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TITLE: Investigating Foetal Maternal Haemorrhage in Stillbirths, Windhoek Central Hospital, Namibia

By

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Declaration

I, Edwig Hauwanga hereby declare that the work contained in the thesis entitled “assessing foetal maternal haemorrhage in stillbirths at Windhoek Central Hospital, Namibia” is my own original work and that I have not previously, in its entirety or in part, submitted it at any university or other higher education institution for the award of a degree.

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Dedication

To my first-born daughter, Gabriella Penelao Shingenge. I love you baby!

Abstract

Background: Two point six million stillbirths occur annually with the majority of cases being from low to middle-income countries (LMICS) of Sub-Saharan Africa. Infections, placental disorders, asphyxia obstructive birth, poor antenatal care and immune-mediated complications are amongst the top contributors of stillbirths in LMICs. Foetal maternal haemorrhage (FMH) is associated with poor pregnancy outcomes such as maternal alloimmunisation, foetal anaemia, haemolytic disease of the foetus and new-born (HDFN) and stillbirths. Although maternal response to FMH is poorly understood, aggravated inflammatory response is suggested and its impact on organ function remains elusive. Therefore, this study primarily aimed to determine whether FMH is associated with inflammation, maternal alloimmunisation and altered hepatorenal function.

Methods: This was a descriptive cross sectional study involving mothers who gave birth to stillborn babies at the Windhoek Central Hospital between August 2019 to February 2020. A total number of 60 samples were randomly collected using the convenient sampling technique. Blood samples were drawn from these patients by a registered nurse. Standard laboratory instruments and validated assays were used to measure and determine the inflammatory profiles, haematological indices, hepatorenal function and antibody screening and identification. The Kleihauer Betke test was used for the qualitative testing of FMH and its quantification, therefore.

Results: The prevalence of FMH in the included patients was at a staggering 25% (n = 15) and of these cases, 29% had massive haemorrhage. Comparisons of FMH positive and negative cases on sociodemographic characteristics (age, gravida, parity, alloimmunisation and Rh factor) were comparable ($p>0.05$). Although there were no significant differences on red cell counts and haemoglobin levels between the two groups ($p>0.05$), the mean cell volume (MCV) (86.10 ± 3.53) and mean cell haemoglobin (MCH) (29.00 ± 1.34) were significantly lower in the FMH positive group in comparison to that of the FMH negative cases (94.03 ± 5.56), $p = 0.0043$ and (31.76 ± 2.07), $p=0068$, respectively. (Assessment of inflammatory profiles revealed high C-reactive protein (CRP) amongst participants, with a CRP mean value of 23.8 [6.9 - 52.6]. Notably, women with FMH had significantly higher levels of WCC ($p=0.0143$) and lymphocyte ($p=0.0237$). Assessment of renal profiles showed

significant differences in sodium, urea, creatinine, and glomerular filtration rate (eGFR) ($p<0.05$) amongst the two groups. The enzyme aspartate aminotransferase (AST) levels were much higher in FMH positive (53.17 ± 29.04) participants as when compared to FMH negative participants (25.33 ± 10.98). The correlation analysis showed association of MCV ($r=0.78$, $p = 0.037$), MCH ($r=0.80$, $p=0.027$), urea ($r=-0.87$, $p=0.01$), and AST ($r=-0.82$, $p=0.046$) to eGFR.

Conclusions: Foetal maternal haemorrhage is associated to poor foetal outcomes, at times even stillbirths. Foetal maternal haemorrhage predisposes expectant mothers to alloimmunisation of significant red cell antibodies such as anti-D and anti-E that can cause serious foetal haemolysis in subsequent pregnancies. Foetal maternal haemorrhage is associated with inflammation that can worsen the anaemic condition in pregnancy. Apart from inflammation, FMH also alters the renal function. Timely and efficient diagnosis in pregnancy after a potentially sensitising events to FMH, can prompt lifesaving intervention for unborn babies. In addition, mothers can be given inflammatory drugs after these events.

Contents

List of figures

Chapter Two: Literature Review

Chapter Four: Results

List of tables

Chapter Two Literature Review

Chapter Four: Results

Abbreviations

Terms/Acronyms/Abbreviations

CRP-C-Reactive Protein

DHS-Demographic Health Survey

eGFR- Glomerular Filtration Rate

EHB- Extreme Hyperbilirubinemia

EPO-Erythropoietin

FC- Flow cytometry

FIRS- Foetal Inflammatory Response Syndrome

FMH- Foetal Maternal Haemorrhage

GA- Gestation Age

HDFN- Haemolytic Disease of Foetus and New-born

IgG- Immunoglobulin G

IL-6- Interleukin 6

KB- Kleihauer-Bekte

LMICs- Low- and Medium-Income Countries

MCH- Mean Cell Haemoglobin

MCHC- Mean Cell Haemoglobin Concentration

MCV- Mean Cell Volume

MOHSS- Ministry of health and social services

RCC- Red Cell Count

Rh- Rhesus

RhIG- Rhesus Immunoglobulin G

UNICEF- United Nations International Children's Fund

WCC- White Cell Count

WCH- Windhoek Central Hospital

WHO- World Health Organization

Chapter One

Introduction

Stillbirth remains one of the untoward birth outcomes that presents a major health care challenge, particularly in developing countries with an estimated 2 million stillbirths having occurred in 2019 (Berhe, Gebreyesus and Teklay, 2019). In fact, over two-thirds of global cases of stillbirth are from low-to-medium income countries (LMIC) (Blencowe *et al.*, 2015). This increased number of cases in these countries is associated with poor antenatal and obstetric care (De Bernis *et al.*, 2016; Berhe, Gebreyesus and Teklay, 2019). Notably, in Sub-Saharan Africa, most of the stillbirth cases are attributed to asphyxia, placenta disorders, maternal hypertensive disorders, infections, cord, and uterus problems (Goldenberg *et al.*, 2015; Aminu *et al.*, 2019). Worrisomely, the causes of approximately a quarter of stillbirth cases are unknown in this region (Madhi *et al.*, 2019). This has prompted the exploration of other potential causes such as immunological aberrancies (Koelewijn *et al.*, 2009). Amongst these is foetal maternal haemorrhage (FMH), a condition characterised by the entry of foetal cells into the maternal circulation occurring during pregnancy or delivery (Wylie and Dalton, 2010; McEwan, 2015)

The introduction of foetal cells into the maternal circulation during FMH evokes maternal immune system to produce antibodies against foreign foetal antigens (Koelewijn *et al.*, 2009; Abbasi, Johnson and Ryan, 2017). These maternal antibodies being mainly IgG can cross the placenta and sensitise foetal cells in subsequent pregnancies causing Haemolytic Disease of the Foetus and New-born (HDFN). This condition manifests in increased destruction of foetal red cells resulting in haemolytic anaemia and an increased foetal bone marrow output. The condition is further exacerbated by the accumulation of unconjugated bilirubin leading to irreversible neurological damage in the neonate (Blaney and Howard, 2013; A. Victor Hoffbrand, 2019; Harmening, 2019). Considering HDFN only manifests from second pregnancy onwards, the risk of the condition increases with gravida status and so does the risk of multiple antibody alloimmunisation (Moinuddin, Fletcher and Millward, 2019). Although Rhesus (Rh) anti-D is responsible for the most severe cases of HDFN (Koelewijn *et al.*, 2008) antibodies of other blood group systems such as ABO, Kell, Kidd, Duffy and other Rh group antibodies are responsible for mild to severe cases of HDNF (Moise, 2005;Fletcher, 2019). Notably, 25% of foetus with anaemia due to Rh alloimmunisation, demonstrated high levels of plasma interlukin-6 (IL-6) concentration indicating a hallmark of the Foetal Inflammatory Response Syndrome (FIRS) caused by

non-infection related factors (Vaisbuch *et al.*, 2011). The FIRS as defined by increased levels of foetal plasma IL-6, is a confirmed risk factor to severe neonatal morbidity and mortality (Gomez *et al.*, 1998; Wolfsberger *et al.*, 2020).

