MATERNAL HAEMOGLOBIN AND OUTCOME OF PREGNANCY

Written by

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DECLARATION

I, Kwaku Poku Amponsah, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgement indicate otherwise) and that neither the whole work nor any part of it has been, or is to be submitted for another degree in this or any other university.

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ABSTRACT

Background: The association between maternal haemoglobin concentration and the outcome of pregnancy has been a source of continual controversy. Preterm delivery and low birth weight are major causes of stillbirths and early neonatal deaths. Pre-eclampsia is a major complication which occurs during pregnancy and leads to significant maternal and fetal morbidity and mortality. This study aims to assess the association between maternal haemoglobin concentration and pregnancy outcome.

Objectives: To assess the association between maternal haemoglobin concentration and pre-eclampsia, preterm birth and low birth weight.

Methods: Retrospective analysis of 191 patients who delivered an infant of 28 weeks gestation or more from 1st to 22nd May 2017 at New Somerset Hospital with documented maternal haemoglobin concentration at 22 – 33 weeks gestation. The maternal haemoglobin concentration used was the lowest documented during 22 – 33 weeks gestation.

Main outcome measures: Development of pre-eclampsia, low birth weight and preterm birth.

Results: The distribution of pre-eclampsia, low birth weight and preterm birth were skewed towards the higher side of the maternal haemoglobin concentration

spectrum. There were statistically significant differences in the development of pre-eclampsia, low birth weight and preterm birth between maternal haemoglobin concentrations =>13g/dl and that of <13g/dl. Pre-eclampsia was associated with relatively lower birth weight, preterm birth and low placental weight independently of the haemoglobin status.

Conclusion: High maternal haemoglobin concentration at 22 – 33 weeks gestation is associated with an increased prevalence of pre-eclampsia, low birth weight and preterm birth.

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ABBREVIATIONS

NS	Not Significant
Hb	Haemoglobin
g/dl	Grams per deciliter
g	Grams
WHO	World Health Organization
n/a	Not applicable
wks	Weeks
n	Number
%	Percentage
BMI	Body Mass Index
msq	Meter square
SD	Standard Deviation
NVD	Normal Vaginal Delivery
C/S	Caesarean Section

1.0 INTRODUCTION

Background:

The association between maternal haemoglobin concentration and the outcome of pregnancy has been a source of continual controversy. Some studies have reported the association of maternal anaemia with adverse birth outcomes (1, 2) with others reporting high maternal haemoglobin concentration's association with adverse birth outcomes (3 - 5).

Preterm delivery and low birth weight are major causes of stillbirths and early neonatal deaths. Preterm birth according to the World Health Organization (WHO) is birth that occurs before 37 completed weeks of gestation (6). Globally, approximately 15 million infants (nearly 12% of all births) are born preterm every year, with the rate of preterm delivery ranging from about 18% in most African countries to about 5% in some European countries (7). The majority of preterm deliveries in the world (over 60%) occurs in South Asia and sub-Saharan Africa (7). Even though many factors are related to prematurity, the actual causes of most spontaneous premature deliveries are not identified (8, 9).

Global reports indicate that in 2016, complications of premature delivery were responsible for the majority of neonatal and early childhood deaths (10). Complications of preterm birth include, among others, intraventricular haemorrhage, periventricular leukomalacia, infections, respiratory distress syndrome and cerebral palsy in addition to gastrointestinal, auditory and ophthalmic problems (11 - 14). The risk of adverse outcomes among babies delivered preterm far exceed that among babies delivered at term (15, 16). Prematurity has been associated with poor performance at school, neurodevelopmental problems and frequent admissions to hospital, among others (17 - 19). Preterm delivery also put significant stress mentally and financially on parents and relatives as well as burdening the health system (20 - 22). Preterm delivery is a huge public health issue globally. Effective ways to reduce preterm deliveries and to manage preterm babies is essential especially in areas with poor resources.

There is an association between birth weight and morbidity and mortality of the fetus, neonate and infant, as well as their childhood growth and development. Low birth weight according to the World Health Organization is weight of a baby at birth

below 2500g (23). Determination of birth weight needs to be done immediately after delivery to avoid appreciable loss of weight post-delivery (24). Birth weight below 2500g is associated with high morbidity and mortality in infancy (25 - 31). Birth weight may reflect the overall health status of the mother close to conception as well as during pregnancy. Low birth weight is a huge burden mostly in developing countries which requires effective interventions to prevent or reduce its occurrence (32).

According to estimates by the World Health Organization/United Nations Children's Fund, about 15.5% (nearly 20 million) of deliveries occurring in the world yearly are infants with birth weight below 2.5kg, with about 95.6% occurring in developing countries (33). The low birth weight incidence rate ranges from as high as 18.8% in a typical developing country to as low as 8.7% in a typical developed country (34). The low birth weight prevalence rate in developing countries (16.5%) is more than double that of developed countries (7%) (35).

It is known that the weight of a child at birth is important in predicting how well the child will grow and survive to adulthood (36, 37). Infants with low birth weight are at a higher risk of getting sick and dying from poor nutrition, failure to grow and infections as well as performing poorly in school (38, 39). Low birth weight infants are also highly prone to developing chronic diseases in adulthood (40 - 43). Several fetal as well as maternal factors contribute to low birth weight, but most of these factors can be modified.

A well-functioning placenta is important for the developing fetus to grow adequately, and the capacity of maternal cardiac output to increase to perfuse the placenta efficiently is essential. Placental development during implantation depends mainly on trophoblastic cell differentiation (trophoblast which differentiate into cytotrophoblast and syncytiotrophoblast). Invasion of the endometrial stroma by the trophoblastic cells (syncytiotrophoblast) occurs to complete implantation. The maternal blood-filled lacunar network develops through enzyme activity to supply nutrients to the embryo. These lacunae fuse to form the intervillous spaces. Maternal blood enters the intervillous space formed from syncytiotrophoblast lacunae through 80 – 100 spiral arteries (44). Various substances are exchanged at the intervillous space between the maternal blood and the fetal blood. The maternal blood and the fetal blood however do not mix.

Endovascular trophoblastic invasion is a physiological adaptation that occurs if pregnancy is to be successful. This process occurs in two stages: first wave and second wave trophoblastic invasion. The first wave trophoblastic invasion occurs at a gestational age of about 8 to 10 weeks and involves invasion of decidual segments of the spiral arteries. The second wave trophoblastic invasion occurs at a gestational age of about 16 to 18 weeks and involves invasion of the myometrial segments of the spiral arteries. Endovascular trophoblastic invasion leads to the conversion of muscular walls of spiral arteries to low pressure capacitance vessels to enhance adequate blood flow to the developing fetus. This ensures adequate supply of nutrients to the developing fetus as well as elimination of waste products from the maternal blood circulation. The placenta also secretes numerous hormones to support pregnancy and enhance growth and development of the fetus. Defective trophoblastic invasion is seen in pre-eclampsia and other severe early-onset diseases including fetal growth restriction where the surface area of the placenta as well as the volume are both reduced (44).

The mechanism by which defective placental development, poor uterine perfusion and associated ischaemia of the placenta leads to generalized endothelial cell dysfunction in pre-eclampsia remains unresolved. Pre-eclamptic women demonstrate intravascular volume contraction, coagulative disorder and vasoconstriction whereas healthy pregnant women demonstrate intravascular volume expansion with vasodilatation. Women who develop pre-eclampsia exhibit features of defective placental development and high resistance to perfusion of the utero-placental unit before clinical symptoms appear. The risk of developing cardiovascular disease later in life is high for pre-eclamptic women.

Anaemia in pregnancy is defined by the World Health Organization as haemoglobin concentration below 11g/dl (45). Anaemia in pregnancy is associated with an increase in fetal and maternal morbidity and mortality and remains a major public health issue (46). Anaemia may be present prior to pregnancy or may develop during pregnancy. The causes of anaemia in pregnancy includes infections such as Tuberculosis, Human Immunodeficiency Virus, intestinal helminthiasis and malaria (47). Anaemia in pregnancy may also be caused by vitamins, folate and iron deficiency. Obstetric complications such as antepartum haemorrhage and HELLP (Haemolysis, Elevated Liver enzymes, Low Platelets) Syndrome may lead to anaemia. Physiological haemodilution in pregnancy due to plasma volume

expansion may cause maternal haemoglobin concentration to fall below 11g/dl which may however not be genuine anaemia.

