Hemoglobin of 9 g/dL in the 3rd trimesters was associated with reduced risk (HR 0.8, 95% CI 0.7, 0.97). Maternal anemia was not associated with neonatal mortality.

Conclusion: Anemia in the first half of pregnancy is associated with increased risk of stillbirth, but not neonatal deaths. Early identification and appropriate intervention to

manage maternal anemia might prove beneficial to improve adverse pregnancy outcomes.

Introduction

Maternal anemia is highly prevalent among pregnant women in both the developing and industrialized countries (1), with a reported prevalence rate of 42% in China (2). If maternal anemia is indeed associated with increased risk for perinatal mortality, this observation will have a significant public health implication. Unfortunately, the association between anemia and perinatal mortality in general, and in developing countries in particular, remains inconclusive.

Although the etiology of maternal anemia remains speculative, 3 major biologic mechanisms supporting the association through which maternal anemia may be associated with increased risk of perinatal mortality has been postulated. These include an hypoxic insult at the maternal-fetal interface, oxidative stress, and maternal infections (3). Hypoxia activates maternal and fetal stress responses through elevation of corticotrophin-releasing hormone or cortisol (3). This, in turn, results in restricted fetal growth (4) and preterm delivery (5;6). Iron deficiency may also increase oxidative stress resulting in damage to erythrocytes and the feto-placental unit (3) – a mechanism commonly observed in studies of preeclampsia and gestational diabetes (7;8). Furthermore, iron deficiency may increase the risk of maternal infections, and infection is one of the main risk factor for preterm labor (3). Products of the activated maternal immune system in the presence of infection may have crossed the placenta and activated the fetal hypothalamic-pituitary-adrenal axis (9).

A few retrospective cohort studies from developing countries have examined the association of maternal anemia with perinatal mortality with equivocal findings.

Increased risks of stillbirth and perinatal mortality in relation to maternal anemia were reported in some (10-12), but not others (13-17), including 3 studies conducted in China (14-16). The few studies examining the association between anemia and stillbirth (18-20) or perinatal death (21;22) in industrialized countries also provide inconsistent results. The inconsistent findings may be the result of variations in the timing of hemoglobin assessment during pregnancy and other limitations afforded by study designs, e.g., failing to adjust for important confounding factors. The associations between maternal anemia and pregnancy outcomes may vary across trimesters, while maternal hemoglobin concentration changes over the course of pregnancy as a result of physiological hemo-dilution (23). Therefore, separate assessment corresponding to the stage of pregnancy may reveal important insights.

In our data, maternal hemoglobin levels were prospectively measured in every trimester before the pregnancy outcomes were determined in a large population-based cohort. This afforded a unique opportunity to carefully disentangle the association between maternal anemia and perinatal mortality both within trimester and across trimesters of exposure throughout gestation.

Methods

Pregnancy Monitoring Program in China 1993-96

The data of this study were prospectively collected through a population-based pregnancy-monitoring system in China. This pregnancy-monitoring system was operated along with the community intervention trial of preventing neural tube defects

with peri-conceptive supplementation of 400 μg folic acid daily in 21 counties of three provinces in China. The original project design and results of the neural tube defects prevention program have already been published (24-26). This project was approved by the institutional review boards of both the Centers for Disease Control and Prevention, Atlanta, GA and the Chinese Ministry of Health, Beijing, China.

Every woman who resided in the project area and became pregnant was registered at her marital registration or first prenatal visit, and was routinely followed up by local health care professionals. Regardless of their folic acid supplementation status, women who were pregnant and delivered between October 1993 and December 1996 were monitored through 42 days postpartum.

The Perinatal Health Care Booklet, as the primary data collection tool, was distributed to all women at registration and was assigned a unique identification number for tracking purposes. The data contained parental demographics; maternal medical history; medical conditions, vital and laboratory measures in each trimester; perinatal health outcomes; and perinatal health care utilization. Hemoglobin values, if available, were ascertained during a pre-marital physical exam before the marital registration, and at the 1st, 2nd, and 3rd trimesters during pregnancy. The blood samples were collected by local health care professionals during the pre-marital exam or prenatal visits. Hemoglobin concentration was determined at each local laboratory using the usual clinical methods, mostly by spectrophotometer. When more than one hemoglobin measurements were conducted in 2nd and 3rd trimesters, only the lowest hemoglobin value of that trimester was recorded. All data recorded in the individual booklets were

computerized by trained staff in each county using a standardized data entry application with built-in data checking function.

Study cohort

In our study, 31,960 women from the Hebei province in North China and 40,431 women from three counties (Fenghua, Yinxian, and Ninghai) in East China were excluded from the total cohort of 241,831 women, due to concerns about the methods of ascertainment and accuracy of hemoglobin measurement. Following these exclusions, there were 172,414 women that delivered a singleton live birth or stillbirth between 20 and 44 completed weeks in the 13 counties of Zhejiang and Jiangsu provinces in East China. Among them, 164,667 women with hemoglobin measures in at least 1 of the 3 trimesters were included in the analysis for stillbirth, and 153,952 women who delivered a live-born infant and were followed up to 28th days were included for the neonatal mortality analysis. Gestational age, in completed weeks, was estimated based on the last menstrual period.

Definitions for maternal anemia and outcomes

Anemia in each trimester was defined as hemoglobin <10 g/dL, which was pre-specified in the original protocol. This hemoglobin cut-off is also a commonly used clinical standard in China (14). Stillbirths were defined as fetal demise at ≥20 weeks of gestation, including those that occurred before the onset of labor (antepartum stillbirth) and during labor (intrapartum stillbirth). Neonatal death was defined as the death of a live-

born infant within the first month, and were stratified as early neonatal (at <7 days) and late neonatal (7-27 days) death. Perinatal deaths included all stillbirths and early neonatal deaths.

Confounding factors

We simultaneously adjusted for a variety of confounders that were considered potentially associated with both maternal hemoglobin level and the mortality outcomes. These included maternal age at delivery (categorized as less than 26 and 26 years or older), maternal education (completion of elementary school or less, junior high school, and high school or above), occupation (farmer, factory worker, and other occupations), parity (nulliparity and multiparity), taking 400 μg daily folic acid supplement some time before pregnancy and during the 1st trimester, pre-pregnancy body-mass index (kg/m^2, and categorized as below 18.5, 18.5 to 24, and 25 or over). These categories correspond to underweight, normal and overweight/obese, respectively, as recommended by the WHO (27). Timing of registration for the project (before or during pregnancy), timing of first prenatal visit (1st, 2nd, or 3rd trimester), and fetal gender, were also considered as other potential confounders.

Statistical analysis

Rates of stillbirth were derived among all eligible singleton births, whereas neonatal mortality rates were derived using live births as the denominator. The perinatal mortality rate was estimated among all stillbirths and live births with known neonatal

survival status. The rates were also estimated by maternal characteristics and fetal gender. To evaluate the effects of maternal anemia on perinatal mortality at a given birthweight or gestational age, we estimated and plotted the birthweight- and gestational age-specific mortality rates for those births of anemic mothers, compared with those of non-anemic mothers. The corresponding distribution of birthweight was illustrated along with the birthweight-specific perinatal mortality curves (on a logarithmic scale). Birthweight was expressed as standardized *z*-scores (standardized to a Gaussian distribution, with mean of 0 and variance of 1). We used the fetuses-at-risk approach (28) to estimate the gestational age-specific perinatal mortality rate for births ≥ 28 weeks of gestation, based on maternal anemia status. Due to concerns of gestational age errors at 42 weeks and beyond (29), all births at 42 weeks or later were censored at 42 weeks in all gestational age-specific analysis.

Associations of hemoglobin in each trimester, as a continuous variable, with stillbirth and neonatal death were assessed based on adjusted hazard ratios derived from multivariable Cox proportional hazards regression models. In these models, events of mortality are associated with time to event, *i.e.,* complete weeks of gestation at delivery for stillbirths and days after birth for neonatal deaths. Women who delivered before 28 completed weeks (regardless of outcome) were excluded in the analyses for exposure in the third trimester. To prevent problems resulting from inappropriate linearity assumption, we fitted one multivariable regression model with the cubic spline function (30;31) of hemoglobin value (g/dL) for each trimester with 4 knots corresponding to the 5^{th}, 35^{th}, 65^{th}, and 95^{th} percentiles of hemoglobin value. Number of

knots was determined by the values of Akaike's information criterion (AIC) of the multivariable proportional hazards regression models (31), and the model with 4 knots resulted with the smallest AIC. The adjusted hazard ratio for mortality by hemoglobin concentrations was estimated; hemoglobin value of 11 g/dL was assigned as the reference point. To evaluate the effects of anemia across trimesters on the outcomes, we classified anemic women according to the timing of anemia occurrence as the 1st, 2nd or 3rd trimester only, or the combinations of any 2 trimesters, or all 3 trimesters, and compared them to women that remained non-anemic throughout pregnancy. The corresponding risks and the adjusted hazard ratios for stillbirth and early neonatal mortality were estimated. For the stillbirth analysis, all live births were censored at the gestation week at delivery. For the neonatal death analysis, all infants alive on the 28th day were censored. The proportional hazards assumption was tested by examining the interaction terms (32) of hemoglobin measures and the time to event, *i.e.,* week of gestation or days after birth, and found to be satisfied.

Potential confounding factors were included in the models as covariates. In the initial analysis, maternal age was modeling using spline transformations; since this did not reveal any additional insights than a model using maternal age in categories, we retained the later analysis. The interactions between folic acid supplementation and maternal hemoglobin level in each trimester were tested and not found to be significant ($P>0.4$). All analyses were conducted using SAS version 9.1 (SAS Institution, Cary, NC).

Sensitivity and subgroup analysis

Although the main analyses were restricted to women with at least 1 hemoglobin measures during pregnancy, we also carried out a concurrent analysis by imputing missing hemoglobin measures. This latter analysis was treated as a multiple imputation problem (33;34) and was accomplished using the MI procedure in SAS (35;36). We first created an indicator variable for the missing value of each covariate except for hemoglobin. The imputation for missing hemoglobin was carried out 5 times to generate 5 complete data sets. We selected the Markov chain Monte Carlo method (37) in our imputation, because of the arbitrary missing pattern of hemoglobin. Multivariable Cox proportional hazards regression models were fit to each of the 5 complete data sets, and results from these models were subsequently combined. From this final analysis, we derived the adjusted hazard ratio and 95% confidence interval describing the association of hemoglobin level with stillbirth and neonatal mortality (37). We also conducted a sub-group analysis restricted to women without pregnancy induced hypertension, to eliminate the effect of this pregnancy complication on the results.

Results

Table 2 – 1 shows the rates of stillbirth and early neonatal death by maternal characteristics and fetal gender. Nearly a third (33%) of the women were diagnosed with anemia (hemoglobin <10 g/dL) at least once during their pregnancy. Table 2 - 2 shows the proportion of women with and without anemia in each trimester as well as over the pregnancy, and the corresponding antepartum, intrapartum, and all stillbirth rates, and

early, late, and all neonatal mortality rates. There were 1,354 stillbirths (8.2 per 1,000 births), of which 80% of them occurring during the antepartum period (n=1,081, 6.6 per 1,000 births). Among 1,116 neonatal deaths (7.2 per 1,000 live births), 861 were classified as early neonatal deaths (5.6 per 1,000 live births), and 255 as late neonatal deaths (1.7 per 1,000 live births). The overall perinatal mortality rate was 14.3 per 1,000 births.

Birthweight- and gestational age-specific perinatal mortality rates based on anemia status are contrasted in Figures 2 - 1 and 2 – 2. While the birthweight distribution of infants born to anemic mothers was shifted slightly to the right, the birthweight-specific perinatal mortality rates for those with maternal anemia were slightly lower or similar to those without anemia. The gestational age-specific perinatal mortality rates for women with anemia were slightly lower than or similar to those without anemia.

The adjusted hazard ratios for perinatal mortality in relation to hemoglobin values in each trimester as compared with hemoglobin value of 11 g/dL were plotted in Figure 2 - 3 for stillbirth and Figure 2 -4 for early neonatal death. In the 3rd trimester hemoglobin levels between 9 and 10 g/dL was associated with 20% reduced risk for stillbirth, while hemoglobin level at 12 g/dL was associated with slightly increased risks for stillbirth (HR 1.1, 95% CI 1.0, 1.2). No association between maternal anemia and intrapartum stillbirth and neonatal mortality was apparent. However, we observed a trend of elevated hemoglobin levels in the 2nd and 3rd trimester associated with slightly increased risks of early neonatal death, with only the 3rd trimester hemoglobin at 12 g/dL was found marginally significant (HR 1.1, 95% CI 1.0, 1.2). When considering the timing

of anemia occurrence, there were 7 categories of anemia across trimesters. We found anemia in both the 1st and 2nd trimesters was associated with a 70% increased risk of stillbirth. (Table 2 - 4). The multiple imputation approach did not substantially change the overall risk patterns (not shown), but the magnitude of the associations was shifted more toward the null. The results derived from the sub-group of women without pregnancy–induced hypertension were similar to those reported.