In 2016, UNICEF reported 2.6 million stillbirths worldwide annually of which 98% of these occurred in low-income countries (LICs) and 75% Sub-Saharan Africa. Additionally, most of these stillbirths result from such as obstetric complications and non-communicable diseases which are preventable through high quality antenatal and intrapartum care (De Bernis *et al.*, 2016). Foetal Maternal Haemorrhage being an obstetric complication, in itself has been attributed to 3-14% of stillbirths (Leary *et al.*, 2015; Flenady *et al.*, 2017). Although foetal consequences stemming from FMH gave been well documented, maternal response to this phenomenon remains severely understudied. With timely diagnosis and proper antenatal management, fatal consequences of FMH can be mitigated. It is therefore imperative to screen and quantify pregnant mothers for FMH following a potentially sensitising event. Furthermore, maternal screening and identification of irregular antibodies, therefore, is imperative in avoiding untoward immune-mediated pregnancy complications. Hence, this study primarily aimed at determining the incidence of FMH in stillbirths.

1.1 Hypothesis

Foetal maternal haemorrhage is a clinically notably condition that contributes to a significant number of stillbirths in Namibia.

1.2 Research questions

- What is the prevalence of FMH amongst stillbirths at the Windhoek State Hospital?
- Are the mothers of stillborn birth babies alloimmunised and what is the distribution of the antibodies?
- Is there any association between FMH, maternal inflammatory state, renal and hepatic function?

1.3 Overall aim

To determine whether FMH is associated with inflammation, maternal alloimmunisation and altered hepatorenal function.

1.4 Objectives

- To assess FMH in still birth mother samples using the KB test
- To quantify the degree of FMH in positive mothers of stillborn babies.

- To identify the clinically significant antibodies in the mothers of stillborn babies. To investigate maternal response to FMH by assessing haematological, inflammatory and hepatorenal function.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Stillbirths have undoubtedly become a cause of public health concern with an estimated 2.6 million stillbirths occurring every year globally (De Bernis *et al.*, 2016). This health crisis is most prevalent in low-to-middle-income countries (LMICs), particularly those in sub-Saharan Africa and Asia which accounts for about 98% of global stillbirths cases (Joy E. Lawn *et al.*, 2016; Aminu *et al.*, 2019). It is these staggering statistics that prompted investigations in the causes of stillbirths especially in LMICs. Infections, placental disorders, asphyxia, maternal hypertensive disorders and obstructed labour are amongst the top causes of stillbirths in LMICs (Aminu, Bar-zeev and Broek, 2017; Madhi *et al.*, 2019). Despite these well-charactered causes, approximately 55% of stillbirth causes are still unaccounted for, thus prompting for more investigation on the contributing factors of stillbirths (Madhi *et al.*, 2019). Notably, the quality of ante-natal care is a strong independent factor on pregnancy outcomes (Saleem *et al.*, 2018)

Stillbirths are characterised by death of a foetus that is at least 28 weeks old of gestation (WHO 2018). Stillbirths are either fresh, whereby the foetus is born dead without signs of skin disintegration and the death is assumed to have occurred less than 8 hours prior to delivery or macerated, with the foetus demonstrating skin changes such as discoloration, peeling or redness and death occurring over 8 hours before delivery (Goldenberg *et al.*, 2015). Stillbirths can further be categorized according to weight and height with the International Classification of Diseases suggestion the following: 1) Late foetal death 1000 g or more or 28 weeks or more or 35 cm or more, (2) Early foetal death 500 g or more or 22 weeks or more or 25 cm or more and (3) miscarriage as a pregnancy loss before 22 completed weeks of gestational (World Health Organisation, 2010). Risk factors associated with stillbirths include, maternal infections such as malaria, non-communicable diseases such as diabetes, nutrition and lifestyle factors, prolonged pregnancies and maternal age (Joy E. Lawn *et al.*, 2016). In Namibia, the major risk factor associated with stillbirths is the quality of antenatal care (Tshibumbu and Blitz, 2016).

Table 2.1 Classification of Stillbirth according to International Disease Classification

Classification	Weeks	Weight
Miscarriage	22 weeks or <	<500g
Stillbirth- Early Foetal Death	22 weeks- 28 weeks	>500g
Stillbirth- Late Foetal Death	28 weeks-41 weeks	>1000g
Neonatal Death	>41 weeks (post-term)	

Foetal Maternal Haemorrhage (FMH) which is the transfer of foetal cells into the maternal circulation, has been reported to account for up to 14% of stillbirths (Silver *et al.*, 2009; Lebrun and JacquemynYves, 2018). FMH is the main basis of maternal alloimmunisation resulting in serious foetal and neonatal complications such as the Haemolytic Disease of The Foetus and new-born (HDFN) which presents with the breaking down of foetal red cells resulting in severe anaemia and extreme hyperbilirubinemia (Webb and Delaney, 2018). Alloimmunisation has been further linked to immunological aberrances such as the foetal immune response syndrome (FIRS) (Vaisbuch *et al.*, 2011) which can further exacerbate foetal anaemia due to an elevated interlukin-6 that increase the release of hepcidin in inflammatory cases.

2.2 Risk Factors for stillbirths

There are several foetal and maternal factors that are associated with increased risk of stillbirths (Tamara *et al.*, 2019). With the majority stillbirths occurring in LMICs, the focus has now moved to these areas (Lawn *et al.*, 2011). The lack of or low quality of intrapartum care such as access to timely referral is the most common factor underlaying most stillbirths in LMICs (Lawn *et al.*, 2016). It is therefore imperative that this situation is addressed to improve foetal and neonatal health outcomes as well as reducing the incident of stillbirths in these regions.