Anaemia in pregnancy is associated with a higher risk of peri-partum, intra-partum and post-partum complications for the mother and a higher risk of preterm birth for the infant. According to Beckert et al. anaemic mothers are more likely to be diagnosed with hypertension, diabetes, placental abruption, chorioamnionitis, or admission to an intensive care unit (48).

The effects of anaemia in pregnancy on maternal health include the aggravation of the risks of childbirth, danger of heart failure and reduction of immune response (49). Maternal anaemia during pregnancy is associated with adverse fetal outcome through growth restriction or perinatal death with an increased risk of infant mortality and morbidity (50). Work done by Nair et al. revealed that iron deficiency anaemia in pregnancy is associated with higher risks of postpartum haemorrhage, small-for-gestational age babies, low birth weight and preterm birth and perinatal death (41).

The relationship between maternal haemoglobin concentration and birth weight has been observed in several studies to be U-shaped with abnormally high haemoglobin concentration indicating poor plasma volume expansion being a risk for low birth weight and preterm delivery. Maternal anaemia before mid-pregnancy is associated with an increased risk of preterm birth.

Effective antenatal care is the key to early detection, diagnosis and treatment of anaemia in pregnancy to reduce associated maternal and fetal morbidity and mortality.

Physiological changes in pregnancy include expansion of the volume of plasma to approximately 3800ml in pregnancy from approximately 2600ml at pre-pregnancy, without much further increase beyond the gestational age of 32 weeks. An increase in the mass of red blood cells also occurs from about 250ml to about 400ml during pregnancy. The volume of plasma however increases proportionately more than the mass of red blood cells, resulting in a reduction of haemoglobin concentration (44, 52). During pregnancy, iron absorption goes up but haemoglobin concentration falls. Plasma volume expansion and the associated fall in haemoglobin appears to be an important maternal response to pregnancy.

There is an association between maternal haemoglobin concentration in pregnancy and pregnancy outcome with respect to preterm birth, low birth weight, perinatal mortality and maternal morbidity. An association of increased risk of adverse pregnancy outcome with high maternal haemoglobin concentration is of concern as this could be a manifestation of a maladaptation to the physiological changes in normal pregnancy.

High blood viscosity has an adverse effect on the intervillous space leading to poor exchange of gas and nutrients between the fetal and maternal circulation (53). Haemodilution occurs in pregnancy to enhance perfusion of the placenta through a reduction in the viscosity of blood. Failure of maternal haemoglobin concentration to fall and maintain the low level until term during pregnancy results in adverse fetal and maternal outcomes (4, 54).

The association between maternal haemoglobin concentration and the outcome of pregnancy is still unresolved. Findings of the association between maternal haemoglobin concentration and the outcome of pregnancy will guide clinicians to identify mothers who are at risk. Investigating the underlying clinical cause and following up such at risk women closely may help improve pregnancy outcomes.

2.0 LITERATURE REVIEW

The Placenta

At term, the overall placental surface area over which exchange of nutrients and gas occurs is about 11 square metres (44). Adequate placental function including

an increase in utero-placental blood flow is important in achieving optimal fetal growth. Therefore, to optimize fetal growth, the placenta undergoes physiological changes by converting the uterine spiral arterioles to wide-bore channel utero-placental vessels to improve blood flow to the developing fetus as pregnancy advances. According to Nijman et al. (55), abnormal placental perfusion is largely responsible for most very and moderate late preterm birth. The surface area and volume of the placenta are significantly reduced in fetal growth restriction (44).

According to Panti et al. (56), at term the weight of the placenta correlates directly with birth weight, though the placental weight and birth weight declines in prolonged pregnancy. Janthanaphan et al. (57) also reported that placental weight increases accordingly with birth weight, with the ratio of placental weight to birth weight decreasing after term in prolonged pregnancy. Placental weight percentile curves developed by Thompson et al. (58) also indicates that the weight of the placenta increases with birth weight until term after which it decreases slightly with prolonged pregnancy. According to Sandra et al., (59) high maternal haemoglobin concentration is associated with a decrease in placental weight. A direct association seems to exist between low maternal haemoglobin concentration and higher placental weights (60 - 62).

Birth Weight

During the first year of life, the child's morbidity and mortality mainly depends on the weight at birth and reflects how functional the placenta was during the course of the pregnancy. Low birth weight according to the World Health Organization is weight of a baby at birth =< 2499g, regardless of the gestation. This can be further classified into very low birth weight representing weight of a baby at birth less than 1500g, and extremely low birth weight representing weight of a baby at birth less than 1000g (63). At term, a birth weight of 2500 – 4200g will be classified as normal birth weight. The main causes of low birth weight are preterm birth (low gestational age at birth) and small for gestational age (slow prenatal growth rate). The latter is also referred to as fetal growth restriction.

A high incidence of low birth weight is a major problem for developing countries with respect to infant mortality and the care of survivors that become physically or mentally challenged. There is a higher chance of developing chronic diseases later

in life when the fetus fails to achieve optimal development in the uterus. Adequate placental perfusion in pregnancy is vital for fetal growth. The physiological changes in pregnancy leading to haemodilution and to reduced viscosity and thus an improvement in placental perfusion is essential. The outcome of pregnancy is poor when the maternal haemoglobin concentration does not fall during pregnancy (54). High maternal haemoglobin concentration at 27-29 weeks gestation is associated with fetal growth restriction and reduced birth weight (64). According to Steer (65), maternal haemoglobin concentrations above 12.0g/dl at the end of the second trimester is associated with a higher risk of pre-eclampsia and fetal growth restriction. Steer also reported that the minimum incidence of low birth weight and preterm birth occurs at maternal haemoglobin concentration of 9.5g/dl – 10.5g/dl.

Preterm Birth

Preterm birth refers to births occurring before 37 completed weeks gestation and can be further classified into late preterm (34W0D – 36W6D), moderate preterm (32W0D – 33W6D), very preterm (28W0D – 31W6D), and extreme preterm (<28W) (63). Prematurity is a major cause of perinatal mortality and morbidity mostly due to organ system immaturity, causing more than one million deaths every year (66 - 68). Globally, about 12% of all births (nearly 15 million babies) are born preterm (7), representing about 1 in 10 babies. In South Africa, nearly 15% of all births are born preterm (7), representing about 1 in 7 babies. The preterm birth rate in South Africa is slightly higher than the world average.

Zhang et al. (69) reported in their study that low maternal haemoglobin concentration, and thus haemodilution, in the third trimester of pregnancy was associated with a lower risk of spontaneous premature birth. According to Steer et al. (70), the degree of fall in maternal haemoglobin concentration during pregnancy is associated with fetal outcome and the failure of maternal haemoglobin concentration to fall results in a higher risk of preterm birth and low birth weight. A low maternal haemoglobin concentration of <13.0g/dl in the first trimester of pregnancy coupled with a high maternal haemoglobin concentration of =>13.0g/dl in the second trimester of pregnancy is associated with a higher risk of preterm birth (71).

Pre-eclampsia

The incidence of hypertension in pregnancy is about 6% (72). Pre-eclampsia refers to new hypertension plus proteinuria which resolves after delivery and is one of the major complications during pregnancy that causes maternal and fetal morbidity and mortality affecting 2%-3% of pregnancies (73). Pre-eclampsia, according to the new American College of Obstetricians and Gynecologists guideline, is persistent elevated blood pressure developing in pregnancy or postpartum period which is associated with significant urine proteins or the new development of thrombocytopenia, renal or liver derangement, pulmonary edema or central nervous system signs such as seizures and/or visual disturbances. Pre-eclampsia occurs as a result of poor placentation with associated placental oxidative stress leading to the release of anti-angiogenic factors by placenta causing diffuse maternal endothelial cell dysfunction (72), and the condition for its development is believed to be set as early as the first trimester (74). Early diagnosis of pre-eclampsia is essential in antenatal care to reduce adverse pregnancy outcomes. Elevated blood viscosity has been observed to be a clue for the diagnosis of pre-eclampsia for many years (75). Maternal haemoglobin concentrations are highly increased close to delivery in women with pre-eclampsia (76). High first trimester maternal haemoglobin concentration is related to the development of hypertensive disorders of pregnancy (77 - 79).