Discussion

Maternal anemia in this Chinese cohort was ubiquitous; a third (33%) of women in this cohort had anemia some time during their pregnancy. We found anemia (hemoglobin <10 g/dL) in both the 1st and 2nd trimesters to be associated with a 70% increased risk of stillbirth, whereas hemoglobin levels between 9 and 10 g/dL in the 3rd trimester was associated with marginally reduced risk for stillbirth. However, maternal anemia was not associated with the risk of neonatal mortality. The association between maternal anemia and perinatal mortality remains equivocal from previous studies (10-22), with the 3 studies conducted in Chinese populations reported no association between anemia and perinatal mortality (14-16). Given its high prevalence, the role of maternal anemia in contributing to adverse outcomes, especially stillbirth, remains a significant public health problem.

Unlike most previous studies, we analyzed hemoglobin levels in each trimester as a continuous variable and accordingly estimated the adjusted hazard ratios using a flexible regression approach. An implicit strength is our estimates are not affected by

arbitrary cut-off values for anemia or anemia severity. In all 3 previous Chinese studies (14-16), in contrast, women were classified as either being anemic or non-anemic using hemoglobin cut-off of ≤10 g/dL. An inherent limitation with this approach is that the effect of anemia could be diluted by combining elevated hemoglobin levels in the non-anemic group. Furthermore, arbitrary categorization of cut-points implicitly assumes that risks are homogeneous within category, and are different at the cut-point (38). We observed a marginally increased risk for stillbirth in relation to elevated hemoglobin in the 3rd trimesters. Stronger associations were reported by other studies (18;19;22), with one study reported elevated hemoglobin associated with 80% increased risk of stillbirth (adjusted odd ratio 1.8, 95% CI 1.0, 3.3) (19).

We found that the risk patterns of maternal anemia in relation to stillbirth were different across trimesters. In particular, anemia in both the 1st and 2nd trimesters was associated with significantly increased risk of stillbirth. Routine prenatal iron or multivitamin supplements were not commonly recommended in China during the time the project was conducted (1993-96) (14;39). However, 56% of the women in our cohort who participated in the peri-conceptive folic acid supplementation program had voluntarily opted to take 400μg folic acid daily sometime before or early in pregnancy. Iron supplement, as a treatment to alleviate anemia, may partially explain the reverse causation of 2nd and 3rd trimester anemia and stillbirth risk. While one previous Chinese study (14) examined associations between anemia at the first prenatal visit or in the 3rd trimester and risk of perinatal mortality; the other 2 (15;16) studies ignored the timing of anemia. All 3 studies, however, reported an absence of association between anemia and

stillbirth or perinatal mortality (14-16), while others that stratified these associations by trimester reported significant findings (20;22). Hemoglobin assessment in each trimester in our large prospective cohort enabled us to evaluate the association in each trimester over the actual span of hemoglobin values without dichotomizing them. Both the sensitivity analysis with missing hemoglobin values imputed, and the subgroup analysis restricted to women without pregnancy-induced hypertension yielded results essentially similar to the ones reported.

When dichotomizing pregnant women as being anemic or non-anemic, regardless of the timing of anemia occurrence, the distributions of birthweight, and birthweight- and gestational age-specific perinatal mortality showed that the babies of anemic mothers were slightly heavier and had slightly lower perinatal mortality, in comparison to those born to women without anemia. Medical and obstetrical intervention may have played a role to some extent in improving overall pregnancy outcomes among anemic women. In such scenarios, women diagnosed with anemia are usually treated and more closely monitored. If so, then this may lead to a paradoxical finding of a reverse-causal association. However, we were unable to examine the treatment effects because treatment data such as iron supplementation were not ascertained as part of the project. Women with insufficient iron stores before pregnancy are likely to present with anemia at earlier gestational ages (40). The fact that 1st and 2nd trimester anemia being associated with increased risk of stillbirth, may underscore that pre-pregnancy and early pregnancy screening and treatment for low hemoglobin may be beneficial. This also suggests that a simple classification of women as being anemic or

non-anemic and disregarding the time in gestation when the anemia episode occurs may likely mask the true association between maternal anemia and perinatal mortality.

Strengths

The strength of our study was that we used the data from a large, prospective, population-based cohort, which provided information on serial hemoglobin measurements during pregnancy. Unlike many other retrospective cohort studies, the prospective nature of our study enabled hemoglobin assessments prior to determining pregnancy outcomes rules out the possibility of differential misclassification of anemia status. The population-based nature also enhances the generalizability of the findings to substantial areas of China. Our study population was relatively homogeneous by race, smoking, alcohol, and geographic altitude. This unique setting enabled us to examine the association of different hemoglobin levels with stillbirth and neonatal death at different stages of pregnancy, with reduced selection and confounding bias.

Limitations

We are aware that our study also carries potential limitations. Since hemoglobin measure was not the primary interest of the original project, it was assessed in local laboratories using the usual clinical methods instead of standardized protocols. Data on hemoglobin in the 2nd and 3rd trimesters pertained to the lowest of the assessments. This may have obscured the association between anemia and perinatal mortality due to the potential confounding of hemo-dilution that may reach the nadir near the end of the 2nd

and early of the 3rd trimesters in normal pregnancy (41). The data on iron supplement use were not collected. At the time of the project, iron was prescribed only for those women who were diagnosed with anemia to be taken until the anemic status was corrected (14). If such an intervention was indeed effective, it may have impacted the associations noted here.

Conclusions

Despite the aforementioned limitations, anemia in the first half of pregnancy is associated with increased risk of stillbirth. Early assessment and appropriate intervention to manage maternal anemia might be able to improve the pregnancy outcomes.

Reference

(1) World Health Organization. Iron deficiency anaemia assessment, prevention, and control a guide for programme managers. WHO/NHD/01 3 2001;1-132.

(2) Liao Qk. [Prevalence of iron deficiency in pregnant and premenopausal women in China: a nationwide epidemiological survey.]. Zhonghua Xue Ye Xue Za Zhi 2004; 25(11):653-657.

(3) Allen LH. Biological mechanisms that might underlie iron's effects on fetal growth and preterm birth. J Nutr 2001; 131(2S-2):581s-589s.

(4) Smith R. Alterations in the hypothalamic pituitary adrenal axis during pregnancy and the placental clock that determines the length of parturition. J Reprod Immunol 1998; 39:215-220.

(5) Sandman CA, Wadhwa PD, Chicz-DeMet A, Dunkel-Schetter C, Porto M. Maternal stress, HPA activity, and fetal/infant outcome. Ann N Y Acad Sci 1997; 814:266-275.

(6) McLean M, Bisits A, Davies J, Woods R, Lowry P, Smith R. A placental clock controlling the length of human pregnancy. Nat Med 1995; 1(5):460-463.

(7) Cester N, Staffolani R, Rabini RA et al. Pregnancy induced hypertension: a role for peroxidation in microvillus plasma membranes. Mol Cell Biochem 1994; 131(2):151-155.

(8) Poranen AK, Ekblad U, Uotila P, Ahotupa M. Lipid peroxidation and antioxidants in normal and pre-eclamptic pregnancies. Placenta 1996; 17(7):401-405.

(9) Falkenberg ER, Davis RO, DuBard M, Parker CRJ. Effects of maternal infections on fetal adrenal steroid production. Endocr Res 1999; 25(3-4):239-249.

(10) Conde-Agudelo A, Belizan JM, az-Rossello JL. Epidemiology of fetal death in Latin America. Acta Obstet Gynecol Scand 2000; 79(5):371-378.

(11) Geelhoed D, Agadzi F, Visser L et al. Maternal and fetal outcome after severe anemia in pregnancy in rural Ghana. Acta Obstet Gynecol Scand 2006; 85(1):49-55.

(12) Watson-Jones D, Weiss HA, Changalucha JM et al. Adverse birth outcomes in United Republic of Tanzania--impact and prevention of maternal risk factors. Bull World Health Organ 2007; 85(1):9-18.

(13) Aimakhu CO, Olayemi O. Maternal haematocrit and pregnancy outcome in Nigerian women. West Afr J Med 2003; 22(1).

(14) Xiong X, Buekens P, Fraser WD, Guo Z. Anemia during pregnancy in a Chinese population. Int J Gynaecol Obstet 2003; 83(2):159-164.

(15) Zhang J, Cai WW. Risk factors associated with antepartum fetal death. Early Hum Dev 1992; 28(3):193-200.

(16) Lao TT, Pun T-C. Anaemia in pregnancy – is the current definition meaningful? Eur J Obstet Gynecol Reprod Biol 1996; 68:53-58.

(17) Lone FW, Qureshi RN, Emanuel F. Maternal anaemia and its impact on perinatal outcome. Trop Med Int Health 2004; 9(4):486-490.

(18) Garn SM, Ridella SA, Petzold AS, Falkner F. Maternal hematologic levels and pregnancy outcomes. Semin Perinatol 1981; 5(2):155-162.

(19) Stephansson O, Dickman PW, Johansson A, Cnattingius S. Maternal hemoglobin concentration during pregnancy and risk of stillbirth. JAMA 2000; 284(20):2611-2617.

(20) Tomashek KM, Ananth CV, Cogswell ME. Risk of stillbirth in relation to maternal haemoglobin concentration during pregnancy. Matern Child Nutr 2006; 2:19-28.

(21) Levy A, Fraser D, Katz M, Mazor M, Sheiner E. Maternal anemia during pregnancy is an independent risk factor for low birthweight and preterm delivery. Eur J Obstet Gynecol Reprod Biol 2005; 122(2):182-186.

(22) Murphy JF, O'Riordan J, Newcombe RG, Coles EC, Pearson JF. Relation of haemoglobin levels in first and second trimesters to outcome of pregnancy. Lancet 1986; 1(8488):992-995.

(23) Xiong X, Buekens P, Alexander S, Demianczuk N, Wollast E. Anemia during pregnancy and birth outcome: a meta-analysis. Am J Perinatol 2000; 17(3):137-146.

(24) Berry RJ, Li Z, Erickson JD et al. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. N Engl J Med 1999; 341(20):1485-1490.

(25) Li S, Moore CA, Li Z et al. A population-based birth defects surveillance system in the People's Republic of China. Paediatr Perinat Epidemiol 2003; 17(3):289-293.

(26) Li Z, Berry RJ, Li S. [Preventing neural tube defects with periconceptional folic acid supplementation: a population-based intervention program in the China]. Zhonghua Yi Xue Za Zhi 2000; 80(7):493-498.

(27) World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO Consultation. 894. 2000. Geneva, World Health Organization. WHO Technical Report Series. Ref Type: Report

(28) Joseph KS. Incidence-based measures of birth, growth restriction, and death can free perinatal epidemiology from erroneous concepts of risk. J Clin Epidemiol 2004; 57(9):889-897.

(29) Kramer MS, Mclean FH, Boyd ME, Usher RH. The validity of gestational age estimation by menstrual dating in term, preterm, and postterm gestations. JAMA 1988; 260(22):3306-3308.

(30) Durrleman S, Simon R. Flexible regression models with cubic splines. Stat Med 1989; 8(5):551-561.

(31) Harrell FE. Regression modeling strategies. New York: Springer-Verlag, 2001.

(32) Allison PD. Survival ananlysis using the SAS system: a practical guide. Cary, NC: SAS Institute Inc., 1995.

(33) Raghunathan TE. What do we do with missing data? Some options for analysis of incomplete data. Annu Rev Public Health 2004; 25:99-117.

(34) Schafer JL. Multiple imputation: a primer. Stat Methods Med Res 1999; 8:3-15.

(35) Yuan, CY. Multiple imputation for missing data: concepts and new development Cary, NC: SAS Institute Inc., 2000.

(36) SAS Institute Inc. SAS OnlineDoc® 9.1.3. Cary, NC: SAS Institute Inc., 2007.

(37) Rubin DB. Multiple imputation for nonresponse in surveys. New York: John Wiley, 1987.

(38) Greenland S. Problems in the average-risk interpretation of categorical dose-response analyses. Epidemiology 1995; 6(5):563-565.

(39) Ren A, Wang J, Ye RW, Li S, Liu JM, Li Z. Low first-trimester hemoglobin and low birth weight, preterm birth and small for gestational age newborns. Int J Gynaecol Obstet 2007; 98(2):124-128.

(40) Casanueva E, Pfeffer F, Drijanski A, Fernandez-Gaxiola AC, Gutierrez-Valenzuela V, Rothenberg SJ. Iron and folate status before pregnancy and anemia during pregnancy. Ann Nutr Metab 2003; 47(2):60-63.

(41) Whittaker PG, Macphail S, Lind T. Serial hematologic changes and pregnancy outcome. Obstet Gynecol 1996; 88(1):33-39.