The factors associated with stillbirths include foetal growth restrictions, congenital abnormalities, and immunopathology (lawn 2016). On the other hand, maternal risk factors include; age, gravida and parity, hypertensive disorders, infections, diabetes, and modifiable factors such as smoking, the use of alcohol, obesity, sleep position and attendance of antenatal care (Joy E Lawn *et al.*, 2016; Tamara *et al.*, 2019). The different disorders associated with stillbirths are summarized in table 2.1

Table 2.2 Foetal and Maternal Factors associated to stillbirths

Foetal Factors	• Congenital Disorders • Gestational Age (prematurity) • Foetal Maternal Haemorrhage • Gender (male)
Maternal Factors	• Infections • Maternal Hypertensive disorders • Infections • Diabetes • Age • Gravida and Parity • Obesity • Substance (smoking, drugs, Alcohol) • Trauma (violence, accidents)

Asphyxia is the leading cause of stillbirths reported in sub-Saharan Africa (Blencowe *et al.*, 2015). Asphyxia is the deprivation of oxygen causing suffocation and death (Goldenberg, Mcclure and Harrison, 2016). In pregnancy, asphyxia is characterised by impaired placental exchange of blood gases progressing to foetal hypoxia with metabolic acidosis (Goldenberg, Mcclure and Harrison, 2016). Asphyxia is rarely identified as primary cause of stillbirth, but rather conditions associated with it such as obstructed labour, foetal distress, placenta abruptio, multiple births and pre-/eclampsia and other conditions that reduce foetal oxygenation such as malaria and other infections (Goldenberg, Mcclure and Harrison, 2016). Similarly, FMH and other consequences arising from it such as HDFN is also listed under asphyxia as the foetus dies from oxygen deprivation due to severe anaemia (Goldenberg, Mcclure and Harrison, 2016). In Namibia, compromised quality antenatal care, is the most common risk factor associated with stillbirths (Tshibumbu and Blitz, 2016). Thus, consenting with the rest of the sub-Saharan Africa in the need for quality and timely antenatal care provision to all expectant mother to lower the stillbirth rates in the region.

2.3 Foetal Maternal Haemorrhage

Foetal maternal haemorrhage (FMH) poses a great concern in obstetrics. Traumatic events such as blunt trauma to the abdomen, invasive procedures such as cordocentesis and amniocentesis, removal of placenta, abruptio and even pre-eclampsia (Wylie and Dalton, 2010; McEwan, 2015) have been described as potentially sensitizing to the occurrence of FMH. Massive FMH has devasting effects on the foetus such as anaemia, brain damage and hydrops fetalis (Stefanovic, 2016). Notably, FMH is the main cause of maternal alloimmunisation that results in the build-up of maternal antibodies that can cross the placenta in subsequent pregnancies, resulting in severe haemolytic anaemia in the foetus. Different thresholds between 10mL and 150mL of foetal haemorrhage have been proposed as massive FMH, however 30ml is most the most commonly used volume to define massive FMH (Fan *et al.*, 2014; Stefanovic, 2016).

2.3.1 Pathophysiology of FMH

Severe haemorrhage results in poor pregnancy outcomes that may be life threatening to the foetus. In early gestation, larger bleeds are more tolerable as compared to late gestation. A large volume lost periodically overtime poses less danger to foetus than a large haemorrhage at once which results in foetal hypotension, ischemia, or acid acidosis (McEwan, 2015). Trauma to the abdomen during pregnancy may result in FMH (Wylie and Dalton, 2010). Other obstetric events such as amniocentesis, cordocentesis, pre-eclampsia, cephalic version, placenta removal, placenta abruption and placental tumours are associated with FMH (McEwan, 2015). Eighty percent of massive FMH cases (greater than 30mL) however, remain unexplained (Wylie and Dalton, 2010). Although the exact mechanism of pathology is not yet well understood, it is postulated that for foetal cells to enter the maternal circulation, there must be a breach in the trophoblast (Wylie and Dalton, 2010). Trophoblast are cells forming the outer layer of blastocytes (embryonic) cells which provide nutrients to the embryo and develop into a large placenta (Saghian *et al.*, 2019) Figure 2.2.

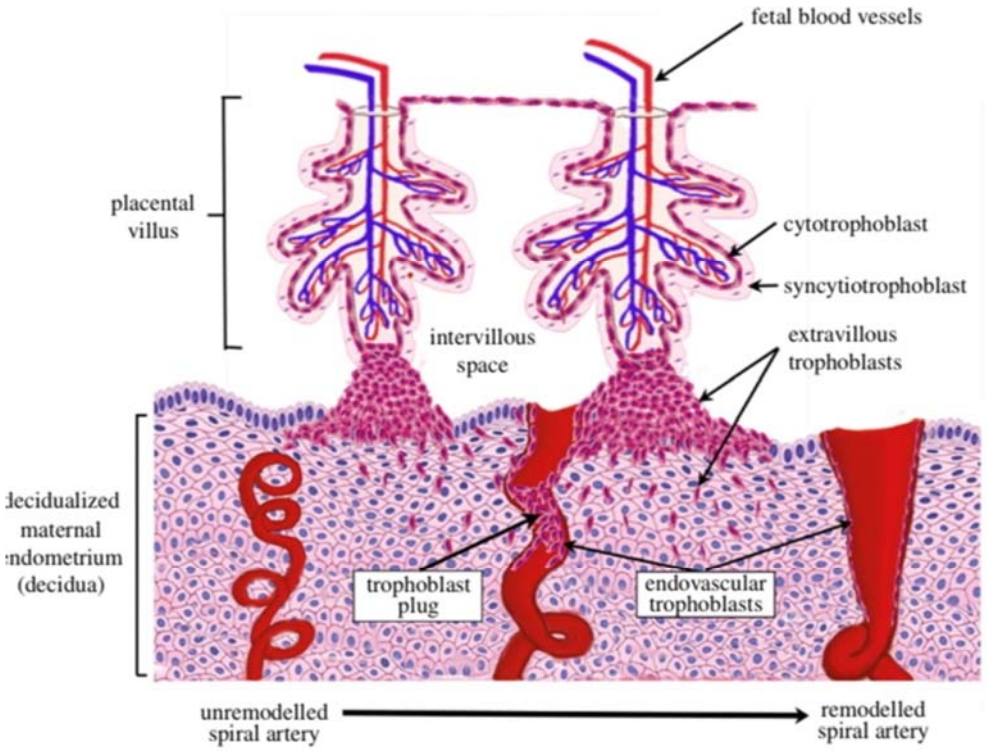

Figure 2.2 Illustration the placental trophoblast. The trophoblast forms plugs that keep foetal cells from flowing into the maternal circulation. A breach in this results in FMH (Saghian *et al.*, 2019)

2.4 Presentation and Outcomes of Foetal Maternal Haemorrhage

Absent or decreased foetal movements is usually the first and most common sign of and it is also indicative of neonatal anaemia (Abbasi, Johnson and Ryan, 2017). Other signs include foetal distress, poor perfusion, neurological injury, growth restrictions which may all result in unplanned caesarean section (Solomonia, Playforth and Reynolds, 2012; McEwan, 2015). Maternal physical outcomes are poorly understood as the mother often presents normally with no signs of bleeding or contractions (Topping, Lett and Thorp, 2019). Additionally, foetal presentations include an abnormal heart rhythm with sinusoidal rhythms indicating severe anaemia and hypoxia, hallmarks of HDFN (Schmit and Duminil, 2019). Severe foetal anaemia results in hydrops foetalis or even death (McEwan, 2015). Should baby survive, FMH may causes cognitive difficulties and poor growth (Ravishankar *et al.*, 2017).