Changes in Maternal Haemoglobin during Pregnancy

In pregnancy, iron absorption goes up and plasma volume increases but maternal haemoglobin concentration falls. The volume of plasma increases faster than the mass of red blood cells resulting in physiological haemodilution. Physiological haemodilution in pregnancy causes maternal haemoglobin concentration to fall. The fall in maternal haemoglobin concentration in pregnancy occurs until approximately 32 weeks gestation with a subsequent slight rise to term (80).

The physiological increase in plasma volume during pregnancy is needed to improve intervillous placental perfusion and therefore enhance oxygen and nutrient supply to the developing fetus for optimal growth occurs by 34 weeks gestation (81). According to Steer (82), a fall in maternal haemoglobin concentration of about 1.0g/dl occurs on the average from before 12 weeks to 24

– 34 weeks gestation, with the steepest fall occurring up to 20 weeks gestation. Maternal haemoglobin concentration measurements taken after 20 weeks gestation will therefore be a fair representation of the pregnancy induced fall in haemoglobin (70).

Huisman and Aarnoudse (4) reported that a high maternal haemoglobin concentration in the mid-trimester is associated with low birth weight. Also, according to Klebanoff et al. (83), mid-trimester very low maternal haemoglobin concentration is associated with spontaneous preterm delivery. A study by Murphy et al. (84) showed that both high and low maternal haemoglobin concentration in pregnancy at booking were associated with adverse pregnancy outcomes. Dunlop et al. (85) also observed a direct relationship between high maternal haemoglobin concentration and small-for-date babies. Work done by Sagen et al. (86) revealed that low maternal haemoglobin concentration at term is associated with large-for-date babies whilst high maternal haemoglobin concentration at term is associated with small-for-date babies.

High maternal haemoglobin concentration in early pregnancy without physiological decrease in maternal haemoglobin concentration as well as low maternal haemoglobin concentration in early pregnancy without subsequent increase in maternal haemoglobin concentration are both associated with adverse pregnancy outcomes (87). In pregnancy a maternal haemoglobin concentration level of 10.5g/dl will not be unusual (44). Haemodilution in normal pregnancy leads to a fall in maternal haemoglobin concentration to 9.5g/dl – 11.5g/dl which should be regarded as optimal for adequate growth of the developing fetus (65). Failure of the maternal haemoglobin concentration to fall below 10.5g/dl in pregnancy is associated with a higher risk of preterm birth and low birth weight (70).

According to Koller et al. (88), an inverse correlation exists between birth weight and lowest maternal haemoglobin concentration reached during pregnancy. Klebanoff et al. (89) concluded in their study that maternal anaemia is not a major cause of prematurity. Yip (90) reported in his study that high maternal haemoglobin concentration is associated with poor pregnancy outcome.

Haemoglobin levels change with altitude. An altitude of more than 1000 meters is associated with an increase in haemoglobin level (91). This phenomenon of elevated haemoglobin at high altitude is the body's way of adapting to the low

oxygen partial pressure at high altitude in order to deliver enough oxygen to the tissues of the body (91). Anaemia can be masked by the elevation in haemoglobin at high altitude if the altitude is not considered (92).

For people living at high altitude, it is recommended by the World Health Organization to correct the cut-off point for anaemia with respect to haemoglobin levels. This correction spans from the addition of up to 0.2g/dl to 4.5g/dl to the cut-off point for residents at 1000 meters and more than 4500 meters above sea level respectively (93). By correcting the haemoglobin cut-off for anaemia at high altitude, the prevalence of anaemia increases as altitude increases (94). Residents at high altitudes lack iron when in fact their iron levels are normal.

In general, high haemoglobin refers to a haemoglobin level of more than two standard deviations above the normal range. Different studies have reported different cut-offs for high haemoglobin concentration which spans from 13.3g/dl to 17.0g/dl (84, 95). The optimal maternal haemoglobin concentration in mid-pregnancy is 9 – 11g/dl (40). According to study done by Murphy et al. (84), the complication of pregnancy increases above maternal haemoglobin concentration of 13.2g/dl. Zhang et al. (71) also reported that the risk of adverse pregnancy outcome increases with maternal haemoglobin concentration =>13.0g/dl in the mid-trimester of pregnancy.

Rationale for the study

Pregnancy is a physiological process which is monitored for risk factors and complications for timely intervention. The outcome of pregnancy is of concern to the patient, the family and the clinician.

The association between maternal haemoglobin concentration and outcome of pregnancy has been a source of continual controversy. Some studies have reported the association of low maternal haemoglobin concentration with adverse birth outcomes with others reporting high maternal haemoglobin concentration's association with adverse birth outcomes.

No previous study of this nature has been undertaken at this study site. Maternal haemoglobin concentration in pregnancy is routinely measured at booking, around 28 weeks gestation and close to term at antenatal clinics, as well as admissions to

the ward. Maternal anaemia receives attention of clinicians but high maternal haemoglobin concentrations are usually overlooked.

Knowledge of the association between maternal haemoglobin concentration in pregnancy and the outcome of pregnancy may help identify mothers who are at risk. This will allow for investigation of the underlying causes and close follow up to improve pregnancy outcome.

Study Question

Is there an association between maternal haemoglobin concentration in pregnancy and the outcome of pregnancy with respect to preterm birth, low birth weight and pre-eclampsia?

Null hypothesis

There is no association between maternal haemoglobin concentration in pregnancy and the outcome of pregnancy.

Assumptions

Results from previous studies indicate that plasma volume expansion and hence haemodilution in pregnancy induces a fall in maternal haemoglobin concentration which occurs by 34 weeks gestation, and that this fall in maternal haemoglobin concentration is steepest up to about 20 weeks gestation and thereafter remain fairly constant after 20 weeks gestation until about 34 weeks gestation from where it rises slightly. Thus, any estimation of maternal haemoglobin concentration undertaken beyond 20 weeks gestation to before 34 weeks gestation will be representative of the fall in maternal haemoglobin concentration induced by pregnancy.

Aims and Objectives

The aim of the study was to assess whether changes in maternal haemoglobin concentration levels from early to middle or late pregnancy are associated with adverse pregnancy outcomes with reference to low birth weight, preterm delivery and pre-eclampsia.

The objectives of the study:

1. To assess the association between maternal haemoglobin concentration in pregnancy and low birth weight.

2. To assess the association between maternal haemoglobin concentration in pregnancy and preterm birth.

3. To assess the association between maternal haemoglobin concentration in pregnancy and pre-eclampsia.

Purpose of the study

The study is being conducted in partial fulfilment of the requirements for the award of MMed degree in Obstetrics and Gynaecology by the University of Cape Town. It will provide information on the association between maternal haemoglobin concentration in pregnancy and the outcome of pregnancy to improve antenatal care in future and form the basis for future research in the area.

3.0 METHODOLOGY

Study Population

Women who delivered an infant of 28 or more weeks gestation at New Somerset Hospital.

Study Site

Department of Obstetrics and Gynaecology, New Somerset Hospital.

Study Period

From 1st May to 22nd May 2017.

Study Design

Retrospective Cohort Study.

Source of recruitment: New Somerset Hospital delivery records book.

Source of data: Maternal record books of recruited patients.

Study Sample

All women who delivered an infant of 28 or more weeks gestation at New Somerset Hospital within the study period with documented maternal haemoglobin concentration at 22 – 33 weeks gestation.

Sampling Procedure

Consecutive recruitment of women who delivered an infant of 28 or more weeks gestation with documented maternal haemoglobin concentration at 22 – 33 weeks gestation from 1st May 2017 until the sample size of 191 was achieved (on 22nd May 2017) at which stage recruitment was stopped.

Maternal records of all recruited patients were evaluated.