Table 2 - 1

Maternal and fetal characteristics and perinatal mortality

Characteristics	Total births	Stillbirth (per 1,000 births)	Early neonatal mortality (per 1,000 live births)
All births	164,667	8.2 (n=164,667)	5.6 (n= 153,952)
Maternal age (yrs)			
<25	102,912	7.9	5.6
25-29	42,650	8.2	5.6
30-34	17,965	9.6	5.3
≥35	1,138	15.8	10.5
Education			
High school/college	18,593	7.2	3.3
Junior high school	98,362	7.9	5.9
Elementary school or less	47,300	9.4	5.9
Occupation			
Farmer	90,525	8.4	5.6
Factory worker	64,956	8.4	5.8
Other	8,987	7.5	4.0
Ethnicity			
Han	163,555	8.2	5.6
Other	917	6.5	9.3

Gravidity			
1	76,652	8.0	6.0
2	48,665	8.1	5.3
≥ 3	39,233	8.8	5.1
Parity			
0	138,713	7.7	5.4
≥1	25,954	10.8	6.6
Folic acid use			
Yes	87,723	7.9	5.4
No	76,944	8.6	5.8
BMI			
Underweight	23,601	8.2	5.5
Normal weight	113,051	8.0	5.4
Overweight/Obese	4,869	11.9	6.6
Registration			
Pre-pregnancy	66,679	8.0	4.9
During pregnancy	97,988	8.4	6.0
First prenatal visit			
<12 weeks	134,090	8.1	5.4
12-27 weeks	23,827	9.5	6.4
≥ 28 weeks	6,750	5.8	6.1
Fetal sex			
Male	84,864	7.0	6.0
Female	79,803	9.6	5.2

Table 2 - 2

The rates of stillbirth and neonatal mortality, by maternal anemia status

Maternal anemia status	Total births	With attribute (%)	Stillbirth rate (per 1,000 total births)			Total live births	Neonatal mortality rate (per 1,000 live births)		
			Total	Ante-partum	Intra-partum		Total	Early (<7 days)	Late (7-27 days)
During pregnancy									
Anemic	53,634	32.6	6.2	4.9	1.3	50,485	6.4	4.8	1.6
Non-anemic	111,033	67.4	9.2	7.4	1.8	103,467	7.7	6.0	1.7
1st trimester									
Anemic	14,043	11.2	8.9	7.3	1.6	13,173	6.8	5.5	1.4
Non-anemic	111,321	88.8	8.2	6.5	1.7	104,269	7.1	5.5	1.6

2nd trimester									
Anemic	27,475	20.1	7.2	6.0	1.2	25,885	6.4	5.0	1.4
Non-anemic	109,158	79.9	7.7	6.1	1.6	102,194	7.3	5.7	1.6
3rd trimester									
Anemic	38,510	26.2	4.3	3.3	1.0	36,323	6.1	4.5	1.6
Non-anemic	108,362	73.8	5.5	4.1	1.4	101,625	7.0	5.4	1.6

Table 2 - 3

Anemia status across trimesters and perinatal mortality

Anemia status across trimesters	Total births	With attribute (%)	All stillbirths		Total live births	Early neonatal mortality (<7 days)	
			Rate (per 1,000)	Adjusted HR (95% CI)		Rate (per 1,000)	Adjusted HR (95% CI)
None	64,663	65.2	5.1	1.0 (Reference)	60,823	5.6	1.0 (Reference)
1st trimester only	1,944	2.0	4.6	0.9 (0.6, 1.7)	1,840	4.9	0.9 (0.4, 1.7)
2nd trimester only	5,290	5.3	4.7	0.9 (0.6, 1.3)	5,033	4.2	0.7 (0.5, 1.2)
3rd trimester only	11,814	11.9	4.1	0.8 (0.6, 1.0)	11,176	3.9	0.7 (0.5, 1.0)
1st and 2nd trimesters	2,370	2.4	8.9	1.7 (1.1, 2.7)	2,212	5.4	1.0 (0.6, 1.7)
1st and 3rd trimesters	937	0.9	5.3	1.0 (0.4, 2.4)	883	4.5	0.8 (0.3, 2.1)
2nd and 3rd trimesters	6,589	6.6	3.5	0.7 (0.4, 1.0)	6,241	3.8	0.7 (0.5, 1.1)
All 3 trimesters	5,607	5.7	5.4	1.0 (0.7, 1.5)	5,308	4.7	0.8 (0.6, 1.2)

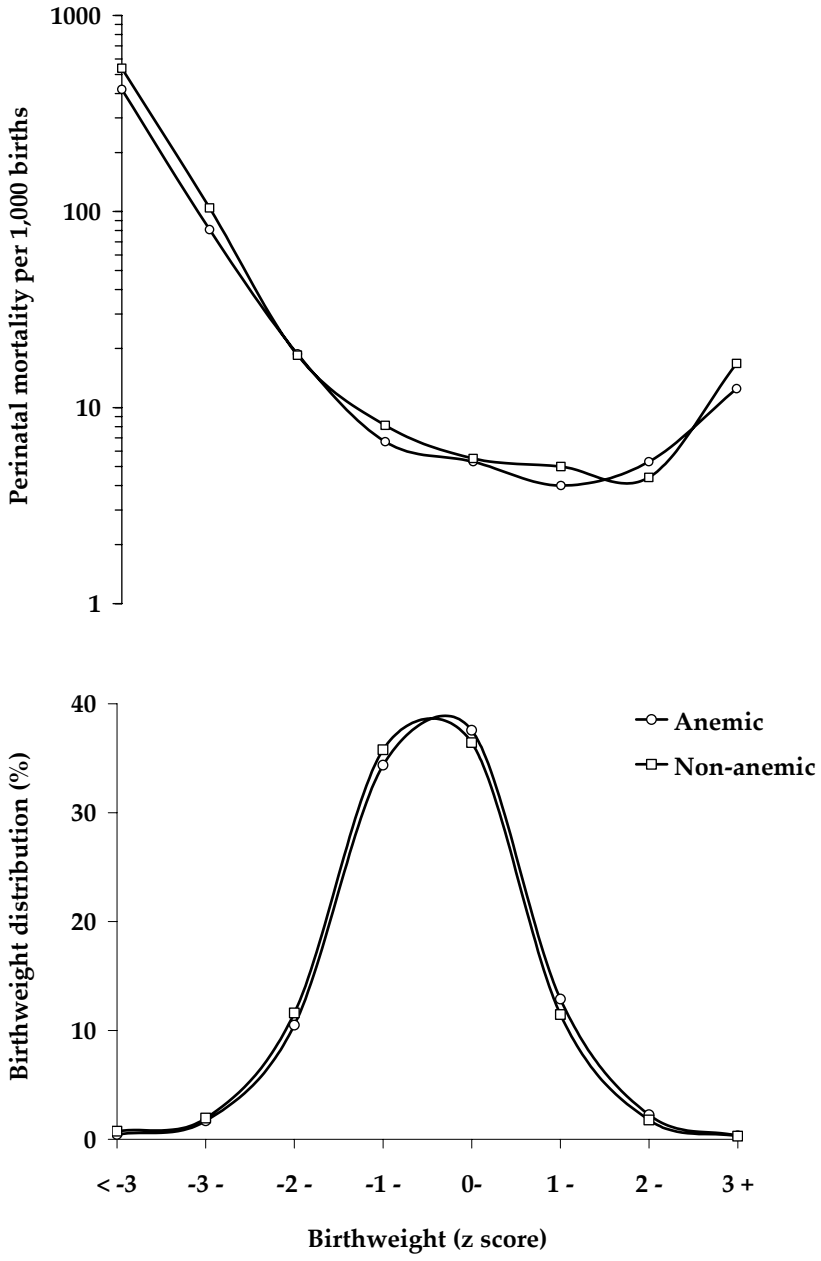


Figure 2 - 1
Birthweight distribution and perinatal mortality rates by anemia status

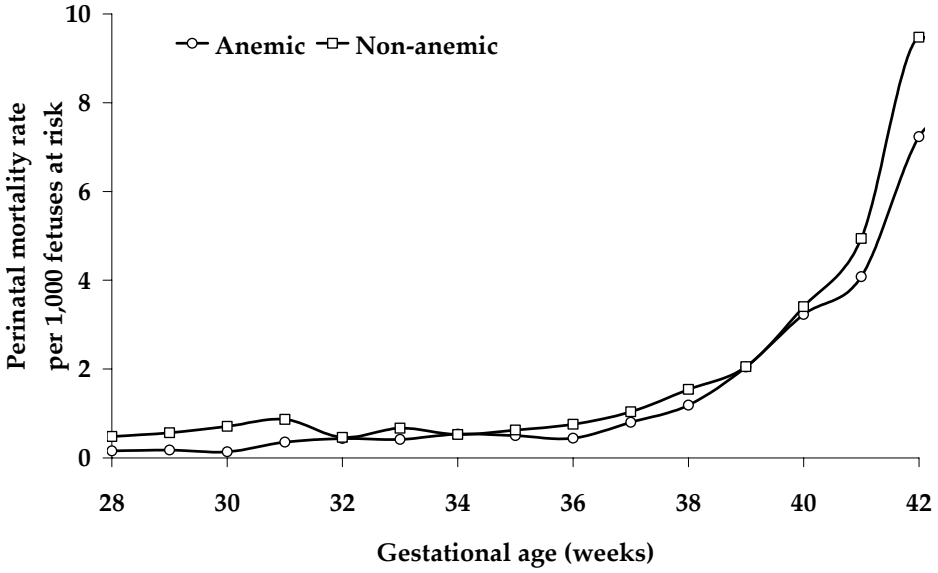


Figure 2 - 2
Gestational age specific-perinatal mortality rates by anemia status

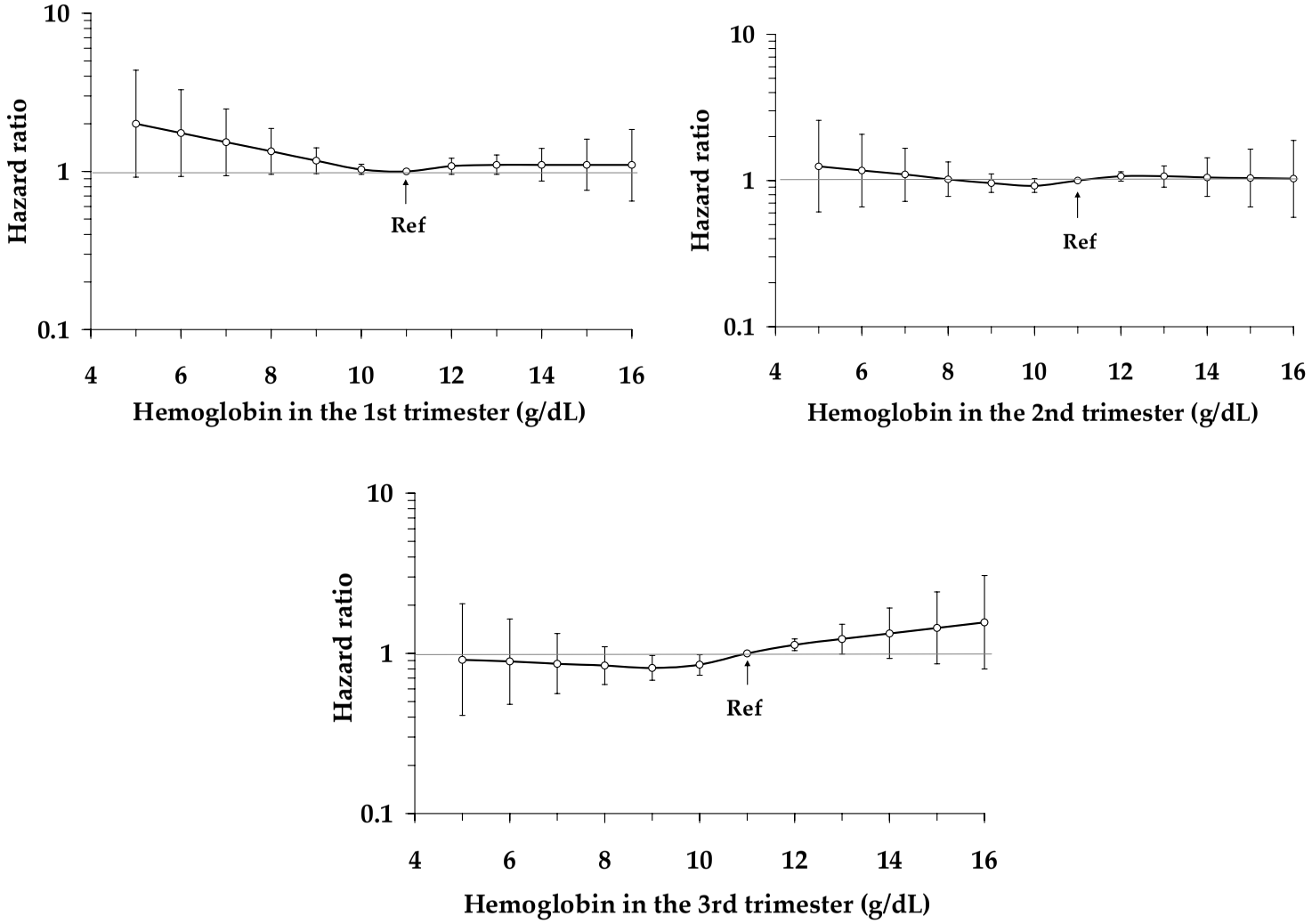
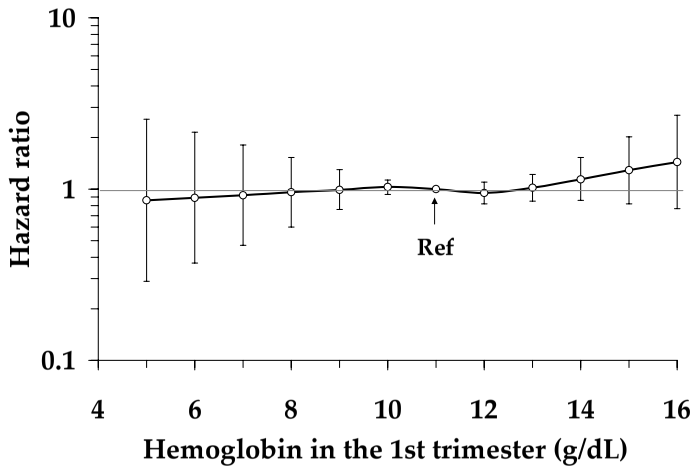
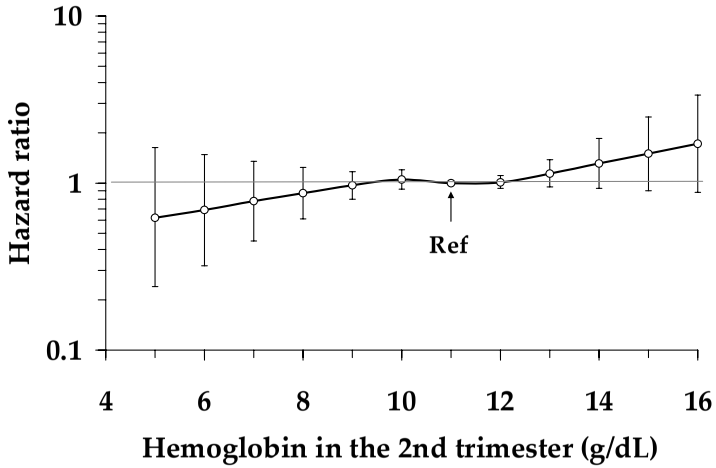