Small volumes of FMH may result in maternal alloimmunisation which causes foetal haemolytic anaemia and can further exacerbate existing anaemia already present from large bleeds (Stroustrup *et al.*, 2016). A haemorrhage of at least 0.1ml is adequate to stimulate alloantibody production (Delaney, Svensson and Lieberman, 2017). Maternal red cell alloimmunisation may result from

previous blood transfusion and FMH from previous pregnancies (Ghesquière *et al.*, 2018). In the context of the latter, Rhesus (Rh) and Kell antibodies are major mediators of severe cases of HDFN (Moise, 2008). Since these antibodies are immunoglobulin (Ig)G, they can freely cross the placenta and attach to the foetal erythrocytes resulting in extravascular haemolysis (Saul Snowise, 2010). If not managed, HDFN may result in hyperbilirubinemia, kernicterus, hydrops foetalis and ultimately foetal death (Zipursky, Bhutani and Odame, 2018). The Rh disease may lead to foetal response syndrome due to persistently high levels of interleukin 6 (IL-6) in neonates with Rh HDFN (Vaisbuch *et al.*, 2011). The exposure of paternal antigens through FMH and exposure to foetal leukocytes may have an implication on the mother's health such as a risk of autoimmune disease (Wylie and Dalton, 2010).

2.4.1 Foetal anaemia

Foetal haemoglobin levels increase gradually during pregnancy, therefore the degree of haemoglobin (Hb) deviation from mean gestational age (GA) is used to define foetal anaemia (Abbasi, Johnson and Ryan, 2017). Haemolytic anaemia resulting from alloimmunisation is the most common form of foetal anaemia (Giancarlo Mari, 2000; Abbasi, Johnson and Ryan, 2017; Prefumo *et al.*, 2019). There are however other causes of foetal anaemia such as infections, and membranes defects (Table 2.1). The cause of foetal anaemia by FMH can be termed as twofold: 1) Red cell destruction by maternal antibodies as a result of alloimmunisation which is a consequence of FMH, 2) Chronic or acute foetal blood loss (McEwan, 2015). The impact of FMH on foetus will depend on gestation and duration over which blood is lost (McEwan, 2015). As previously mentioned, larger bleeds will be tolerated less well at earlier gestation and chronic blood loss occurring over a period in small volumes is better tolerated than acute blood loss over short period of time. Delivery is the most common time for FMH to occur, however there will be no consequence if bleeding occurs once the cord has been clamped already.

Reduced foetal movements and poor perfusion are usually the first signs of foetal anaemia and should prompt immediate diagnosis to prevent detrimental consequence (Topping, Lett and Thorp, 2019). Diagnosis is made by calculating the peak-velocity of systolic blood in the middle cerebral artery (PSV-MCA) by doppler ultrasonography (Prefumo *et al.*, 2019; Schmit and Duminil, 2019). Other very important diagnostic methods include the Kleihauer-Bekte (KB) test for FMH and maternal/paternal blood grouping to determine possible incompatibilities and maternal antibodies screening, identification, and titration (Abbasi, Johnson and Ryan, 2017).

Foetal anaemia is associated with severe complications such as foetal hypotension, acidosis, ischaemia, hydrops foetalis and even intrauterine death (McEwan, 2015; Prefumo *et al.*, 2019). Accurate and prompt identification of degree of anaemia is crucial and lifesaving in foetus at risk. The

demonstration and quantification of FMH can allow for life saving management and treatment regimens of anaemia and prevent sensitisation by maternal antibodies which results in severe haemolytic anaemia (Coe, 2019). Intrauterine transfusions have been proved to significantly improve prognosis and survival rate in most cases (Giancarlo Mari, 2000). Anaemia resulting from alloimmunisation can be prevented by giving prophylaxis in the case of anti-D alloimmunisation (Porter, T. F. and Holmgren, C. 2018).

Table 2.3: Classification of causes of foetal anaemia

Mechanism	Cause
Increased Red cell Breakdown	• Red cell alloimmunization • Membrane defects
Reduced Red cell Production	• Infections: parvovirus • Bone marrow disorders • Metabolic Disorders
Increased Red Cell Loss	• Foetal Maternal Haemorrhage • Placenta Previa • Multiples (co-twins)

2.4.2 Alloimmunisation

Alloimmunisation is a production of antibodies in response non-self-antigens on the surface of red cells and is a consequence of FMH. It occurs in response to antigen exposure through pregnancy and blood transfusion products and antibodies may be clinically significant leading to transfusion reactions and HDFN (Moise, 2005; Hendrickson, Tormey and Haven, 2016). The incidence of alloimmunisation in healthy patients ranges from 1%-3% and much higher chronically transfused patients (Hendrickson, Tormey and Haven, 2016). The most common antibodies implicated in alloimmunisation in transfused patients in sub–Saharan Africa are of the Rh group with anti-E being the most frequent followed by anti-D (Ngoma *et al.*, 2016). Kell group antibodies are the second most common with anti-K coming in overall 2nd most implicated after anti-E (Ngoma *et al.*, 2016).

Alloimmunisation may also occur during pregnancy due to FMH and as previously mentioned, it is the main cause of foetal anaemia (Giancarlo Mari, 2000). Foetal maternal haemorrhage exposes the mother to non-self-antigens on the foetal cells, provoking her immune system to build up antibodies against these red cells (Ghesquière *et al.*, 2018). A pregnant woman may also be immunised from transfusions prior to or during pregnancy or intra-uterine transfusions (Haas *et al.*, 2015; Hendrickson and Delaney, 2016). These antibodies are commonly of IgG nature, thus making them clinically significant since they are able to cross the placenta and cause haemolysis to foetal red cells in subsequent pregnancies (Saul Snowise, 2010). The global prevalence of antibodies in pregnant women is estimated to be at 12% worldwide with blood groups from the Rh (anti-D, anti-E and anti-c) leading the pack followed by anti-K of the Kell blood group system (Webb and Delaney, 2018; Moinuddin, Fletcher and Millward, 2019). Haemolysis occurs extravasculary via phagocytosis of IgG-bound foetal cells by the macrophages of the spleen and liver (reticuloendothelial system) (Saul Snowise, 2010). This results in severe anaemia, causing hyperbilirubinemia, hydrops fetalis and even foetal death (Haas *et al.*, 2015). Should foetus survive birth, neonates are born with unmanageable levels of bilirubin leading to irreversible brain damage (Delaney and Matthews, 2015). The antibody anti-D is the most common culprit of severe HDFN with up to 85% case being reported, followed by antibodies from the Duffy, other Rh and Kell blood group systems respectively (Gottvall and Filbey, 2008; Moise, 2008).It is therefore imperative to screen pregnant women for clinically significant antibodies in order to mitigate any outward pregnancy outcomes.