The lowest maternal haemoglobin concentration documented at 22 – 33 weeks gestation was recorded on the data collection sheet.

The highest blood pressure documented with proteinuria after 20 weeks gestation was recorded on the data collection sheet

Birth weight at delivery was recorded on the data collection sheet.

Gestational age at delivery was recorded on the data collection sheet.

Data collection sheets were completed for all recruited patients. The data collection sheet attached as Appendix A.

Inclusion Criteria:

. Women who delivered at New Somerset Hospital with documented maternal haemoglobin concentration at 22 – 33 weeks gestation.

. Singleton pregnancy

. Pregnancy of 28 or more weeks gestation at delivery.

Exclusion Criteria:

. Multiple pregnancy

. Congenital anomaly

. Women with known haemoglobinopathies

Sample Size:

The sample size was calculated using a 95% confidence interval (2-sided) and a power of 90%. Because consecutive cases will be included in the sample the proportion of patients with low or normal haemoglobin will be greater than those with high haemoglobin. The high haemoglobin is defined as 13.0g/dl and above. Therefore, a ratio of 2:1 will be used based on the predicted proportion of 2 patients with normal haemodilution to 1 patient with failure of haemodilution.

The main outcome variable will be pre-eclampsia. Evidence indicates that 5% of patients with a normal or low haemoglobin will develop pre-eclampsia compared with 20% of patients with a raised haemoglobin, i.e. failure of haemodilution. Cases are defined as haemoglobin of 13.0g/dl and above. Controls are defined as a haemoglobin of less than 13.0g/dl. This assumption correlates with a sample size

of 191 using the statistical calculator (approximately 64 cases - High Hb and approximately 127 controls – Normal Hb). Using these assumptions and the statistical calculator, a sample size of 191 will result in a power of 90%. We find this additional power important when using this study design. See the table below (96).

Sample Size: X-Sectional, Cohort, & Randomized Clinical Trials

Two-sided significance level (1-alpha):	95
Power (1-beta, % chance of detecting):	90
Ratio of sample size, Normal/High Hb	2
Percent of Normal Hb with pre-eclampsia:	5
Percent of High Hb with pre-eclampsia:	20
Odds Ratio:	4.8
Risk/Prevalence Ratio	4
Risk/Prevalence difference:	15

	Kelsey
Sample Size – High Hb	64
Sample Size – Normal Hb	127
Total Sample Size	191

Ethical considerations

Description of risks and benefits

This was a retrospective study. Measurements for evaluation were those which were already done and were in the archives of the hospital, therefore no additional risk to the study patients. No new measurements were made in the course of the study. There were no additional visits and no additional tests. Data remained anonymous. Data was stored in a password protected file that could only be accessed by Dr. Amponsah.

Informed consent process

Ethics approval was obtained from the Human Research Ethics Committee, Health Sciences Faculty of the University of Cape Town (Appendix B). Institutional consent was granted to investigate the hospital records of the identified patients (Appendix C). Individual patient consent was not required.

Privacy and confidentiality

No patient names were used in the study or during preparation of the final study report. Paper based records were kept in a secure location and was accessible to only persons involved in the study. Electronic records were anonymised and stored in a password protected computer that was accessed only by the researcher. All patient related data were always anonymised to ensure confidentiality and compliance with the Helsinki Declaration (97).

Emergency care and insurance for research-related injuries

This was a retrospective study and no study subject was exposed to any injury during the period

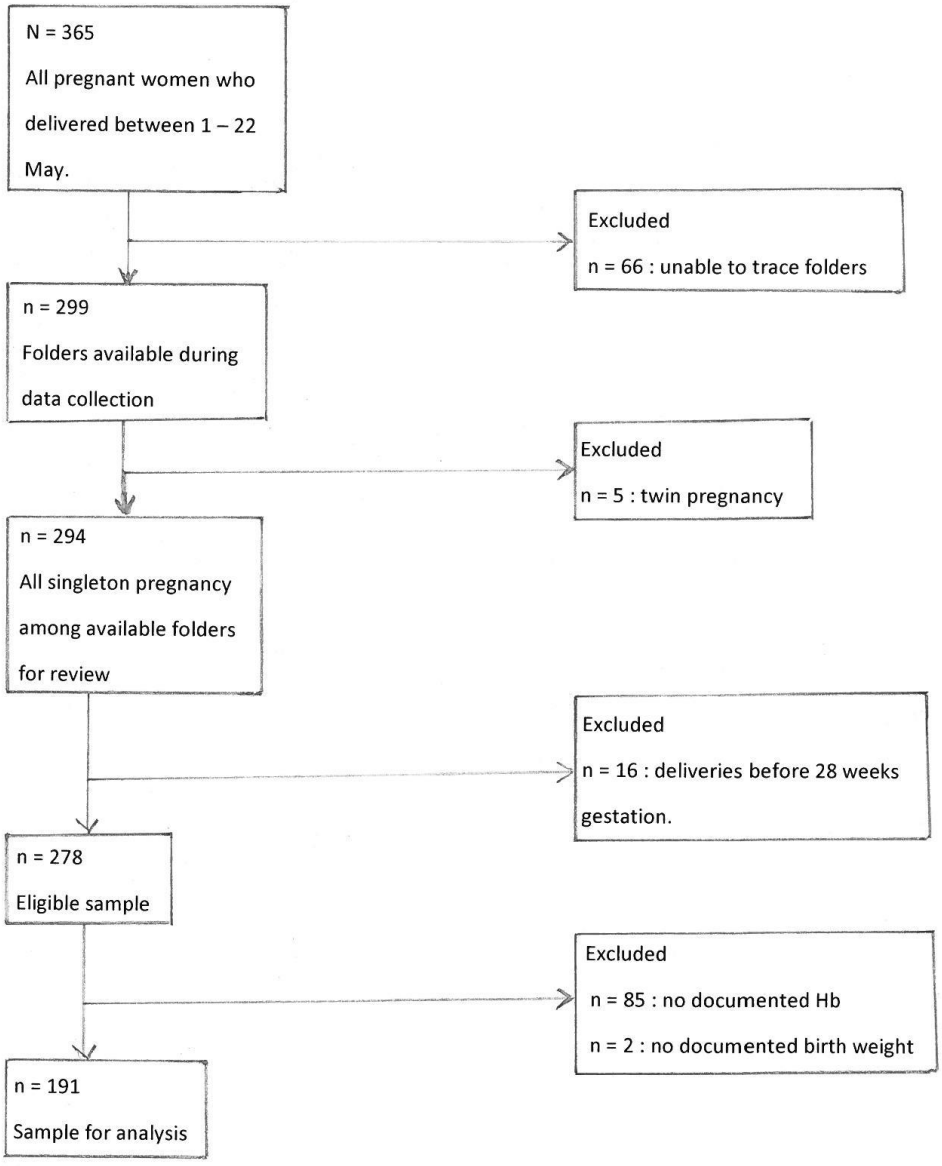
Funding

All the data reviewed in the study were those that have been previously acquired as part of the routine care of pregnant women at antenatal period or at delivery in the hospital. No funding was required for the acquisition of any new data. There was no cost to the hospital during the study. All cost emanating from purchase of stationery were catered for by the researcher's personal funds.

MATERIALS AND METHODS

The aim of the study was to recruit 191 patients to meet the minimum requirements for the sample size. The flow diagram (figure 1) outlines the recruitment and attrition of patient folders.

FIGURE 1: Participant flow chart



A total of 365 consecutive deliveries of patients who delivered between the 1st and 22nd of May 2017 were reviewed. Out of the 365 patient folders, 174 patient folders were excluded. There were 66 missing folders, 5 twin pregnancies, 16 deliveries before 28 weeks gestation, 85 deliveries with no documented haemoglobin at 22 – 33 weeks gestation, and 2 deliveries with no documented birth weight. This left 191 deliveries for inclusion in the analysis.

Data extracted from the patient folders included maternal age in years, parity, body mass index, lowest documented haemoglobin concentration at 22 -33 weeks gestation in g/dl, birth weight in grams, gestation at delivery in weeks, placental weight in grams, development of pre-eclampsia, HIV status, smoking status, rhesus status, syphilis status, alcohol use, mode of delivery, stillbirth and Apgar scores at 1 and 5 minutes as recorded in the folders. The data extraction sheet is attached as Appendix A.