Figure 2 - 3. Adjusted hazard ratio of stillbirth and 95% confidence interval for hemoglobin level in each trimester





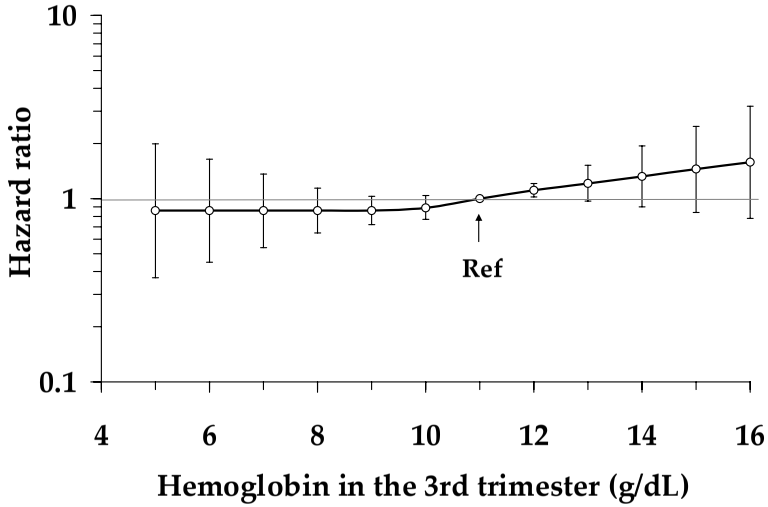


Figure 2 - 4. Adjusted hazard ratio of early neonatal mortality and 95% confidence interval for hemoglobin level in each trimester

Chapter 3

Maternal Anemia on Preterm Birth Clinical Subtypes:

A Prospective Cohort Study

Abstract

Objective: Despite numerous studies, the role of maternal anemia in preterm birth remains poorly defined, and the association between anemia and preterm birth clinical subtypes remain unclear. We examined if maternal anemia exposure both within and across trimesters during gestation is associated with preterm birth and its clinical subtypes.

Methods: This was a secondary analysis of a prospective cohort study of data from the population-based pregnancy-monitoring system in 13 counties of East China region (1993-96). All singleton live births delivered at 20-44 weeks to women with at least 1 hemoglobin measures during pregnancy were included (n=160,700). Infants with unknown birthweight or implausible birthweight-gestational age combinations were excluded. Preterm birth rates were estimated by clinical subtypes, namely, preterm premature rupture of membranes (PROM), preterm birth following spontaneous onset of labor and preterm birth resulting from medical interventions. Hemoglobin changes across trimesters were assessed as proxy of hemo-dilution (hemoglobin increase ≥1 g/dL) and hemo-concentration (hemoglobin decrease >1 g/dL). Associations were expressed by hazard ratios (HR) derived from Cox proportional hazards regression models after adjusting for potential confounders.

Results: Overall preterm birth (<37 weeks) rates were 4.1% for anemic and 5% non-anemic women (P<0.05). In comparison to women with hemoglobin of 11 g/dL (reference), values <11 g/dL in the 1st trimester was associated with increased risk for preterm PROM. Women with hemoglobin ≤5 g/dL were at highest risk for preterm

PROM (HR 3.3, 95% confidence interval (CI) 1.4, 7.7) with progressively declining risk with increasing hemoglobin levels. In contrast, the association between hemoglobin in the 3rd trimester and spontaneous preterm labor was reversed, with lower hemoglobin levels being associated with reduced risk. Anemia in all 3 trimesters was associated with a moderately increased risk for only spontaneous preterm labor. Anemia was not associated with medically indicated preterm birth. Hemo-dilution, but not hemo-concentration, over gestation was associated with up to 23% reduced risk for preterm birth.

Conclusion: Anemia exposure in early pregnancy is associated with increased risk for preterm PROM, while exposure in late pregnancy is associated with reduced risk for spontaneous preterm labor. Adequate physiological hemo-dilution during mid- and late pregnancy may be associated with reduced risk for preterm birth.

Introduction

Preterm birth has remained as one of the strongest predictors of perinatal mortality and morbidity (1;2). Despite numerous studies, the association between maternal anemia and preterm birth remains equivocal. Anemia was found to be associated with increased risks for preterm birth in some (3-8), but not other (9-12) studies. The findings from Chinese studies (4;8;9;13) on the relationship between anemia and preterm birth were also inconsistent. In addition, a "U"-shaped relationship between anemia and preterm birth has been reported (13-17). Studies have also suggested that the association between anemia and preterm birth may vary based on the timing of anemia during gestation (12).

Preterm birth may occur through multiple etiologic pathways, with maternal infection, hypoxia, and oxidative stress being the 3 major postulated biological mechanisms by which anemia and iron deficiency could cause preterm birth (18). Iron deficiency may increase the risk of maternal infections, and low hemoglobin may cause a state of hypoxia that induces maternal and fetal stress. Activated immune system of infected mothers (19) and corticotrophin-releasing hormone or cortisol that are released following a stress responses, can activate the maternal or fetal hypothalamic-pituitary-adrenal axis (20). This, in turn, initiates the events of preterm labor (18). Finally, iron deficiency may also increase oxidative stress resulting in damage to erythrocytes and the feto-placental unit (21;22). This latter mechanism is associated with preeclampsia and gestational diabetes (18), which are strong risk factors for preterm birth (23;24).

Considerable etiologic heterogeneity persists among preterm birth clinical subtypes, namely, spontaneous preterm labor (spontaneous onset of labor or following

preterm PROM) and medically indicted preterm birth (1;25-28). However, few studies have attempted to evaluate if associations between anemia and preterm birth are largely driven by associations with one particular subtype (29). If maternal anemia is indeed associated with one preterm birth subtype, and not others, the association with preterm birth as an entity may be attenuated (30). Furthermore, such patterns may reveal interesting clues to etiology and biologic mechanisms.

We hypothesized that maternal anemia may be associated with increased risk of preterm birth, and that an examination of associations within preterm birth clinical subtypes may reveal interesting clues to biologic mechanisms. We carried out the analysis by utilizing data that were prospectively ascertained in a large population-based cohort.

Methods

Data source

We designed a secondary analysis of data from a prospective, population-based cohort. Data were obtained from a population-based pregnancy-monitoring system established through a community intervention trial to prevent neural-tube defects in 21 counties of 3 provinces in China. Every woman who resided in the project area and became pregnant was registered at her marital registration or first prenatal visit. Women who were pregnant and delivered between October 1993 and December 1996 were identified. At the time of registration, a campaign program of peri-conception folic acid supplementation of 400 μg daily during pre- and early pregnancy was offered to non-

pregnant women or those in their 1st trimester. The project was approved by the institutional review boards of both the Centers for Disease Control and Prevention, and the Chinese Ministry of Health (31;32).

The data were documented on a Perinatal Health Care Booklet that was issued to every woman at registration with a unique identification number. The information in the booklet contained parental demographics and family history, maternal medical history and obstetric conditions during the pregnancy, perinatal health outcomes, and perinatal health care utilization. Hemoglobin levels were determined at the pre-marital physical exam when applicable, and repeated at each trimester by health care professionals, using the usual clinical methods. For women that had more than 1 hemoglobin assessment in the 2nd and 3rd trimesters, only the lowest value was recorded. All data recorded in the individual booklets were computerized by trained staff in each county using a standardized data entry application with built-in data checking filters.

Study cohort

This study was restricted to data from 13 counties of Zhejiang and Jiangsu provinces, a relatively wealthy region, in East China. Of the 170,885 women that delivered a singleton live birth between 20 and 44 completed weeks of gestation, 163,313 women had at least 1 hemoglobin value measured during pregnancy. We excluded 2,186 births with missing birthweight and 427 births with implausible birthweight-gestational age combinations (33). After all exclusions, 160,700 singleton live births remained for analysis.

The population in these 13 counties is relatively homogeneous with respect to ethnicity, smoking, alcohol, and geographic altitude. During the time of the project, women were not recommended to routinely take prenatal iron supplements; instead, iron supplements were prescribed only when anemia was diagnosed (4;9). However, information on iron supplement use was collected in this project.

Definition of maternal anemia, hemoglobin change, and preterm birth

Anemia in each trimester was defined as hemoglobin <10 g/dL, which was pre-specified in the original protocol and has been also a commonly used cut-off in China (9). We classified women with anemia as follows: 1st trimester only, 2nd trimester only, 3rd trimester only, 1st and 2nd trimesters, 1st and 3rd trimesters, 2nd and 3rd trimesters, and all 3 trimesters.

Hemoglobin changes were calculated as the differences of the 1st and 2nd trimesters; the 2nd and 3rd trimesters; and the 1st and 3rd trimesters. We reclassified the difference as hemo-dilution when change ≥1 g/dL or hemo-concentration when <-1 g/dL.

Gestational age, in completed weeks, was estimated based on the last menstrual period. Preterm birth was defined as live births delivered before 37 completed weeks. We further categorized preterm birth as very preterm (<32 weeks), moderately preterm (32-33 weeks), and mild preterm (34-36 weeks). We also examined preterm birth based on its 3 underlying clinical subtypes (30) in the following hierarchy: preterm PROM (spontaneous rupture of chorioamniotic membranes prior to the onset of labor and delivery before 37 weeks), medically indicated preterm birth (preterm birth following

labor induction and/or cesarean performed before onset of labor for impending *in utero* fetal compromise), and spontaneous preterm labor (the remainder of all preterm births).

Confounding factors

The data provided a variety of confounders that were considered potentially associated with both maternal hemoglobin level and preterm birth, and not known on the disease pathways. Selected maternal characteristics included age at delivery, education (completion of elementary school or less, junior high school, and high school or above), occupation (farmer, factory worker, and other occupations), parity (nulliparity and multiparity), pre-pregnancy body-mass index (kg/m^2, and categorized as below 18.5, 18.5 to 24, and 25 or over) and infant sex. We also controlled for prenatal care utilization, using timing of registration for the project (either before or during pregnancy) and timing of first prenatal visit (1^{st} or 2^{nd} trimester). Finally, we adjusted for folic acid use (400 μg daily) either before pregnancy or during the 1^{st} trimester.

Statistical analysis

Rates of all preterm birth, as well as by of each clinical subtype were estimated among singleton live births. The distribution of gestational age at delivery was contrasted for women with and without anemia during pregnancy. The proportions of clinical subtypes among preterm births were estimated based on anemia status and preterm severity. For women who had hemoglobin assessments in all 3 trimesters we calculated

the preterm birth rates for the 7 maternal anemia status groups as described earlier, both by preterm birth severity and by its clinical subtype.