2.4.3 Haemolytic Disease of the Foetus and New-born

Haemolytic disease of the foetus and new-born (HDFN) is a condition resulting from clinically significant maternal IgG antibodies formed in response to alloimmunisation (Saul Snowise, 2010). These antibodies cross the placenta and sensitize foetal red cells and leading to their destruction (Wintjens, Walther and Lopriore, 2008). This manifests in severe haemolytic anaemia, hyperbilirubinemia resulting in kernicterus and even more severe outcomes such as hydrops foetalis or foetal demise (Saul Snowise, 2010; Hendrickson and Delaney, 2016). In 2013 18% of 134 million live births where at risk of neonatal hyperbilirubinemia with adverse of the mortality occurring in Sub-Saharan Africa and Asian countries (Bhutani *et al.*, 2013). Furthermore, there was a 24% risk of death with from the hyperbilirubinemia with 3 quarters of which results from sensitization from anti-D antibodies of the Rh blood group system, was the leading cause of the hyperbilirubinemia in these regions (Bhutani *et al.*, 2013). Apart from anti-D, other antibodies of the Rh blood group system such

as anti-c and from other blood group system such as Kell, Duffy and Kidd also cause severe HDFN (Fasano, 2015).

2.4.3.1 Pathogenesis of HDFN

Foetuses express red cell antigens inherited both maternally and paternally as from a month after gestation (Saul Snowise, 2010). The paternally inherited antigens may be recognised as foreign by the maternal immune system which response by producing IgG antibodies. IgG antibodies are effectively transported across the placenta mainly by the neonatal Fc receptor and bind to antigen positive foetal red cells causing extravascular haemolysis by the macrophages of the liver and spleen (Hendrickson and Delaney, 2016; Borghi *et al.*, 2020). The worsening of the anaemia results in a compensatory increase in haematopoiesis releasing immature progenitor red cells (erythroblasts), splenomegaly and hepatomegaly due to extramedullary haematopoiesis (Haas *et al.*, 2015).

The extramedullary mechanism of haematopoiesis leads to decreased plasma protein (mainly albumin) production by the liver leads to cardiac failure with generalized oedema and ascites in foetal cavities (Ghesquière *et al.*, 2018). Hydrops foetalis which refers to a manifestation of pulmonary effusion, pericardial effusion, significant ascites, and severe anaemia is usually the end stage of the disease in foetuses (Saul Snowise, 2010; Li and Blaustein, 2017). The foetal liver being immature, is unable to fully conjugate bilirubin which results from the release of haemoglobin as red cells break down resulting in hyperbilirubinemia (Haas *et al.*, 2015). After birth, neonatal hyperbilirubinemia persists leading to kernicterus which is characterised by the deposition of bilirubin in the brain (Sabhubrata and Rennie, 2017). Acute bilirubin toxicity has been associated with hypercarbia, acidosis, sepsis, hypoxia and disturbances in the blood brain barrier (Wennberg *et al.*, 2015). Furthermore, kernicterus is a cause of neuro-developmental impairment with cerebral palsy, hearing difficulties and psychomotor handicaps (Slootweg *et al.*, 2018).

Conversely, IgM blood group antibodies such as those from Lewis, I and P blood group systems do not cross the placenta to cause HDFN (Saul Snowise, 2010). Anti-D, anti-E, anti-c which are all from the Rh blood group system and anti-K from the Kell blood group system are the most common cause of severe HDFN (Webb and Delaney, 2018). Other antibodies from Duffy, Kidd, MNSs and ABO blood group systems have also been reported to cause moderate to severe HDFN (Koelewijn *et al.*, 2008). It is therefore advised that pregnant woman be typed for ABO and Rh groups and be screened for unexpected blood group antibodies (Porter, T. F. and Holmgren, C., 2018). With the administration of prophylactic anti-D immunoglobulin, HDFN cases caused by anti-D have significantly decreased

putting a spotlight on other antibodies (Fasano, 2015). Figure 2.3 summarizes the pathogenesis of HDFN.

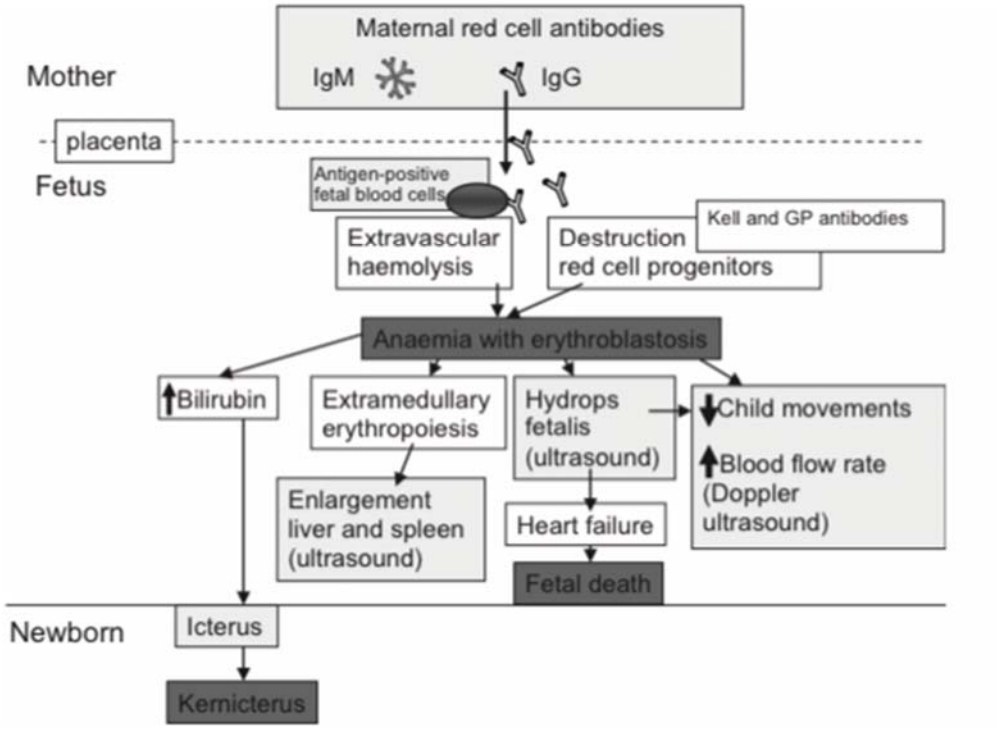

Figure 2.3 The pathogenesis of the Haemolytic disease of foetus and new-born (adapted from de Hass et al, 2015). Maternal IgG antibodies are produced in response to exposure to red cells by Foetal Maternal Haemorrhage (FMH) or blood transfusions. These IgG antibodies cross the placenta in subsequent pregnancies and haemolyse foetal cells.