Low birth weight was defined as less than 2500 grams at birth; preterm birth as before 37 completed weeks gestation at birth; and pre-eclampsia as hypertension (blood pressure => 140/90 mmHg) with proteinuria from 20 weeks gestation. Gestational age was determined by the best estimate using menstrual history and early ultrasound measurements where this was performed.

For ease of interpretation, maternal haemoglobin concentration values were classified into categories each spanning 1g/dl. A total of nine categories were created with corresponding data on maternal and neonatal characteristics. Histograms were constructed to illustrate the relations between maternal haemoglobin concentration and pre-eclampsia, birth weight and preterm birth.

In addition, the study patients were divided into two categories: those with maternal haemoglobin =>13.0g/dl at 22 – 33 weeks gestation considered as the group with failed physiological haemodilution in pregnancy, and those with maternal haemoglobin <13.0g/dl at 22 – 33 weeks gestation considered as the group who achieved physiological haemodilution in pregnancy. The demographic characteristics between these two groups were compared (Table 1). The following covariates were examined: maternal age (years), parity, body mass index, development of pre-eclampsia, smoking status, alcohol use, HIV status, rhesus status, syphilis status, mode of delivery, birth weight (g), gestation at birth (weeks), placental weight (g), stillbirth, and Apgar score.

STATISTICAL ANALYSIS

Student's t-tests were used to analyse continuous variables. Chi-square tests were used to compare categorical parameters in the analysis. Descriptive statistics were reported as percentages for categorical variables and means and standard deviations for continuous variables. Statistical significance was set at $p<0.05$. All data analysis was performed using Stata version 13.1. (Copyright 1985-2013 StataCorp LP).

4.0 RESULTS

Table 1 provides the demographic characteristics of the study sample.

Table 1: Demographic characteristics.

Characteristic	Total (N = 191)	Group without haemodilution (Hb =>13g/dl) (n=50)	Group with haemodilution (Hb <13 g/dl) (n=141)	p-value
Maternal				
Age mean (SD)	26.86 (5.83)	27.10 (5.60)	26.78 (5.93)	p=0.74
Parity mean (SD)	1.07 (1.03)	1.00 (1.12)	1.09 (0.99)	p=0.59
BMI mean (SD)	28.99 (6.77)	29.67 (7.86)	28.75 (6.36)	p=0.41
Development of pre-eclampsia n (%)				
Yes	17 (8.90)	13 (26.00)	4 (2.84)	
No	174 (91.10)	37 (74.00)	137 (97.16)	p<0.001
Smoking n (%)				
Yes	13 (6.81)	5 (10.00)	8 (5.67)	
No	178 (93.19)	45 (90.00)	133 (94.33)	p=0.30
HIV n (%)				
Negative	152 (79.58)	40 (80.00)	112 (79.43)	
Positive	39 (20.42)	10 (20.00)	29 (20.57)	p=0.93
Alcohol n (%)				
Yes	4 (2.19)	2 (4.17)	2 (1.48)	
No	179 (97.81)	46 (95.83)	133 (98.52)	p=0.27

Rhesus n (%) Positive Negative	 185 (96.86) 6 (3.14)	 50 (100.00) 0 (0.00)	 135 (95.74) 6 (4.26)	 p=0.14
Syphilis n (%) Negative Positive	 190 (99.48) 1 (0.52)	 50 (100.00) 0 (0.00)	 140 (99.29) 1 (0.71)	 p=0.55
Mode of delivery n (%) NVD C/S	 150 (78.53) 41 (21.47)	 33 (66.00) 17 (34.00)	 117 (83.98) 24 (17.02)	 p=0.01
Newborn				
Birth weight mean (SD)	3132.12 (618.93)	2823.70 (651.66)	3241.49 (570.30)	p<0.001
Low Birth Weight (<2500g) n (%) Yes No	 27 (14.14) 164 (85.86)	 18 (36.00) 32 (64.00)	 9 (6.38) 132 (93.62)	 p<0.001
Gestation at birth mean (SD)	38.57 (2.30)	37.42 (2.74)	38.97 (1.97)	p<0.001
Preterm Birth (<37 weeks) n (%) Yes No	 26 (13.61) 165 (85.86)	 17 (34.00) 33 (66.00)	 9 (6.38) 132 (93.62)	 p<0.001
Placental weight mean (SD)	621.25 (140.64)	572.56 (145.32)	638.64 (135.26)	p=0.004
Stillbirth n (%)	2 (1.05)	1 (2.00)	1 (0.71)	NS
Apgar score mean (SD) 1-minute 5-minute	 8.56 (1.35) 9.80 (1.10)	 8.50 (1.44) 9.65 (1.48)	 8.60 (1.32) 9.86 (0.93)	 p=0.57 p=0.27

Table 1 shows that there were statistically significant differences in mean birth weight (p<0.001), low birth weight (p<0.001), mean gestation at birth (p<0.001), preterm birth (p<0.001), development of pre-eclampsia (p<0.001), placental weight (p=0.004) and mode of delivery (p=0.01) between the failed haemodilution group (Hb=>13) and physiological haemodilution group (Hb <13). The group of failed haemodilution (Hb =>13) was associated with a relatively lower birth weight, higher preterm birth, and pre-eclampsia rate, and low placental weight compared to the group of physiological haemodilution (Hb <13). A higher proportion of the failed haemodilution group were delivered by Caesarean section (34% vs 17%). However, there were no statistically significant differences in maternal age, parity, body mass index, smoking status, HIV status, alcohol use, rhesus status, syphilis status, stillbirth and Apgar scores at 1 and 5 minutes between the two groups.

Table 2 shows the distribution of mean birth weight and the proportions of low birth weight, preterm births and mothers with pre-eclampsia for each of the haemoglobin concentration category spanning 1g/dl.

Table 2: Distribution of mean birth weight and proportions of low birth weight (<2500g at birth), preterm births (<37 weeks at birth), and mothers with pre-eclampsia (high blood pressure + proteinuria) by haemoglobin concentration in categories.

Haemoglobin concentration (g/dl)	Number of pregnancies (N)	Mean birth weight (g)	Proportion of babies <2500g at birth n (%)	Proportion of babies <37wks at birth n (%)	Proportion of mothers with pre-eclampsia n (%)
<8.6	4	3056	0 (0.00)	0 (0.00)	0 (0.00)
8.6 – 9.5	16	3437	0 (0.00)	0 (0.00)	0 (0.00)
9.6 – 10.5	30	3159	2 (6.67)	1 (3.33)	1 (3.33)
10.6 – 11.5	36	3220	2 (5.56)	3 (8.33)	1 (2.78)
11.6 – 12.5	40	3323	3 (7.50)	3 (7.50)	1 (2.50)
12.6 – 13.5	28	3055	5 (17.86)	5 (17.86)	4 (14.29)
13.6 – 14.5	17	3116	3 (17.65)	4 (23.53)	3 (17.65)
14.6 – 15.5	9	2620	4 (44.44)	3 (33.33)	3 (33.33)
>15.5	11	2360	8 (72.73)	7 (63.64)	4 (36.36)
Total	191	n/a	27 (14.1)	26 (13.61)	17 (8.90)

Table 2 shows that the prevalence of low birth weight in the study sample was 14.1%. The distribution of low birth weight was skewed toward patients with failed haemodilution. There were no cases of low birth weight in maternal haemoglobin concentrations of <9.6g/dl. The highest proportion of low birth weight (72.73%) occurred at maternal haemoglobin concentration of >15.5g/dl. The distribution of low birth weight and birth weight =>2500g for each of the maternal haemoglobin category is illustrated below in figure 2.

Figure 2: Distribution of low birth weight (<2500g) and birth weight =>2500g by haemoglobin category (g/dl).