Associations of hemoglobin in each trimester, as a continuous variable, with preterm birth and its clinical subtypes were assessed based on adjusted hazard ratios derived from multivariable Cox proportional hazards regression models. Each preterm birth clinical subtype was compared with term births. Women who delivered before 28 completed weeks (regardless of outcome) were excluded in the analyses for exposure in the third trimester. We modeled hemoglobin concentrations following transformations based on restricted cubic spline functions (34) with 4 knots. This approach avoids the undesirable property of analyzing data after arbitrary categorizations. The optimal number of knots was determined by that provided the minimum value of Akaike's information criterion (AIC) of the Cox proportional hazards models (35). The adjusted hazard ratio for preterm birth by hemoglobin concentrations was estimated with hemoglobin 11 g/dL as the reference.

To evaluate the effects of anemia across trimesters on preterm birth, all live births delivered at ≥37 weeks of gestation were censored in the Cox proportional hazards models. The proportional hazards assumption was tested by examining the interaction terms (36) of hemoglobin measures and week of gestation and found to be satisfied.

Potential confounding factors were included in the models as covariates. As with hemoglobin, a restricted cubic spline function of maternal age with 4 knots was applied after we detected the violation of linearity assumption. Interactions between folic acid

supplementation and maternal hemoglobin level in each trimester were tested and found to be insignificant (P>0.15).

The effects of hemo-dilution (hemoglobin change ≥ 1 g/dL) and hemo-concentration (> -1 g/dL) were assessed by the multiple Cox proportional hazards models that included the corresponding indicator variables for hemoglobin changes, the 1st trimester hemoglobin value as the baseline level, and confounding factors. All analyses were conducted using SAS version 9.1 (SAS Institution, Cary, NC).

Results

The overall preterm birth (<37 weeks) rate of singleton live-born infants in this cohort was 4.7%, with 4.1% for anemic and 5 % for non-anemic pregnancy women (P<0.05). The most prevalent preterm birth clinical subtype was spontaneous preterm labor (3.6%), accounting for 77% of all preterm births. Table 3 – 1 shows the rates of preterm birth and its clinical subtypes by maternal characteristics and fetal gender. Nearly one third of women (32.7%) had anemia (hemoglobin <10 g/dL) sometime during their pregnancy, with the prevalence of anemia being 11%, 20%, and 26% in the 1st, 2nd, and 3rd trimesters, respectively. The distribution of gestational age at delivery for women with and without anemia was similar (Figure 3 – 1). Distribution of preterm birth rates based on severity and clinical subtype in relation to maternal anemia status are shown in Table 3 – 2. There were relatively more preterm PROM cases among very preterm births (<32 weeks) and among moderate preterm births (32-33 weeks) of anemic women, as shown in Figure 3 – 2.

Associations between hemoglobin levels in each trimester and risk of preterm birth, preterm PROM and spontaneous preterm labor are shown in Figures 3 – 3, 3 – 4 and 3 – 5, respectively. Hemoglobin 9-10 g/dL in the 1st trimester, but not <9 g/dL was associated with slightly increased risk for all preterm births. Hemoglobin < 11 g/dL in the 1st trimester was particularly associated with increased risks for preterm PROM. Women with lowest hemoglobin levels were at highest risk for preterm PROM (adjusted hazard ratio 3.3, 95% CI 1.4, 7.7 for hemoglobin ≤5 g/dL) with progressively declining risk with increasing hemoglobin levels up to 10 g/dL. In contrast, the association

between hemoglobin in the 3rd trimester and preterm birth was reversed, with low hemoglobin values (≤10 g/dL) being associated with reduced risks for all preterm births and spontaneous preterm labor. As shown in Table 3 – 3, anemia in all 3 trimesters was associated with increased risks for all preterm birth and spontaneous preterm labor.

Medically indicated preterm was not associated with maternal hemoglobin (not shown). We also observed a stronger association between 1st trimester anemia and very to moderate preterm birth (<34 weeks) (not shown).

Hemoglobin increased 1 g/dL or more from previous trimester, as the proxy of hemo-dilution, were associated with reduced risks for all preterm birth (Figure 3 – 6).

Discussion

Most studies on the association between maternal anemia and adverse reproductive outcomes have produced inconsistent findings. This is largely mitigated by the fact that maternal anemia has been analyzed as an aggregated exposure such as "any anemia during pregnancy". Although informative, it is likely that anemia diagnosed early in pregnancy may exert stronger associations on pregnancy outcomes than anemia diagnosed later in gestation. Equally, studies on preterm birth have paid little attention to its heterogeneous underpinnings (1), thereby combining etiologically distinct endpoints as being homogeneous, and perhaps leading to attenuated association measures (1;30;37). Finally, little attention has been devoted as to how anemia affects the risk for preterm birth clinical subtypes, including previous Chinese studies (4;8;9;13). Most studies have exclusively focused on spontaneous preterm births (29;38). Our study

was designed to overcome all these aforementioned limitations. In addition, we explored the potential effects of physiological hemo-dilution on preterm birth.

We found anemia in the 1st trimester was associated with slightly increased risks for all preterm birth. These associations were considerably stronger for preterm PROM. However 3rd trimester anemia was associated with reduced risk for all preterm birth, largely confined to spontaneous preterm labor. Medically indicated preterm birth, on the other hand, was not associated with anemia. These results underscore the strong heterogeneity in the risk profile for preterm birth based on underlying clinical subtypes, as well as by exposure window (*i.e.*, trimester in pregnancy when anemia was diagnosed). Differences in exposure assessment as regards the timing of anemia assessment, and associations being driven by preterm birth clinical subtypes (i.e., preterm PROM) may have contributed to inconsistent findings across earlier studies.

Our findings on all preterm birth were consistent with a meta-analysis (39) that concluded that early pregnancy anemia was associated with slightly increased risk for preterm birth and late pregnancy anemia was inversely associated with preterm birth. All 4 previous Chinese studies (4;8;9;13) conducted in the same or nearby regions as the present study, examined the association between anemia and preterm birth without distinguishing its clinical subtype. While some reported anemia in the 1st trimester to be associated with increased risk of preterm birth (4;8), others did not (9). The last study reported that only the 3rd trimester hemoglobin <7 g/dL (severe anemia) was associated with a marginally increased risk for preterm birth (13). Table 1 – 5 shows 5 studies (11;29;37;38;40) that reported associations between maternal anemia and preterm birth

clinical subtypes. All these earlier studies either dichotomized pregnant women as anemic or non-anemic using one cut-off point (9;38;40), or categorized them into multiple groups using several cut-off points (4;8;13;29;37;40). Categorization of a skewed-distributed exposure like anemia, assuming within-category homogeneity, degrades continuous exposure information and tends to be less accurate than spline analysis (41). We analyzed hemoglobin concentrations as a continuous variable based on flexible spine transformation to account for nonlinear effects.

Disaggregating preterm birth into more homogeneous subtypes revealed considerably heterogeneity in their associations with anemia. Anemia present in all 3 trimesters was associated with 20% increased risks for spontaneous preterm labor, while anemia in mid- and late pregnancy was associated with reduced risk. There were trends of increased risks for preterm PROM in relation to anemia present in early half or all 3 trimesters. Anemia in early pregnancy or throughout pregnancy may represent pre-existing, or early onset and persistent iron deficiency. Iron deficiency anemia, in turn, could induce maternal infection, hypoxia, and oxidative stress and trigger the spontaneous onset of preterm labor (18).

Previous studies mentioned the concern that normal physiological hemo-dilution during pregnancy, that usually reaches the nadir at the end of the 2nd trimester and early the 3rd trimester, might mask the true association between anemia and preterm birth (9;12;42;43). However the association between hemo-dilution in the 2nd and 3rd trimesters and preterm birth had not yet been assessed. In our study, we used the hemoglobin reduction across trimesters as the proxy of hemo-dilution, controlling for the 1st

trimester hemoglobin level as the baseline. We found hemo-dilution was associated with reduced risk for preterm birth. This may partially explain the inverse association between the 3rd trimester anemia and preterm birth.

The overall preterm birth rate in this Chinese cohort was fairly low (4.7%) with spontaneous preterm labor (77%) being the most common clinical subtype. This rate is consistent with earlier findings for other cohorts in this region (4;9;13); however, it is considerably lower than most other industrialized countries (1;30). In the United States, for instance, the rate is above twice as higher (1) with nearly a third of singleton preterm births being medically indicated (44). Rates of obstetrical interventions at preterm and term gestations were 11.6% and 16.7%, respectively, in our cohort. This suggests that the threshold for intervention in the presence of impending *in utero* fetal compromise is far higher than in most industrialized societies. It is therefore likely that differences in practice and threshold for intervention may play an important but yet unidentified, role in our findings. In addition, in our data the gestational age was estimated based on last menstrual period without ultrasound or clinical verification, therefore falsely high gestational age due to late ovulation (and late conception) was not corrected. It could lead to a lower preterm birth rate than a ultrasound-based estimate (23).

The observed association between preterm birth and anemia in the 1st trimester was less likely affected by iron supplementation as iron supplement was given after the diagnosis of anemia. However anemic women in latter pregnancy were more likely to take iron supplement as a treatment and receive more medical attention. Besides the effects of the normal physiologically hemo-dilution (9), the observed inverse association

between late pregnancy anemia and spontaneous preterm labor, might be a complex artifact partially due to the benefits of medical interventions. Whether early prevention and prompt treatment of maternal anemia can reduce the risks for spontaneous preterm labor and preterm PROM warrants further investigation.

Limitations and strengths

The preterm birth incidence rate in our cohort is different from some regions in China (45) and some other countries (1;30). Therefore, the findings from our study may not be generalizable to those populations with different preterm birth rate. In addition, hemoglobin was assessed in local laboratories using the usual clinical methods without standardized protocols. Hemoglobin values in the 2nd and 3rd trimesters pertained to the lowest of the assessments, which may blend real anemia with the physiological hemo-dilution that reaches the nadir near the end of the 2nd and early of the 3rd trimesters in normal pregnancy (46). Therefore, the observed association between maternal anemia and preterm birth may have been attenuated. Lastly, information on individual iron supplementation was not available. However it is likely that iron was prescribed to anemic women as a treatment (4;9), and this may have resulted in an attenuation of the associations noted here.

The ability to separate preterm births by its clinical subtypes and to examine associations within more homogeneous groups in relation to maternal anemia are some of the strengths of our study. Hemoglobin values of women in our large, population-based cohort were prospectively ascertained before pregnancy outcomes were

determined, which eliminates the possibility of differential misclassification of anemia. Our study population was relatively homogeneous, and less likely to be affected by selection and confounding biases.

Conclusions

In summary, maternal anemia early in pregnancy is associated with increased risk of preterm PROM and anemia throughout pregnancy is associated with increased risk of spontaneous preterm labor. Early prevention and prompt treatment of maternal anemia may be one avenue for intervention, and may be a topic worthy for further investigation.

Reference

(1) Ananth CV, Vintzileos AM. Epidemiology of preterm birth and its clinical subtypes. J Matern Fetal Neonatal Med 2006; 19(12):773-782.

(2) Slattery MM, Morrison JJ. Preterm delivery. Lancet 2002; 360(9344):1489-1497.

(3) Levy A, Fraser D, Katz M, Mazor M, Sheiner E. Maternal anemia during pregnancy is an independent risk factor for low birthweight and preterm delivery. Eur J Obstet Gynecol Reprod Biol 2005; 122(2):182-186.

(4) Ren A, Wang J, Ye RW, Li S, Liu JM, Li Z. Low first-trimester hemoglobin and low birth weight, preterm birth and small for gestational age newborns. Int J Gynaecol Obstet 2007; 98(2):124-128.

(5) Scanlon KS, Yip R, Schieve LA, Cogswell ME. High and low hemoglobin levels during pregnancy: differential risks for preterm birth and small for gestational age. Obstet Gynecol 2000; 96(5 Pt 1):741-748.

(6) Scholl TO, Hediger ML, Fischer RL, Shearer JW. Anemia vs iron deficiency: increased risk of preterm delivery in a prospective study. Am J Clin Nutr 1992; 55(5):985-988.

(7) Lieberman E, Ryan KJ, Monson RR, Schoenbaum SC. Association of maternal hematocrit with premature labor. Am J Obstet Gynecol 1988; 159(1):107-114.

(8) Zhou LM, Yang WW, Hua JZ, Deng CQ, Tao XG, Stoltzfus RJ. Relation of hemoglobin measured at different times in pregnancy to preterm birth and low birth weight in Shanghai, China. Am J Epidemiol 1998; 148(10):998-1006.

(9) Xiong X, Buekens P, Fraser WD, Guo Z. Anemia during pregnancy in a Chinese population. Int J Gynaecol Obstet 2003; 83(2):159-164.

(10) Lao TT, Pun T-C. Anaemia in pregnancy – is the current definition meaningful? Eur J Obstet Gynecol Reprod Biol 1996; 68:53-58.

(11) Lu ZM, Goldenberg RL, Cliver SP, Cutter G, Blankson M. The relationship between maternal hematocrit and pregnancy outcome. Obstet Gynecol 1991; 77(2):190-194.