2.4.3.2 Rh HDFN

Maternal anti-D alloimmunisation always been the most common cause of HDFN but however with the implementation of prophylaxis anti-D immunoglobulin (RhIG), the incidence of Rh HDFN, has decline over the years (Moinuddin, Fletcher and Millward, 2019). In LMICs however, Rh HDFN continues to be a public health problem that is responsible for thousands of stillbirths and neonatal deaths annually (Zipursky and Paul, 2011). Furthermore, Rh HDFN is mainly responsible for hyperbilirubinemia that leads to serious neurological damage causing disabilities in children (Bhutani *et al.*, 2013). In Uganda, 4 out of the 38 alloimmunizations where identified as anti-D, that is 10.5% (Mbalibulha *et al.*, 2015). Haemolytic disease of foetus and new-born due to anti-D alloimmunisation is also referred to as the Rh disease and although there are several antibodies of the Rh blood group system, this term is used specifically used in HDFN caused by anti-D antibodies.

Rh antibodies are not naturally occurring and are produced in response to exposure to an antigen (Dunbar, 2020). Rhesus negative mothers who lack Rh D antigens can be exposed to these antigens either from blood transfusion of Rh D antigen positive blood or from FMH resulting from carrying a foetus with paternal Rh D positive antigens (Mcbain, Crowther and Middleton, 2015; Dunbar, 2020). Antibody development occurs in approximately 15% of pregnancies in which the foetus is Rh positive and the mother is Rh negative (Joy E. Lawn *et al.*, 2016). Since the initial pregnancy only plays a role in stimulating the mother to produces anti-D, it is not affected (Mcbain, Crowther and Middleton, 2015). Rhesus disease only manifest from the 2nd pregnancy onwards, therefore gravida status plats a role in the development of this disease.

It is important to diagnose this disease timely to prevent untoward consequences. Fortunately, the development of the prophylaxis RhIG significantly ameliorated the alloimmunisation of D-negative mothers exposed D antigens (Zipursky, Bhutani and Odame, 2018; Pegoraro *et al.*, 2020). However, the prevalence of alloimmunisation in LMIC still continues partly due to poor antenatal health care (Zipursky, Bhutani and Odame, 2018). It is recommended that all pregnant women be immunophenotyped for the D antigen and in Rh negative women, screening of irregular antibodies and titre monitoring of anti-D should be monitored through pregnancy before administration of RhIG at 28 weeks of gestation (White *et al.*, 2016). The KB test can be used not only to screen for FMH but also to calculate the dose of RhIG needed to prevent Rh disease in anti-D positive mothers (Kim and Makar, 2012). The prevention of Rh disease can further prevent other immunological aberrances associated with Rh disease such as the Foetal Response Syndrome (FIRS) (Vaisbuch *et al.*, 2011).

Foetal Inflammatory Response Syndrome in Resus Disease

Foetuses with HDFN mediated by rhesus alloimmunisation have are at risk of developing FIRS (Vaisbuch *et al.*, 2011), a condition that is characterised by elevated levels of IL-6 in the foetal plasma and systemic inflammation (Gotsch, Romero and Pedro, 2007). Notably, FIRS is the foetal form of the systemic inflammatory response syndrome (SIRS) in adults (Jung *et al.*, 2020). The FIRS is associated with preterm labour and severe neonatal morbidity and mortality outcomes(Jung *et al.*, 2020). Severe respiratory destress syndrome, sepsis, pneumonia, bronchopulmonary dysplasia and brain injury are the major morbidities associated with FIRS (Gotsch, Romero and Pedro, 2007; Vaisbuch *et al.*, 2011)

Interleukin 6 is a cytokine which play a critical role in the control of inflammation by regulating the adaptive, immune and growth and differentiation of the haematopoietic cells (Gotsch, Romero and Pedro, 2007). A high foetal IL 6 plasma level of greater than 11 pg is used to define FIRS (Vaisbuch *et*

al., 2011). Interleukin 6 levels in plasma of foetuses with Rh disease and a low haematocrit from the anaemia is significantly higher than those without the Rh disease (Vaisbuch *et al.*, 2011). High levels of IL 6 induce the production of hepcidin which negatively inhibits erythropoiesis by reducing the uptake of iron from the intestinal mucosa and its release from body store which in consequence exacerbates the haemolytic anaemia already present from the Rh disease (Hoppe, Hulthén and Samuelson, 2018).

2.4.3.3 HDFN by Rh antibodies other than anti-D

Apart from the D antigen, the Rh blood group system is composed of other antigens namely the E ,e ,C ,c , cw and g (Flores-bello *et al.*, 2018). These antigens have corresponding antibodies of the afore-mentioned antigens are IgG thus making them obstetrically significant (Negi and Dushyant, 2012). Anti-E and anti-c are also frequently associated with HDFN and some studies even suggest them to have now surpassed anti-D in causing the disease due to the use of RhIG (Ghesquière *et al.*, 2018; Agrawal, Hussain and Kumar, 2020). Amongst all Rh antibodies, anti-c is associated with the most severe foetal outcomes (Stefanovic, 2016; Slootweg *et al.*, 2018). In Sub-Saharan Africa, anti-E is ranked the most common antibody in previously transfused patients (Ngoma *et al.*, 2016).

Other remaining non-anti-D Rh antibodies, anti-C, anti-e and anti-C^w are not exempted from causing HDFN. Although uncommon due to low frequency, anti-C results in severe HDFN necessitating intra-uterine transfusion (Negi and Dushyant, 2012). Although C^w is a low frequency antigen, the fact that most of the population lacks it, it means that they may easily build up anti-C^w after exposure. The few cases of HDFN owing to anti-C^w reported only mild to moderate disease (Malik and Moiz, 2012). These antibodies are demonstrated by screening and identification using the indirect antiglobulin test (IAT) (Delaney, Svensson and Lieberman, 2017). Pregnant women should be screened for irregular antibodies during their initial antenatal visit in the first trimester and then again at 28 weeks of gestation (White *et al.*, 2016; Munik, Clarke and Lieberman, 2020). In the event that irregular antibodies are detected, they should be identified and if clinically significant, their titre should be monitored every 2 to 4 weeks (Munik, Clarke and Lieberman, 2020).

2.4.3.4 HDFN from other unexpected antibodies

Irregular antibodies are antibodies that unlike the ABO antibodies from the blood groups, are not naturally occurring but requires exposure to antigen for formation (Fan *et al.*, 2014). Other IgG

antibodies that are capable of crossing the placenta and cause HDFN belongs to the MNS, Kidd, Lutheran, Lewis, Duffy and Kell blood groups systems (Kim *et al.*, 2013). Cold antibodies such as those from the Lewis, P, and H blood group systems can be ignored since they are predominantly IgM (Delaney and Matthews, 2015). Antibodies from the MNS system are implicated mild to moderate cases of HDFN while anti-M is rarely implicated since its predominantly IgM, it develops as IgG and cause HDFN which can range from mild to severe (Saul Snowise, 2010).