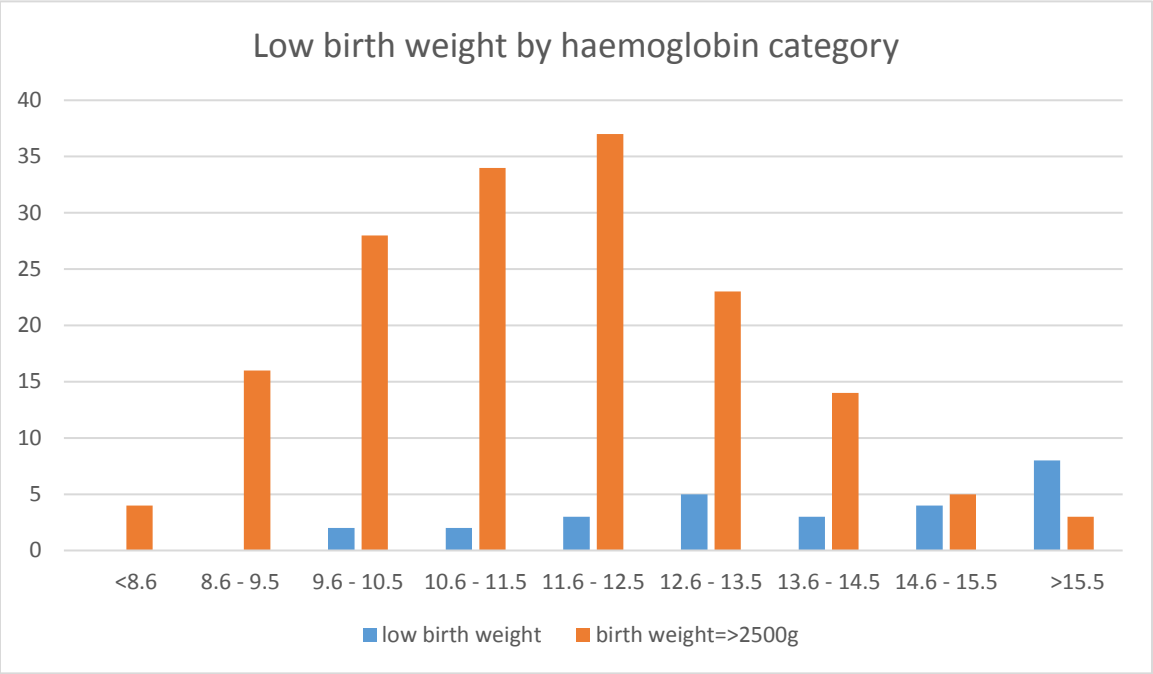
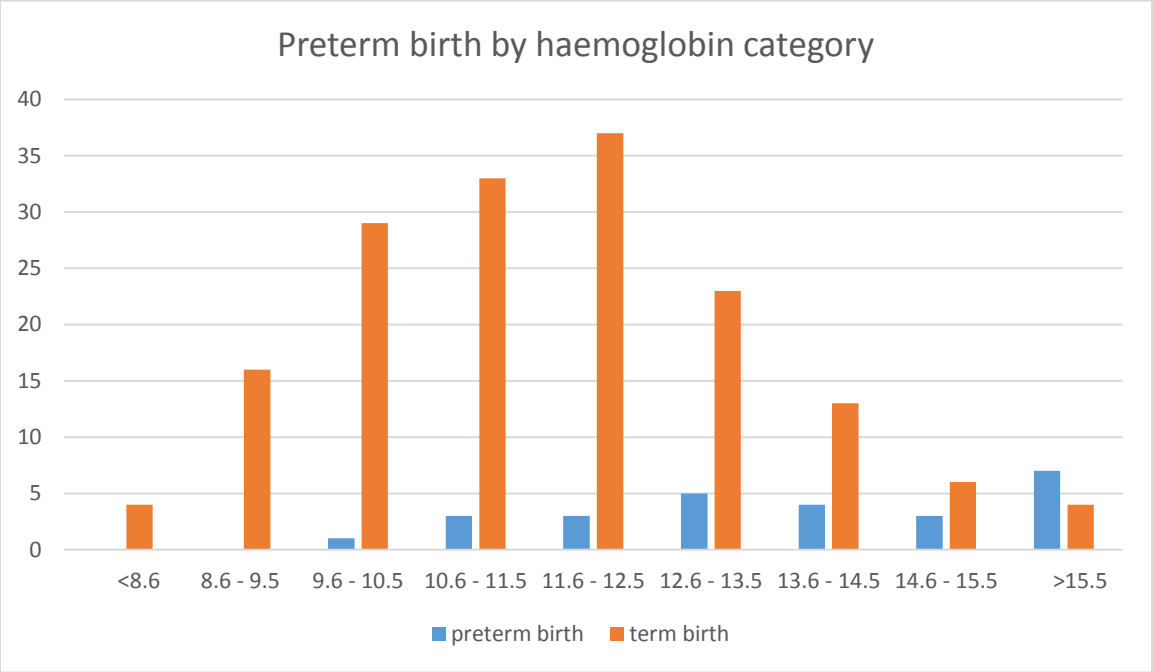


Table 2 shows that prevalence of preterm birth in the study sample was 13.61%. The distribution of preterm birth was skewed toward patients with failed haemodilution. There were no cases of preterm birth in maternal haemoglobin concentration of <9.6g/dl. The highest proportion of preterm birth (63.64%) occurred at maternal haemoglobin concentration of >15.5g/dl. The distribution of preterm birth and term birth for each of the maternal haemoglobin concentration category is illustrated below in figure 3.

Figure 3: Distribution of preterm birth and term birth by haemoglobin concentration category (g/dl).



Note: preterm birth = gestation at birth < 37 weeks; term birth = gestation at birth => 37 weeks

Table 2 shows that prevalence of pre-eclampsia in the study sample was 8.9%. The distribution of pre-eclampsia was skewed towards patients with failed haemodilution. There were no cases of pre-eclampsia in maternal haemoglobin concentration of <9.6g/dl. The highest proportion of pre-eclampsia (36.36%) occurred at maternal haemoglobin concentration of >15.5g/dl. The distribution of the development of pre-eclampsia for each of the maternal haemoglobin concentration category is illustrated below in figure 4.

Figure 4: Distribution of the development of pre-eclampsia by haemoglobin concentration category (g/dl).

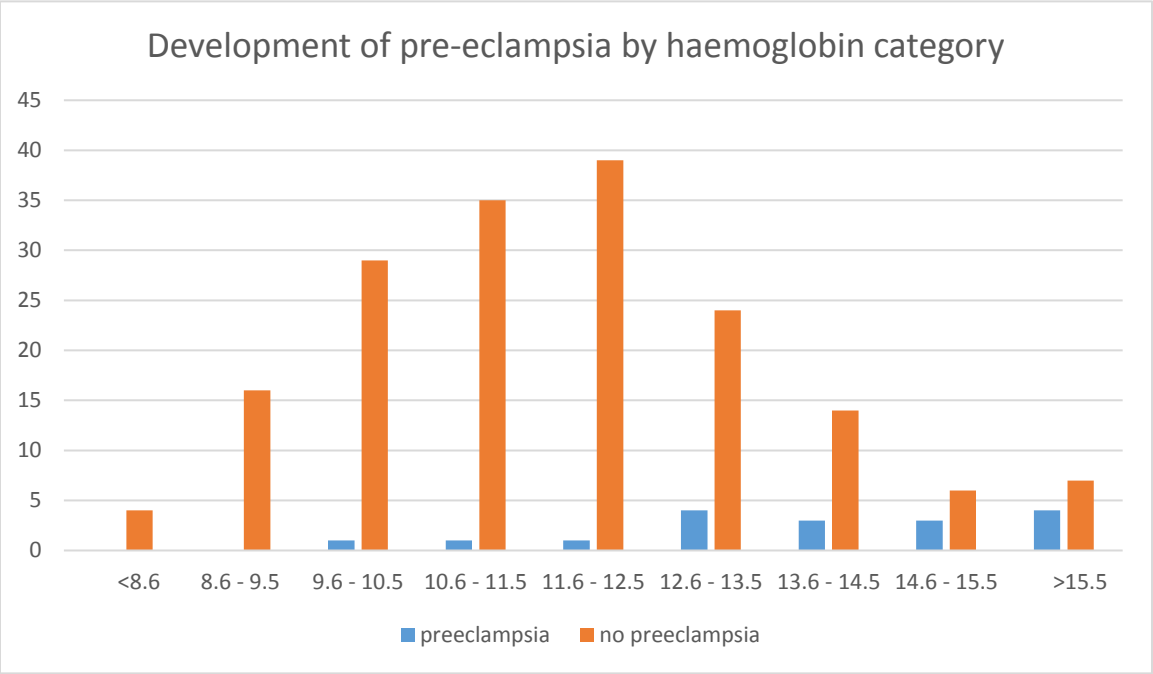


Table 2 shows that highest mean birth weight of 3437g occurred in the 8.6 – 9.5g/dl maternal haemoglobin concentration band. The lowest mean birth weight of 2360g occurred in the maternal haemoglobin concentration of >15.5g/dl.