(12) Klebanoff MA, Shiono PH, Berendes HW, Rhoads GG. Facts and artifacts about anemia and preterm delivery. JAMA 1989; 262(4):511-515.

(13) Wang J, Ren Ag, Ye Rw et al. [Study on the third trimester hemoglobin concentrations and the risk of low birth weight and preterm delivery]. Zhonghua Liu Xing Bing Xue Za Zhi 2007; 28(1):15-18.

(14) Chang SC, O'Brien KO, Nathanson MS, Mancini J, Witter FR. Hemoglobin concentrations influence birth outcomes in pregnant African–American adolescents. J Nutr 2003; 133(7):2348-2355.

(15) Knottnerus JA, Delgado LR, Knipschild PG, Essed GG, Smits F. Haematologic parameters and pregnancy outcome. A prospective cohort study in the third trimester. J Clin Epidemiol 1990; 43(5):461-466.

(16) Murphy JF, O'Riordan J, Newcombe RG, Coles EC, Pearson JF. Relation of haemoglobin levels in first and second trimesters to outcome of pregnancy. Lancet 1986; 1(8488):992-995.

(17) Steer P, Alam MA, Wadsworth J, Welch A. Relation between maternal haemoglobin concentration and birth weight in different ethnic groups. BMJ 1995; 310(6978):489-491.

(18) Allen LH. Biological mechanisms that might underlie iron's effects on fetal growth and preterm birth. J Nutr 2001; 131(2S-2):581s-589s.

(19) Falkenberg ER, Davis RO, DuBard M, Parker CRJ. Effects of maternal infections on fetal adrenal steroid production. Endocr Res 1999; 25(3-4):239-249.

(20) Smith R. Alterations in the hypothalamic pituitary adrenal axis during pregnancy and the placental clock that determines the length of parturition. J Reprod Immunol 1998; 39:215-220.

(21) Cester N, Staffolani R, Rabini RA et al. Pregnancy induced hypertension: a role for peroxidation in microvillus plasma membranes. Mol Cell Biochem 1994; 131(2):151-155.

(22) Poranen AK, Ekblad U, Uotila P, Ahotupa M. Lipid peroxidation and antioxidants in normal and pre-eclamptic pregnancies. Placenta 1996; 17(7):401-405.

(23) Kramer MS. Preventing preterm birth: are we making any progress? Yale J Biol Med 1997; 70(3):227-232.

(24) Kramer MS, Mclean FH, Eason EL, Usher RH. Maternal nutrition and spontaneous preterm birth. Am J Epidemiol 1992; 136(5):574-583.

(25) Berkowitz GS, Blackmore-Prince C, Lapinski RH, Savitz DA. Risk factors for preterm birth subtypes. Epidemiology 1998; 9(3):279-285.

(26) Pickett KE, Abrams B, Selvin S. Defining preterm delivery – the epidemiology of clinical presentation. Paediatr Perinat Epidemiol 2000; 14(4):305-308.

(27) Zhang J, Savitz DA. Preterm birth subtypes among blacks and whites. Epidemiology 1992; 3(5):428-433.

(28) Tucker JM, Goldenberg RL, Davis RO, Copper RL, Winkler CL, Hauth JC. Etiologies of preterm birth in an indigent population: is prevention a logical expectation? Obstet Gynecol 1991; 77(3):343-347.

(29) Siega-Riz AM, Adair LS, Hobel CJ. Maternal hematologic changes during pregnancy and the effect of iron status on preterm delivery in a West Los Angeles population. Am J Perinatol 1998; 15(9):515-522.

(30) Savitz DA, Blackmore CA, Thorp JM. Epidemiologic characteristics of preterm delivery: etiologic heterogeneity. Am J Obstet Gynecol 1991; 164(2):467-471.

(31) Berry RJ, Li Z, Erickson JD et al. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. N Engl J Med 1999; 341(20):1485-1490.

(32) Li Z, Berry RJ, Li S. [Preventing neural tube defects with periconceptional folic acid supplementation: a population-based intervention program in the China]. Zhonghua Yi Xue Za Zhi 2000; 80(7):493-498.

(33) Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. Obstet Gynecol 1996; 87(2):163-168.

(34) Harrell FE, Lee KL, Pollock BG. Regression models in clinical studies: determining relationships between predictors and response. J Natl Cancer Inst 1988; 80(15):1198-1202.

(35) Harrell FE. Regression modeling strategies. New York: Springer-Verlag, 2001.

(36) Allison PD. Survival analysis using the SAS system: a practical guide. Cary, NC: SAS Institute Inc., 1995.

(37) Meis PJ, Michielutte R, Peters TJ et al. Factors associated with preterm birth in Cardiff, Wales. II. Indicated and spontaneous preterm birth. Am J Obstet Gynecol 1995; 173(2):597-602.

(38) Klebanoff MA, Shiono PH, Selby JV, Trachtenberg AI, Graubard BI. Anemia and spontaneous preterm birth. Am J Obstet Gynecol 1991; 164(1 Pt 1):59-63.

(39) Xiong X, Buekens P, Alexander S, Demianczuk N, Wollast E. Anemia during pregnancy and birth outcome: a meta-analysis. Am J Perinatol 2000; 17(3):137-146.

(40) Adams MM, Sarno AP, Harlass FE, Rawlings JS, Read JA. Risk factors for preterm delivery in a healthy cohort. Epidemiology 1995; 6(5):525-532.

(41) Greenland S. Problems in the average-risk interpretation of categorical dose-response analyses. Epidemiology 1995; 6(5):563-565.

(42) Scholl TO, Hediger ML. Anemia and iron-deficiency anemia: compilation of data on pregnancy outcome. Am J Clin Nutr 1994; 59(2 Suppl):S492-S501.

(43) Scholl TO. Iron status during pregnancy: setting the stage for mother and infant. Am J Clin Nutr 2005; 81(5).

(44) Ananth CV, Joseph KS, Oyelese Y, Demissie K, Vintzileos AM. Trends in preterm birth and perinatal mortality among singletons: United States, 1989 through 2000. Obstet Gynecol 2005; 105(5):1084-1091.

(45) Mi J, Lin L, Liu Y, Zhang X, Cao L. A national sampling survey on birth weight in 1998 in China: mean value and standard deviation. Zhonghua Yu Fang Yi Xue Za Zhi 2002; 36(3):154-157.

(46) Whittaker PG, Macphail S, Lind T. Serial hematologic changes and pregnancy outcome. Obstet Gynecol 1996; 88(1):33-39.

Table 3 - 1

Maternal and neonatal characteristics and preterm birth (<37 weeks) rates by clinical subtypes

Characteristics	Total infants	All	Preterm birth clinical subtypes (%)		
			Preterm PROM	Spontaneous preterm	Medically indicated
All live born infants	160,700	4.7	0.6	3.6	0.5
Maternal age (yrs)					
<25	100,385	5.0	0.6	4.0	0.4
25-29	41,685	4.1	0.6	3.0	0.5
30-34	17,525	3.7	0.6	2.5	0.6
≥35	1,103	5.8	0.9	3.4	1.5
Education					
High school/college	18,262	4.1	0.7	2.7	0.7
Junior high school	96,162	4.8	0.6	3.7	0.5
Elementary school or less	45,875	4.9	0.6	3.8	0.5
Occupation					
Farmer	88,051	4.7	0.6	3.7	0.4
Factory worker	63,633	4.7	0.7	3.5	0.5
Other	8,821	4.1	0.8	2.5	0.8
Ethnicity					
Han	159,619	4.7	0.6	3.6	0.5
Other	895	5.7	0.6	4.4	0.7

Gravidity					
1	74,826	5.5	0.6	4.4	0.5
2	47,512	4.1	0.6	3.0	0.5
≥ 3	38,260	4.0	0.7	2.7	0.6
Parity					
0	135,504	4.7	0.6	3.6	0.5
≥1	25,196	4.4	0.6	3.3	0.5
Folic acid use					
Yes	85,782	4.4	0.6	3.3	0.5
No	74,918	5.0	0.6	3.9	0.5
BMI					
Underweight	23,076	4.9	0.7	3.7	0.5
Normal weight	110,394	4.6	0.6	3.5	0.5
Overweight/Obese	4,717	4.5	0.5	3.5	0.5
Registration					
Pre-pregnancy	65,206	3.7	0.6	2.7	0.4
During pregnancy	95,494	5.3	0.6	4.2	0.5
First prenatal visit					
<12 weeks	131,001	4.6	0.6	3.5	0.5
12-27 weeks	23,150	5.5	0.6	4.3	0.6
≥28 weeks	6,549	4.3	0.4	3.7	0.2
Fetal sex					
Male	82,978	4.9	0.7	3.7	0.5
Female	77,722	4.5	0.6	3.4	0.5

Table 3 - 2

Maternal anemia and preterm birth (<37 weeks) rates by severity and clinical subtypes

	Total Live-born infants	With attribute (%)	All preterm births (%)	Preterm birth severity (%)			Preterm birth clinical subtypes (%)		
				<32 wks	32-33 wks	34-36 wks	Preterm PROM	Spontaneous preterm	Medically indicated
All women	160,700	-	4.7	0.3	0.5	3.9	0.6	3.6	0.5
Maternal anemia status	97,612								
None	63,586	65.1	4.4	0.2	0.4	3.8	0.6	3.4	0.5
1st trimester only	1,917	2.0	4.6	0.1	0.4	4.1	0.6	3.6	0.4
2nd trimester only	5,204	5.3	3.5	0.04	0.3	3.2	0.5	2.6	0.4
3rd trimester only	11,627	11.9	2.9	0.1	0.3	2.5	0.4	2.2	0.3
1st and 2nd trimesters	2,318	2.4	3.7	0.1	0.2	3.4	0.8	2.6	0.3
1st and 3rd trimesters	927	0.9	3.2	-	0.4	2.8	0.2	2.6	0.4
2nd and 3rd trimesters	6,505	6.7	3.5	0.1	0.3	3.1	0.5	2.5	0.4
All 3 trimesters	5,528	5.7	5.0	0.3	0.6	4.1	0.7	4.0	0.4

Table 3 - 3

Adjusted hazard ratio for preterm birth (<37 weeks) and its clinical subtypes in relation to maternal anemia

Anemia status during pregnancy	Adjusted hazard ratio (95% confidence interval) for preterm birth			
	Total preterm births	Preterm PROM	Spontaneous preterm labor	Medically indicated preterm birth
None	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
1st trimester only	1.1 (0.9, 1.3)	1.0 (0.6, 1.8)	1.1 (0.9, 1.4)	0.9 (0.5, 1.9)
2nd trimester only	0.8 (0.7, 0.9)	0.8 (0.6, 1.3)	0.8 (0.7, 0.9)	0.9 (0.5, 1.3)
3rd trimester only	0.7 (0.6, 0.8)	0.7 (0.5, 0.9)	0.7 (0.6, 0.8)	0.7 (0.5, 0.9)
1st and 2nd trimesters	0.8 (0.7, 1.0)	1.4 (0.8, 2.2)	0.8 (0.6, 1.0)	0.7 (0.3, 1.4)
1st and 3rd trimesters	0.7 (0.5, 1.1)	0.4 (0.1, 1.5)	0.8 (0.5, 1.2)	0.9 (0.4, 2.5)
2nd and 3rd trimesters	0.8 (0.7, 0.9)	0.9 (0.6, 1.3)	0.8 (0.7, 0.9)	0.9 (0.6, 1.4)
All 3 trimesters	1.1 (1.0, 1.3)	1.2 (0.9, 1.7)	1.2 (1.0, 1.3)	0.8 (0.5, 1.3)