Anti-K is an unexpected antibody that requires special consideration. Kell antigens are well developed at birth and found on foetal red cells at around 10 to 11 weeks of gestation (Goldman *et al.*, 2015). After anti-D, it has been reported as the second most common cause of HDFN after the antibodies of the Rh blood group (Koelewijn *et al.*, 2008; Slootweg *et al.*, 2018; Moosavi *et al.*, 2020). Anti-K causes severe HDFN requiring intra-uterine transfusion and has been implicated in several foetal death cases (Moise, 2008; Munik, Clarke and Lieberman, 2020). Instead of haemolysis and hyperbilirubinemia, anti-K supresses the production of red cells (Goldman *et al.*, 2015) leading to a more aplastic rather than haemolytic anaemia. Due to disease severity resulting from this antibody, prevention of anti-K mediated HDFN should be a priority. As already mentioned, pregnant women are screened at initial visit and then again at 28 weeks for unexpected antibodies. Monitoring of anti-K titre is of vital importance as anti-K causes more severe HDFN than anti-D at lower titres, with hydrops fetalis being reported at titres as low as 8 (Moise, 2005).

Other unexpected antibodies are not exempted from causing the HDFN. Duffy antibodies although result in mild to moderate disease, have also been implicated in HDFN (Saul Snowise, 2010). The Fy(a-b-) phenotype is the most predominant phenotype in Africans, meaning a higher probability of making both anti-Fya and anti-Fyb after exposure due to a lack of both antigens (Lukasik). The kid blood group system consists of 3 main antigens JKa, JKb and JK3 and antibodies towards these 3 antigens cause mild HDFN as they are of IgG nature (Lawicki, Covin and Powers, 2016). Of the MNS blood group system, although anti-M is predominantly IgM it has a IgG component which can cause HDFN. Anti-S, anti-s and anti-U are IgG and result in mild HDFN, while anti-N being IgM, does not result in HDFN (Aeschlimann and Westhoff, 2019).

2.4.4 Maternal Outcomes

The consequences of FMH does not end with the foetus or neonate but the mother is also impacted however remains poorly understood. Due to the traumatic and inflammatory nature of sensitising events, placental inflammation has been associated to the occurrence of FMH (Scholz *et al.*, 2012).

Long term presence of foetal leukocytes in the maternal circulation may result in michrochimerism (Figure 2.4) and is associated with as autoimmune disorders (Wylie and Dalton, 2010). Foetal antigens that are paternally inherited may be recognised as foreign and activate the maternal immune system (Than *et al.*, 2019). Feto-maternal chimerism due to persistent foetal antigens has also been associated to pre-eclampsia which is a pregnancy disease characterised by hypertension, proteinuria, and multi-organ failure and can be life threatening (Hahn *et al.*, 2019).

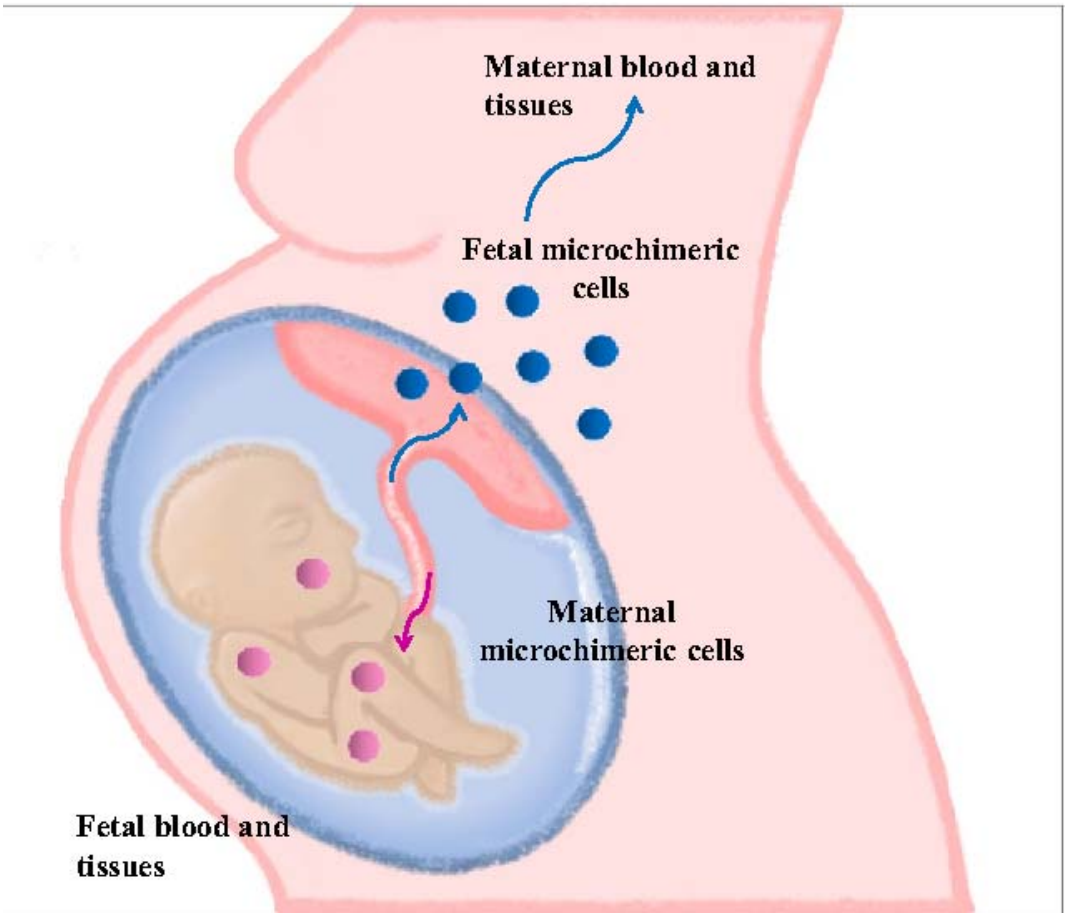

Figure 2.4 Foetal cells passed into the maternal circulation during pregnancy can persist for years. This is known as michrochimerism (adapted from Shanon Hunt, 2019). Michrochimerism has been implicated in autoimmune disorders in women.

2.5 Antenatal screening, diagnosis, and management of FMH related conditions

Although a staggering 7100 stillbirths are occurring worldwide every day, half of these are highly preventable with quality care both before and at birth (De Bernis *et al.*, 2016). Early diagnosis and management of FMH can be beneficial in preventing the manifestation of associated complications such as hydrops fetalis and stillbirths. Foetal maternal haemorrhage is assessed using flow cytometry or KB method (Kim and Makar, 2012). Testing for FMH should preferably be performed in pregnant woman after potentially sensitising events (Kim and Makar, 2012). The KB test is used in determining accurate dose of prophylactic anti-D RhIG which is used in preventing anti-D alloimmunization in Rh-negative mothers (Lebrun and JacquemynYves, 2018). If foetal anaemia is suspected, the evaluation of peak systolic through the foetal mid-cerebral artery by ultrasound has proven to be valuable in the identification of anaemic foetuses (Martinez-Portilla *et al.*, 2019).