Table 3 provides some demographic characteristics between the group that developed pre-eclampsia and the group that did not develop pre-eclampsia.

Table 3: Characteristics between the groups with and without pre-eclampsia.

Characteristic	Total (N = 191)	Group without pre-eclampsia (n = 174)	Group with pre-eclampsia (n = 17)	p-value
Maternal				
Age mean (SD)	26.86 (5.83)	26.95 (5.79)	25.94 (6.35)	p=0.50
BMI mean (SD)	28.99 (6.77)	28.98 (6.87)	29.05 (5.88)	p=0.97
Smoking n (%) Yes No	 13 (6.81) 178 (93.19)	 11 (84.62) 163 (91.57)	 2 (15.38) 15 (8.43)	 p=0.40

Neonatal				
Birth weight mean (SD)	3132.12 (618.93)	3175.92 (607.29)	2683.82 (571.85)	p=0.002
Birth gestation - mean (SD)	38.57 (2.30)	38.69 (2.22)	37.29 (2.71)	p=0.016
Placental weight - mean (SD)	621.25 (140.64)	629.06 (137.81)	541.76 (148.81)	p=0.01
Mode of delivery NVD C/S	 150 (78.53) 41 (21.47)	 139 (92.67) 35 (85.37)	 11 (7.33) 6 (14.63)	 p=0.146

Table 3 shows that there were no statistically significant differences in maternal age, body mass index, smoking status and mode of delivery between those that developed pre-eclampsia and those that did not develop pre-eclampsia. However, there were statistically significant differences in birth weight (p=0.002), gestation at birth (p=0.016) and placental weight (p=0.01) between the two groups: with and without pre-eclampsia. Pre-eclampsia was associated with relatively lower birth weight, preterm birth and low placental weight. This association was independent of the haemoglobin status.

5.0 DISCUSSION

The study has shown an association between pre-eclampsia, low birth weight and preterm delivery and high maternal haemoglobin concentration. The proportion of pre-eclampsia, low birth weight and preterm delivery fell as maternal haemoglobin concentration fell. The highest mean birth weight of 3437g was associated with haemoglobin concentrations of 8.6 – 9.5g/dl which is much lower than commonly appreciated.

These findings are consistent with other studies that have reported the association of high maternal haemoglobin concentration with adverse outcome of pregnancy. Zhang et al. (69) reported in their study the association of low third trimester maternal haemoglobin concentration with lower risk of spontaneous preterm labour. Steer et al. (70) also reported an increase in the incidence of preterm delivery and low birth weight with high maternal haemoglobin concentration and stated that maternal haemoglobin concentration of 9.5g/dl was associated with optimal fetal growth. Huisman and Arnoudse (4) reported elevated mid-trimester

haemoglobin concentration within nulliparous women with fetal growth restriction. Also, a higher rate of preterm birth, low birth weight as well as the development of hypertension among women with high maternal haemoglobin concentration in the first and second trimesters was reported by Murphy et al. (84). According to work done by Cordina et al. (64), high maternal haemoglobin at 27-29 weeks gestation is associated with a reduction in birth weight and adverse fetal outcome.

Pre-eclampsia is a hypertensive disorder in pregnancy that causes significant mortality and morbidity in both the pregnant woman as well as the fetus. The precise aetiology of pre-eclampsia remains elusive. Current research suggests that the functional changes associated with pre-eclampsia is related to abnormal defective invasion of the decidual and uterine spiral vessels by trophoblastic cells with failure of conversion of uterine spiral arterioles to wide-bore channel utero-placental vessels responsible for carrying blood to the intervillous space. The resulting appreciable decline in the flow of blood through the utero-placental unit and hence poor oxygen supply leads to tissue hypoxia with subsequent release of vasoactive substances by placental tissues into circulation causing generalized vasoconstrictive disorder and endothelial cell dysfunction affecting all organs of the body resulting in high blood pressure, proteinuria and thrombocytopenia. An increase in the permeability of capillary endothelium occurs with significant serum protein loss leading to a reduction in intravascular volume and elevated maternal haemoglobin concentration. In women with pre-eclampsia, the volume of blood does not increase resulting in a relatively high haemoglobin concentration (90).

Early features of pre-eclampsia (before clinical symptoms) such as vasoconstriction and the associated increase in resistance to blood flow through the utero-placental unit can be demonstrated on uterine artery Doppler ultrasonography in mid-gestation as notching or high resistance index.

Even though blood flow through the utero-placental unit is significantly reduced in pre-eclampsia, the body compensate to enhance blood flow to the developing fetus, but in severe cases resistance to blood flow becomes abnormal and can be demonstrated on umbilical artery Doppler ultrasonography as high pulsatility index and in extremely severe cases absent end diastolic flow or even reversed end diastolic flow velocity.

The process through which growth of the fetus is affected by maternal haemoglobin concentration in pregnancy is not well understood but is believed to be due to the harmful effect of haemoconcentration. The exchange of materials between the fetal circulation and maternal circulation is negatively affected by the damaging action of hyper viscosity at the intervillous space (53). Higher metabolic activity occurs in pregnancy hence the need for a mechanism to adequately excrete the waste metabolites from maternal circulation and enhance the delivery of nutrients and oxygen to the developing fetus as well as the mother. The body achieves this through cardiac output increase of 35% - 50%; and haemodilution as a result of an expansion in the volume of plasma by 50% - 60% and an increase in the mass of red blood cells by 20% - 30%, termed physiological haemodilution (63). Failure of physiological haemodilution to occur in pregnancy is associated with adverse pregnancy outcome (53).

One possible mechanism of the association between hyper viscosity and fetal growth restriction is high viscosity of blood resulting in poor flow of blood and hence decreased nutrient and oxygen supply to the developing fetus. There is an interplay between viscous forces and kinetic forces with respect to circulation in blood vessels with viscous forces playing a major role in smaller blood vessels such as in the placenta where the velocity of flow is low (98). In bigger vessels kinetic forces play a major role in blood flow. High viscosity with associated higher viscous forces and the resultant poor blood flow in the placenta leads to poor supply of oxygen and nutrients to the developing fetus and hence restriction in its growth.

Another possible mechanism is that poor blood flow through the placenta due to high viscosity may result in deficiency of substrate (nutrients) on which hormones produced by the placenta acts to stimulate growth (99). The main factor affecting the metabolism of insulin-like growth factors which are essential for fetal growth is substrate deficiency (99), which may be caused by reduced flow of blood to the intervillous space due to hyper viscosity (100).

High maternal haemoglobin's association with fetal growth restriction may be explained by the effect of haemoglobin on nitric oxide causing nitric oxide inactivity. Nitric oxide is a smooth muscle relaxant in the vascular endothelium that causes vasodilatation (101). Nitric oxide deficiency causes vasoconstriction. Haemoglobin can bind to nitric oxide causing vasoconstriction (102). Free

haemoglobin in circulation has a negative effect on the vascular endothelium through an oxidant activity resulting in vasoconstriction although its level is low in the plasma (103). High haematocrit and low oxygen saturation increases the permeability of red blood cell to nitric oxide (104). Thus, a high haemoglobin concentration may result in excessive nitric oxide binding with resultant vasoconstriction and hence poor placental perfusion leading to growth restriction.