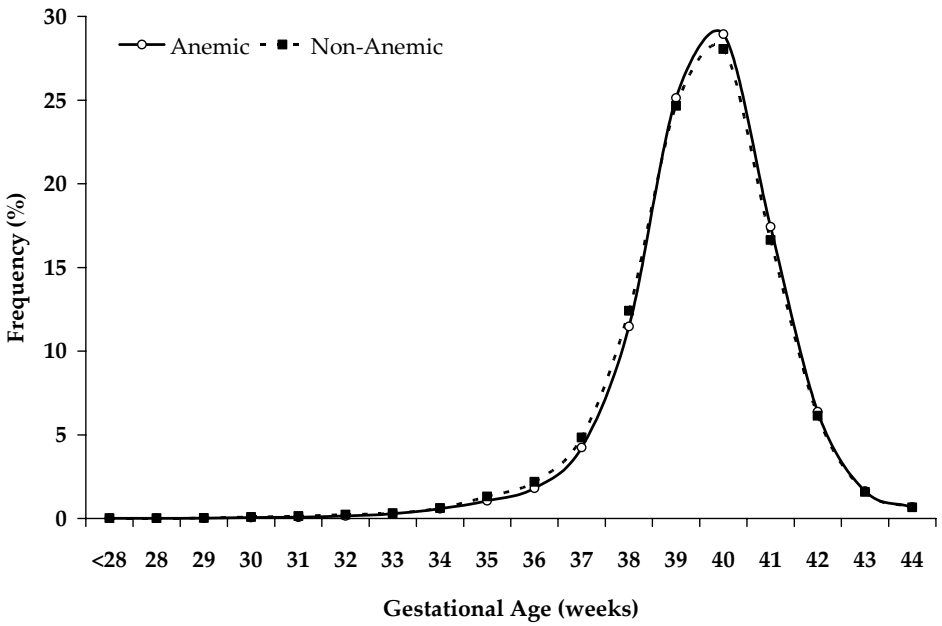


Figure 3 - 1
Distribution of gestational age by anemia status

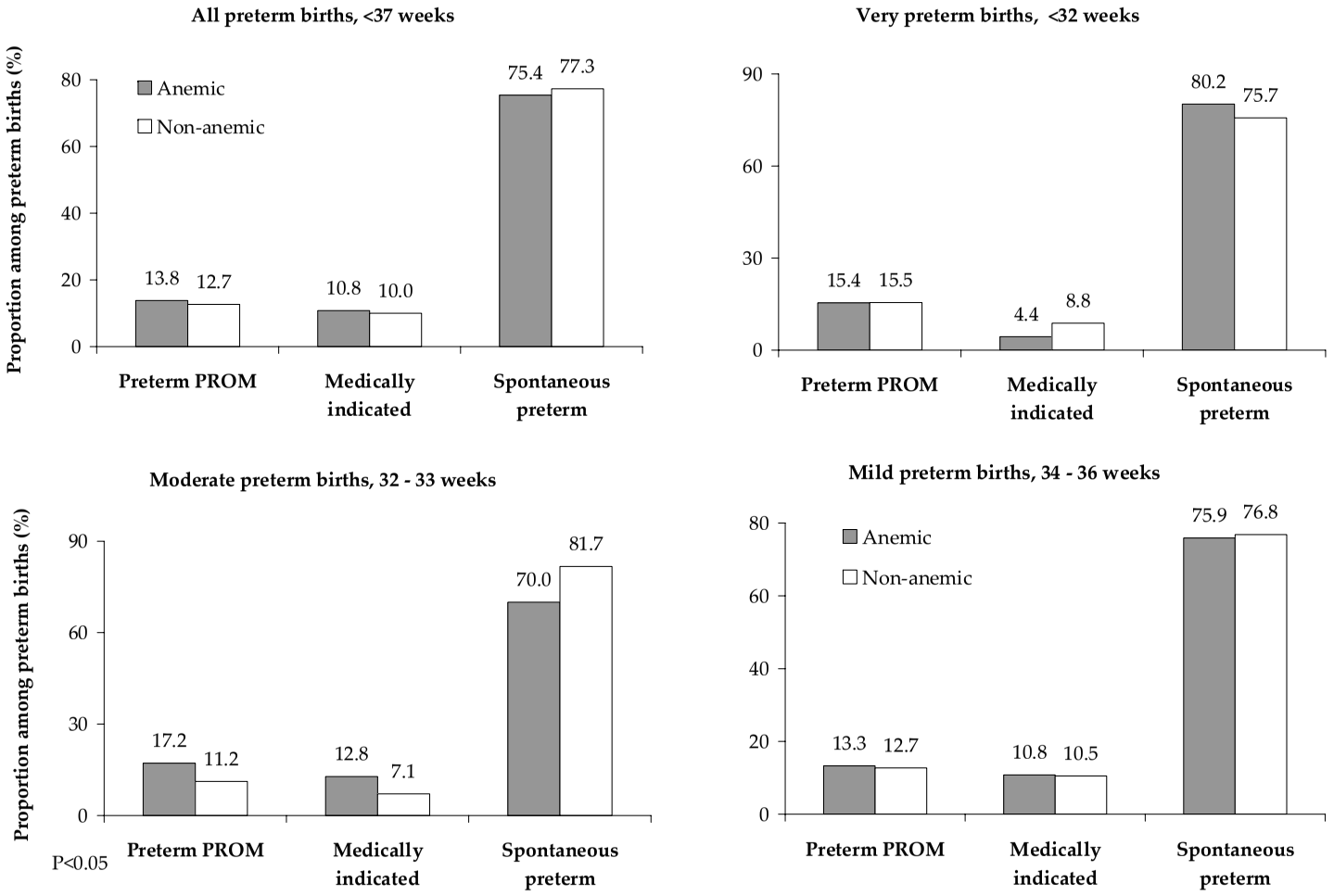


Figure 3 - 2
Proportion of clinical subtypes among preterm births by preterm severity and maternal anemia status

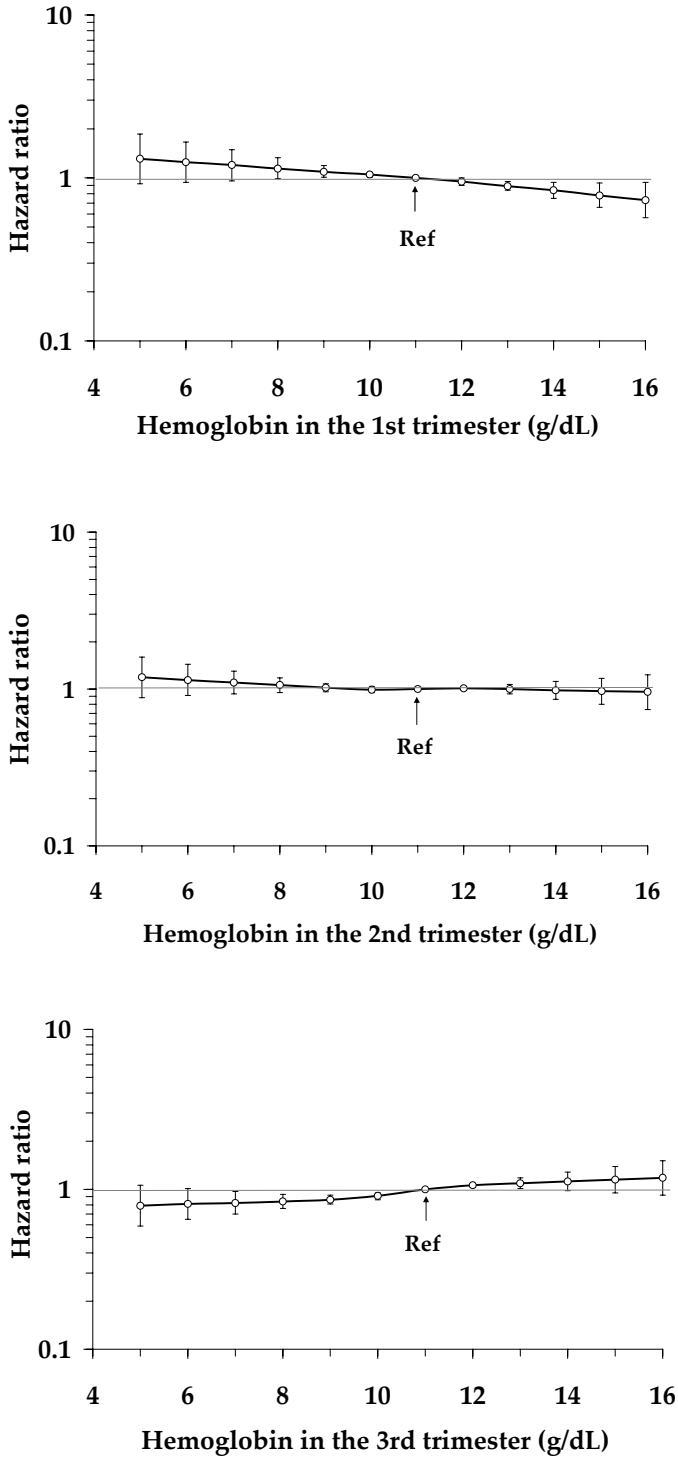


Figure 3 - 3
Adjusted hazard ratio of preterm birth (< 37 weeks) and 95% confidence interval for hemoglobin level in each trimester

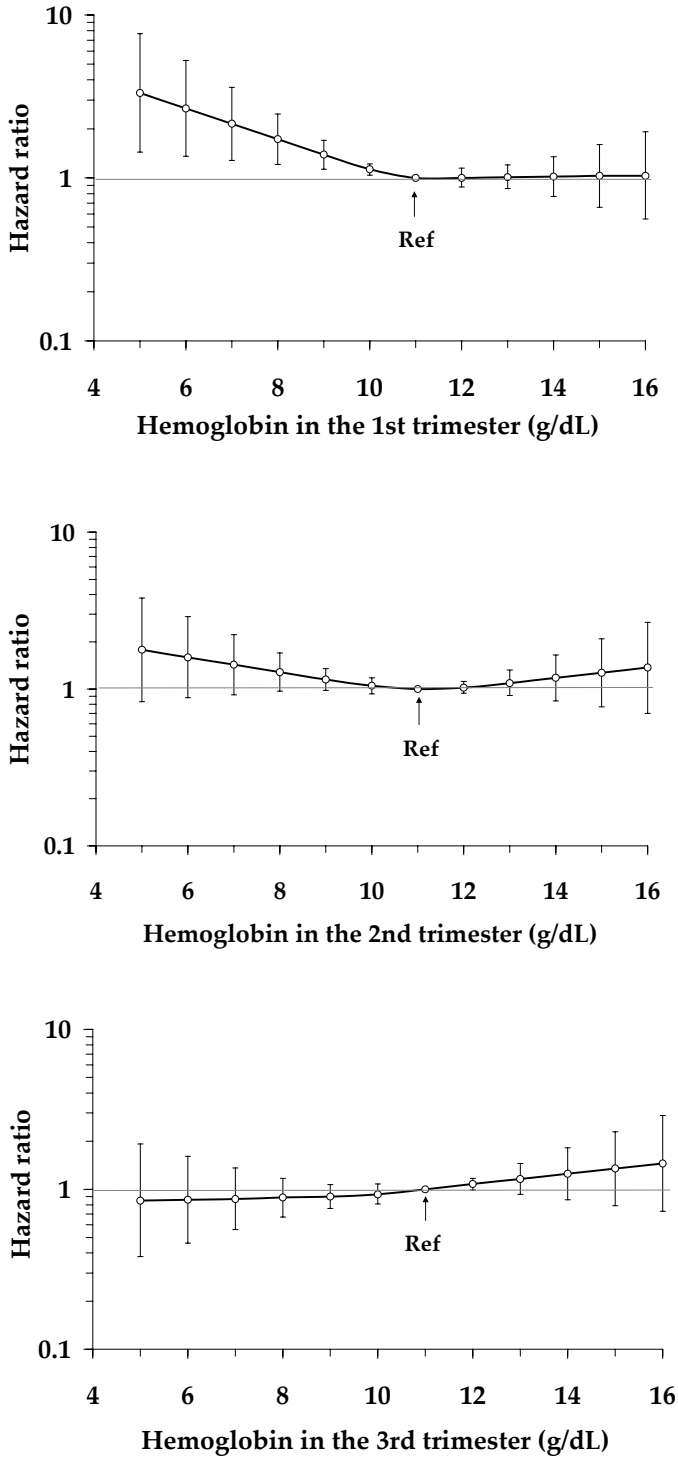


Figure 3 - 4
Adjusted hazard ratio of preterm premature rupture of membranes (< 37 weeks) and 95% confidence interval for hemoglobin level in each trimester

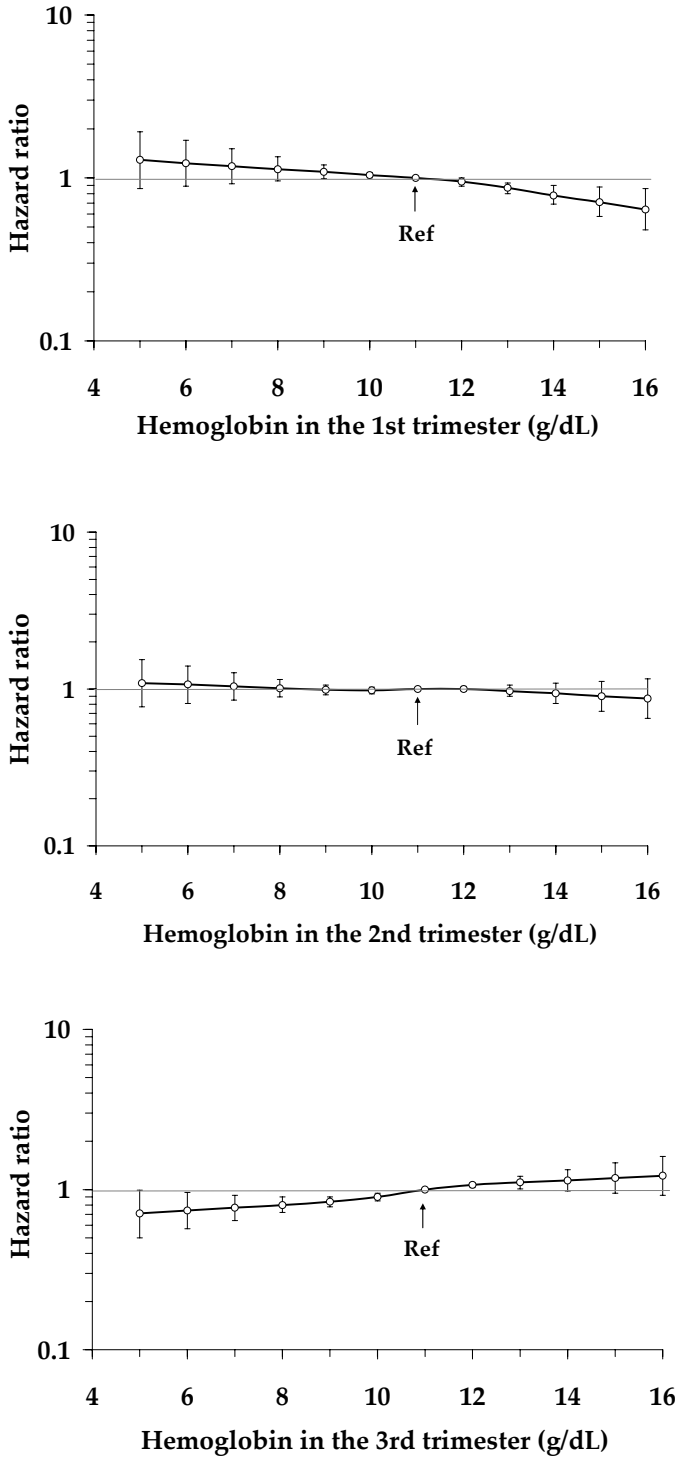


Figure 3 - 5
Adjusted hazard ratio of spontaneous preterm labor (<37 weeks) and 95% confidence interval for hemoglobin level in each trimester

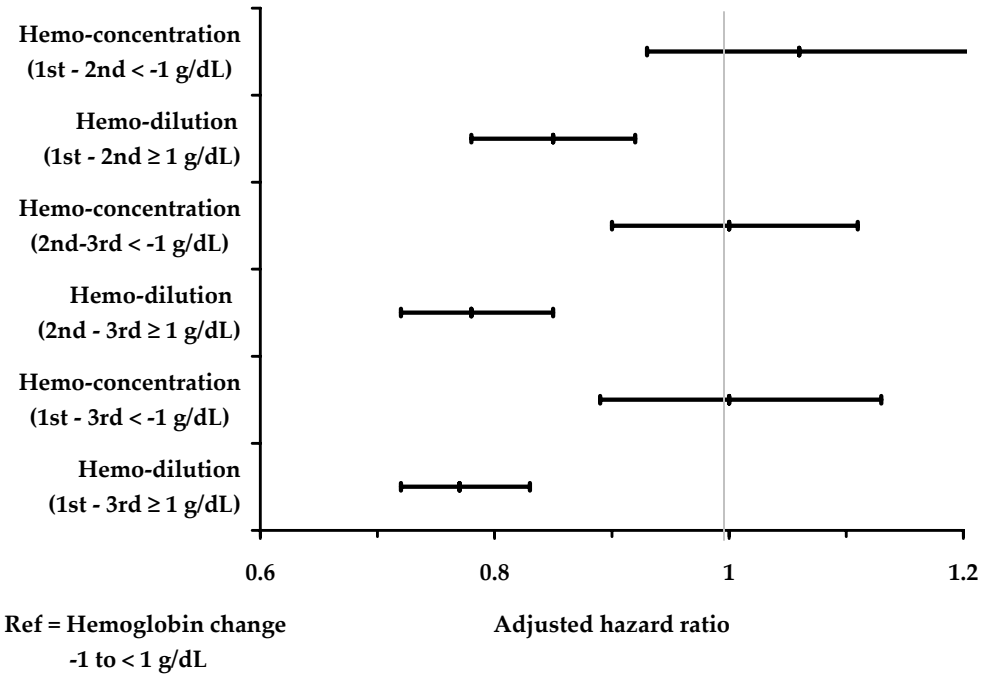


Figure 3 - 6
Hemoglobin change and preterm birth (<37 weeks)

Summary

Maternal anemia is a ubiquitous pregnancy complication associated with an array of adverse pregnancy outcomes, including preterm birth, restricted fetal growth, and perinatal death (1). Because of its high prevalence, the impact of maternal anemia on fetal development remains an important public health concern. Despite numerous reports on associations between maternal anemia and adverse perinatal outcomes, the findings remain largely inconclusive. To some extent, this is mitigated by inadequacies in study designs, e.g., failing to adjust for important confounders, failure to distinguish profiles of anemia across trimesters within pregnancy, and aggregating heterogeneous exposures and outcomes.

The lack of consistency in findings across studies may also be due to variations in the timing of hemoglobin measurement. In mid- and late pregnancy, it may difficult to distinguish physiological hemo-dilution and true iron deficiency anemia (2), therefore, separate assessment corresponding to the stage of pregnancy may reveal important insights of the associations.

To our knowledge, all previous studies have categorized hemoglobin values using one or multiple cut-offs, instead of examining risks on a continuum of hemoglobin values. Categorization of a skewed-distributed exposure like anemia, assuming within-category homogeneity, degrades continuous exposure information and tends to be less accurate than the spline analysis (3) applied in this dissertation.

Considerable etiologic heterogeneity has been reported among preterm birth. Preterm birth can be broadly classified based on underlying sequence of clinical presentations as spontaneous preterm birth (spontaneous onset of labor or following

preterm premature rupture of membranes) and medically indicted preterm birth (4-8). However, little has been done to evaluate if the associations between anemia and preterm birth vary among its different clinical subtypes (9).

This dissertation, using data from a large, prospective, population-based cohort of pregnant women in 13 counties of East China, addressed 3 specific topics: (i) The epidemiology of maternal anemia; (ii) Association of maternal hemoglobin/anemia and perinatal mortality; and (iii) Association of maternal anemia and hemoglobin profiles and preterm birth clinical subtypes. Hemoglobin levels during pregnancy were stratified by the stage of gestation and examined as a continuous measure. A variety of confounding factors were measured and adjusted. Hemoglobin changes across trimesters were quantified and examined as proxy of hemo-dilution.

About one third of the pregnant women of our cohort (n=164,667) had anemia (hemoglobin <10 g/dL) during pregnancy. Older maternal age, lower education, farm occupation, delayed prenatal care, pregnancy-induced hypertension and preeclampsia were associated with increased risk for anemia, whereas peri-conceptional folic acid use was associated with reduced risk for 1st trimester anemia. Anemia in the first half of pregnancy was associated with increased risk of stillbirth (adjusted hazard ratio (HR) 1.7, 95% confidence internal (CI) 1.1, 2.7), but not neonatal deaths. Anemia in the 1st trimester was associated with increased risk for preterm premature rupture of membranes (PROM). Women with hemoglobin ≤5 g/dL were at highest risk (HR 3.3, 95% CI 1.4, 7.7) with progressively declining risk with increasing hemoglobin levels. In contrast, anemia in the 3rd trimester was associated with reduced risk for all preterm birth and

spontaneous preterm labor. Anemia was not associated with medically indicated preterm birth. Hemo-dilution over gestation was associated with up to 23% reduced risk for preterm birth.

The findings of this dissertation underscore that early prevention and prompt treatment of maternal anemia may be one avenue for intervention, and may be a topic worthy for further investigation. Basic studies are needed to further clarify if maternal anemia is a biomarker or an independent condition on the biologic pathways in relation to adverse perinatal outcomes. The efforts of physiologic hemo-dilution during pregnancy should be considered and appropriately controlled for in anemia related studies.

References

(1) Rasmussen K. Is there a causal relationship between iron deficiency or iron-deficiency anemia and weight at birth, length of gestation and perinatal mortality? J Nutr 2001; 131(2S-2):5905-603s.

(2) Scholl TO. Iron status during pregnancy: setting the stage for mother and infant. Am J Clin Nutr 2005; 81(5).

(3) Greenland S. Problems in the average-risk interpretation of categorical dose-response analyses. Epidemiology 1995; 6(5):563-565.

(4) Ananth CV, Vintzileos AM. Epidemiology of preterm birth and its clinical subtypes. J Matern Fetal Neonatal Med 2006; 19(12):773-782.

(5) Berkowitz GS, Blackmore-Prince C, Lapinski RH, Savitz DA. Risk factors for preterm birth subtypes. Epidemiology 1998; 9(3):279-285.

(6) Pickett KE, Abrams B, Selvin S. Defining preterm delivery – the epidemiology of clinical presentation. Paediatr Perinat Epidemiol 2000; 14(4):305-308.

(7) Zhang J, Savitz DA. Preterm birth subtypes among blacks and whites. Epidemiology 1992; 3(5):428-433.

(8) Tucker JM, Goldenberg RL, Davis RO, Copper RL, Winkler CL, Hauth JC. Etiologies of preterm birth in an indigent population: is prevention a logical expectation? Obstet Gynecol 1991; 77(3):343-347.

(9) Siega-Riz AM, Adair LS, Hobel CJ. Maternal hematologic changes during pregnancy and the effect of iron status on preterm delivery in a West Los Angeles population. Am J Perinatol 1998; 15(9):515-522.

Curriculum Vita

Qiaoyi Zhang

Education

2001-08 Doctor of Philosophy (Major in Epidemiology)
University of Medicine and Dentistry of New Jersey
School of Public Health, *Piscataway, New Jersey*

1997-99 Master of Science in Biostatistics
University of Illinois at Chicago
School of Public Health, *Chicago, Illinois*

1983-89 Bachelor of Medicine (MD equivalent)
Beijing Medical University, Beijing, China

Professional Experience

Merck & Co., Inc.
Global Outcomes Research and Health Technology Assessment
Whitehouse Station, New Jersey

May 2007– present Associate director
July 2004 – Apr 2007 Manager
Mar 2001 – June 2004 Associate manager
Mar 1999 – Feb 2001 Research Analyst

Searle
Global Health Outcomes, Global Medical Marketing
Skokie, Illinois

Jan 1998 – Dec 1998 Research Associate

University of Illinois at Chicago
Chicago, Illinois

Aug 1997 – Jan 1999 Teaching Assistant
Division of Epidemiology and Biostatistics

July 1996 – Dec 1997 Research Assistant
Department of Nutrition and Dietetics

Beijing Medical University
National Center for Maternal and Infant Health Care
Department of Health Care Epidemiology, *Beijing, China*

Oct 1994 – Dec 1995 Assistant Professor
July 1989 – Sept 1994 Research Associate

Publications

1. Dai K, **Zhang Q**, Fan T, Sen SS, Team OB. Estimation of resource utilization associated with osteoporotic hip fracture and level of post-acute care in China. Current Medical Research and Opinion. 2007; 23(12): 2937-2943.

2. Hanley DA, **Zhang Q**, Meilleur MC, Mavros P, Sen SS. Prescriptions for vitamin D among patients taking antiresorptive agents in Canada. Current Medical Research and Opinion. 2007; 23(6): 1473-1480.

3. **Zhang Q**, Thomas M, Wisniewski T, Sazonov Kocevar V, Price D. Treatment and outcomes in patients with asthma and allergic rhinitis in the United Kingdom. International Archives of Allergy and Immunology 2007; 142(4):318-28.

4. Van Ganse E, Antonicelli L, **Zhang Q**, et al. Asthma-related resource use and cost by GINA classification of severity in three European countries. Respiratory Medicine 2006; 100(1):140-147.

5. Price D, **Zhang Q**, Kocevar VS, Yin DD, Thomas M. Effect of a concomitant diagnosis of allergic rhinitis on asthma-related health care use by adults. Clinical and Experimental Allergy 2005; 35(3):282-287.

6. Thomas M, Kocevar VS, **Zhang Q**, Yin DD, Price D. Asthma-related health care resource use among asthmatic children with and without concomitant allergic rhinitis. Pediatrics 2005; 115(1):129-134.

7. Antonicelli L, Bucca C, Neri M De Benedetto F, Sabbatani P, Bonifazi F, Eichler HG, **Zhang Q**, Yin DD. Asthma severity and medical resource utilisation. European Respiratory Journal 2004; 23(5):723-729.

8. Langman M, Kong SX, **Zhang Q**, Kahler KH, Finch E. Safety and patient tolerance of standard and slow-release formulations of NSAIDs. Pharmacoepidemiology and Drug Safety 2003; 12(1):61-66.

9. Gerth WC, McCarroll KA, Santanello NC, Vandormael K, **Zhang Q**, Mannix LK. Patient satisfaction with rizatriptan versus other triptans: direct head-to-head comparisons. International Journal of Clinical Practice 2001; 55(8):552-6.

10. Langman M, Kahler KH, Kong SX, **Zhang Q**, Finch E, Bentkover JD, Stewart EJ. Drug switching patterns among patients taking non-steroidal anti-inflammatory drugs: a retrospective cohort study of a general practitioners database in the United Kingdom. Pharmacoepidemiology and Drug Safety 2001; 10(6):517-524.

11. Price DB, Ben-Joseph RH, **Zhang Q**. Changes in asthma drug therapy costs for patients receiving chronic montelukast therapy in the UK. Respiratory Medicine 2001; 95(1):83-89.