Adequate placental support is necessary to achieve optimal growth of the developing fetus. The weight of the placenta may determine the outcome of a pregnancy. A low haemoglobin concentration may lead to a reduction in the supply of oxygen to tissues and thus low haemoglobin in the utero-placental circulation with associated decreased oxygen supply to the placenta may lead to poor growth and development of the fetus (105). Under such circumstances the body may undergo adaptation to ensure adequate placental support (adequate supply of oxygen) for optimal fetal growth and development. One of such body's adaptation is the increase in new blood vessels development in the placenta (106). This form of adaptation has been observed in the placenta of pregnancies where the maternal haemoglobin concentration is low (107). In pregnancies where maternal haemoglobin concentration is low, an increase in the size of the placenta may be explained by numerous new blood vessels development in the placenta which occurs through adaptation. Reports indicate that pregnancies of people living in high altitude where partial pressure of oxygen is low are associated with large placentas likely due to adaptation (108). High haemoglobin concentration leading to high blood viscosity may possibly cause the small blood vessels of the placenta to be congested or occluded resulting in poor oxygen supply and hence suboptimal growth of the placenta (109).

There are several ways through which spontaneous preterm delivery may occur with the major causes being oxidative stress, hypoxia and maternal infection (110). A possible explanation of the relationship between high maternal haemoglobin concentration and preterm delivery may be the effect of hyper viscosity. High viscosity of the blood results in poor blood supply with associated poor oxygen supply to the placenta and developing fetus leading to hypoxia and oxidative stress.

Many researchers have reported the association of adverse pregnancy outcome including fetal growth restriction and low birth weight with high maternal

haemoglobin concentration (84, 87, 88, 90, 95, 109, 111 – 114). High mid-trimester maternal haemoglobin concentration is a major risk factor of delivering a baby of weight below the 10th centile (112). Hypertensive disorders in pregnancy have been found by many researchers to be strongly related to haemoconcentration (4, 84, 115, 116).

A fall in maternal haemoglobin concentration in the second trimester which probably reflects adequate plasma volume expansion seems to be essential with respect to pregnancy outcomes, both fetal and maternal. The process through which adequate plasma volume expansion improves growth and development of the developing fetus remains unresolved. However low viscosity blood may enhance perfusion of the utero-placental unit promoting fetal nourishment and growth. The risk of adverse pregnancy outcome including low birth weight, preterm delivery and pre-eclampsia increases if maternal haemoglobin concentration fails to fall in pregnancy (83, 89). Concerns have been raised by many authors regarding high as opposed to low maternal haemoglobin concentrations during pregnancy (83, 84, 89), though genuine anaemia according to Godfrey et al. (117) may have adverse effect on pregnancy outcome.

STUDY LIMITATIONS

This is a retrospective study and data was collected from existing information in old folders which may be subject to errors during the time of recording.

Due to the late gestation of patients at entry into the maternity services we were not able to calculate individual changes in haemoglobin concentrations for each patient. We used the lowest recorded maternal haemoglobin in late pregnancy as an indicator of haemodilution.

The sample size covered a period of less than a month.

There were no neonatal follow up data in the maternal records.

The study was done in a specialist referral hospital but not a tertiary centre, so more severe cases of pre-eclampsia from the same population may not be included

6.0 CONCLUSION AND RECOMMENDATIONS

This study has shown that high maternal haemoglobin concentration at 22 – 33 weeks gestation is associated with an increased prevalence of pre-eclampsia, low birth weight and preterm birth.

Physiological haemodilution may have a beneficial effect on placental function and hence the outcome of pregnancy. Identifying and investigating the underlying clinical cause of high maternal haemoglobin concentration in pregnancy, as well as monitoring the pregnant women at risk closely throughout the pregnancy may help improve pregnancy outcomes.

It is recommended that clinicians attending to pregnant women consider the effect of physiological haemodilution in pregnancy and look out for elevated maternal haemoglobin concentrations for evaluation and close follow up such as frequent antenatal visits with blood pressure monitoring and urinalysis, haematological and biochemical analysis as well as ultrasound scanning with dopplers when necessary.

Attention should also be paid to the use of drugs that increase haemoglobin concentration, nutrition and chronic medical conditions in the management of patients with high maternal haemoglobin concentration.

With further research, maternal haemoglobin levels may be used as part of risk stratification during antenatal care

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APPENDICES

APPENDIX A - DATA COLLECTION SHEET

FOLDER NUMBER:……. STUDY ID:……..

AGE/DOB:……

BMI:…… SMOKING: YES [ ] NO [ ] UNKNOWN [ ]

ALCOHOL: YES [ ] NO [ ]

SUBSTANCES: YES [ ] NO [ ]

PARITY: G…….. P…….. M……..

HIV…….. (if + CD4/VL……..)

RPR……. RHESUS…….. (Antibodies……)

MEDICAL HISTORY:

HAEMOGLOBIN CONCENTRATION:

1st Documented HB: NHLS…… WARD……

Gestation……..

Date……..

HB @ 22 – 33 weeks: NHLS…….. WARD……..

Gestation……

Date……..

HB @ Labour/Delivery: NHLS…….. BEDSIDE……

Gestation…….

Date…….

DATE OF DELIVERY:…….

MODE OF DELIVERY: NVD…… C/S…….

ASSISTED……..

BIRTH WEIGHT:…….

GESTATION AT DELIVERY:……

LIVEBIRTH [ ] STILLBIRTH [ ]

APGAR SCORE:

PLACENTAL WEIGHT…….

INDICATION FOR DELIVERY:……

BP…….

PROTEINURIA……

INTERVENTION FOR PRE-ECLAMPSIA……..

APPENDIX B

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

10 June 2019

HREC REF: 317/2019

Dr G Petro
Division of Obstetrics and Gynaecology
New Somerset Hospital

Dear Dr Petro

PROJECT TITLE: MATERNAL HAEMOGLOBIN CONCENTRATION AND PREGNANCY OUTCOME (MMED CANDIDATE: DR KP AMPONSAH)

Thank you for your response letter dated 02 June 2019, addressing the issues raised by the Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 June 2020.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student: Dr Kwaku Amponsah will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007

APPENDIX C

Western Cape Government
Health

Health Impact Assessment
Health Research sub-directorate
Health.Research@westerncape.gov.za
tel: +27 21 483 0866: fax: +27 21 483 9895
5th Floor, Norton Rose House, 8 Riebeek Street, Cape Town, 8001
www.capegateway.gov.za)

REFERENCE: WC_201906_020
ENQUIRIES: Dr Sabela Petros

University of Cape Town

Anzio Road

Observatory

Cape Town

7925

For attention: Dr Poku Amponsah

Re: **Maternal haemoglobin and outcome of pregnancy**

Thank you for submitting your proposal to undertake the above-mentioned study. We are pleased to inform you that the department has granted you approval for your research.

Please contact the following person to assist you with any further enquiries in accessing the following sites:

New Somerset Hospital	**Dr Donna Stokes**	**021 402 2463**

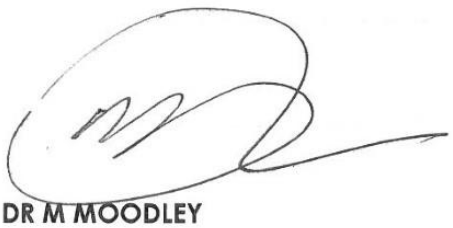
Kindly ensure that the following are adhered to:

1. Arrangements can be made with managers, providing that normal activities at requested facilities are not interrupted.
2. By being granted access to provincial health facilities, you are expressing consent to provide the department with an electronic copy of the final feedback (**annexure 9**) within six months of completion of your project. This can be submitted to the provincial Research Co-ordinator (Health.Research@westerncape.gov.za).

3. In the event where the research project goes beyond the *estimated completion* date which was submitted, researchers are expected to complete and submit a progress report (**Annexure 8**) to the provincial Research Co-ordinator (Health.Research@westerncape.gov.za).

4. The reference number above should be quoted in all future correspondence.

Yours sincerely



DR M MOODLEY

DIRECTOR: HEALTH IMPACT ASSESSMENT

22-08-2014