It is recommended that pregnant woman be tested for ABO and Rh blood groups and screened for the presence of unexpected antibodies at their initial and even subsequent antenatal visits (White *et al.*, 2016; Porter, T. F. and Holmgren, C., 2018). This is carried out to detect not only anti-D but all other antibodies such as anti-K, anti-C, anti-E etc that are of obstetric significance and are culprits in the HDFN and is a very crucial step in preventing HDFN. In the case of a positive antibody screen, further testing for the identification of antibody specificities is carried out (White *et al.*, 2016). After antibody identification, the antibody is quantified to determine the titre or strength of the antibody. This should ideally be done every 2 to or weeks to monitor the titre of the antibody (Munik, Clarke and Lieberman, 2020). Anti-K as previously mentioned is has been shown to result in severe HDFN even at low titres (Moise, 2008).

Early detection and monitoring of FMH and its potential consequences, gives way to implement lifesaving preventions and therapies resulting in better foetal outcomes. Intra-uterine transfusions (IUTs) are indicated for foetal anaemia resulting directly from FMH or alloimmunisation amongst other condition and has a survival rate of up to 80% (Lindenburg, Kamp and Oepkes, 2014). It is generally indicated in cases with a foetal haematocrit of less than 30% and an HB less then 10g/dL (Abbasi, Johnson and Ryan, 2017). Fresh group O Rh negative leukodepleted, irradiated and cytomegalovirus negative unit is used (Abbasi, Johnson and Ryan, 2017). UITs are effective not only in replenishing anaemic red cells but preventing further destruction of the red cells by the maternal antibodies. Postnatal interventions such as exchange transfusions, albumin and phototherapy are amongst a few used to alleviate the effects of the anaemia such as hyperbilirubinemia (Wintjens, Walther and Lopriore, 2008; Ree *et al.*, 2017).

2.5.1 Prevention of Anti-D alloimmunisation

Amongst all antibody alloimmunisations in pregnant woman, anti-D is preventable, this is thanks to the introduction of prophylaxis anti-D. WHO recommends that all Rh-negative women be given the prophylaxis at 28 and 34 weeks to prevent anti-D alloimmunisation (WHO, 2016). The prophylactic treatment has proven to be effective in high-income countries reducing the incidence of the Rh disease significant. Conversely though, the Rh disease continues to be a public health concern in LMICs causing stillbirths amongst other detrimental foetal and neonatal effects (Zipursky, Bhutani and Odame, 2018). Poor or no Rh alloimmunisation prevention has been noted in Sub-Saharan Africa following events that are off potentially sensitising nature (Osaro and Charles, 2010). Reasons for the latter are attributed to, poor understanding of the Rh disease by population (healthcare workers includes), improper use of prophylaxis and poor data management such as missing laboratory data or information from previous pregnancies (Osaro and Charles, 2010; Zipursky, Bhutani and Odame, 2018).

Anti-D immunoglobulin (RhIG) is a sterile solution manufactured from pooled plasma of donors with high levels of anti-D IgG (Mcbain, Crowther and Middleton, 2015). Anti-D immunoglobulin is ideally given as a prophylaxis to all Rh-negative woman and to those with positive FMH. In some countries however, it is given postpartum after delivery of ideally the first Rh positive baby (Delaney, Svensson and Lieberman, 2017). Anti-D immunoglobulin has been proven to supress antibody response to RBC antigens in animal models (Brinc and Lazarus, 2009). Although not clearly understood, a few theories for how exactly the RhIG works in humans, It is postulated that RhIG mediates clearance of foetal antigens preventing maternal immune response and production of maternal antibody by B cells (Brinc and Lazarus, 2009; Mcbain, Crowther and Middleton, 2015). RhIG is not effective in already immunised mothers as it is given as a preventative method.

It is without a doubt that the introduction of RhIG has proven to be a significant evolution in prenatal care cutting the rate of prenatal mortality because of Rh HDFN by 50% (Wintjens, Walther and Lopriore, 2008). This is a previously mentioned with reference to high income countries. With LMIC however, implementation of rh disease programmes remain a challenge and will require efforts. The measurement of FMH using the KB test is not only a cheaper alternative to detecting FMH but also used in calculating the dose of RhIG needed according to the degree of foetal cells passed on through the mother to effectively prevent all immunisations (Kim and Makar, 2012). Knowledge and

collaboration between all healthcare workers and relevant stakeholders is essential in providing protection to both mother and child in LMIC in this regard.

2.6 Conclusion

Stillbirth rates are the highest in Sub-Sharan Africa and LMICs with factors such as poor antenatal care and management contributing significantly to the occurrence of stillbirths. The deprivation of oxygen to the foetus known as asphyxia has been identified as the leading cause of stillbirths. Several conditions such as obstructed labour, foetal distress, placenta abruptio, multiple births and pre-/eclampsia and FMH are known causes of asphyxia. Foetal maternal haemorrhage is the release of foetal blood cells into the maternal circulation and is without both foetal and maternal consequences. Large bleeds lead to foetal anaemia, while even minute bleeds result into alloimmunisation of the mother. Maternal antibodies resulting from alloimmunisation, cross the placenta and cause in HDFN which has detrimental consequences to the foetus and new-borns and can be fatal. Maternal antibodies attach to and destroying foetal red cell resulting in haemolytic anaemia, dangerous levels of bilirubin and hydrops foetalis. Antibodies from the rhesus blood group as anti-D, anti-E are most implicated in severe HDFN cases. Maternal consequences resulting from the exposure of the maternal immune system such as inflammation and development of autoimmune disorders in women have also been reported, however systemic effects of FMH on maternal hepatic or renal systems have not been documented posing a great knowledge gap in these areas.

CHAPTER THREE

METHODOLOGY

3.1 Study design, setting, population and sampling

This was a descriptive cross-sectional study involving mothers who gave birth to stillborn babies. The study was conducted at the maternity section of Windhoek Central Hospital (WCH) located in Windhoek the capital city of Namibia in the Khomas Region. The study was conducted between August 2019 and February 2020. The WCH is a public hospital that also serves as a referral hospital for patients from other regions. The post-natal ward forms part of the maternity section of the hospital and accommodates mothers who have given birth and their babies, this also includes those who have given birth to stillbirths.

Nonprobability convenient sampling was the sampling technique of choice for this study. Convenience sampling is a type of nonprobability or non-random sampling where members of the target population that meet certain practical criteria, such as easy accessibility, geographical proximity, availability at a given time, or the willingness to participate are included for the purpose of the study, it is also known as haphazard or accidental sampling (Dörnyei, Z. 2007). All available patients who gave their consent by filling in and signing the consent forms were considered. Ethical clearance for the study was sought and granted by the following institutions: Namibia University of Science and Technology (NUST) Research Ethics Committee (see appendix 1), Ministry of Health and Social Services (MOHSS) (see appendix 2) and Namibia Blood Transfusion Services (NAMBTS) (see appendix 3).

3.1.1 Inclusion Criteria

Adult women who gave birth to a stillbirth during the data collection period who have consented to take part in the study.

3.1.2 Exclusion Criteria

Women that have not given birth to stillbirths and those that have not given their consent to participate in the study including minors.

3.1.3 Sample size

Sample size was calculated using the following formular: $n=Z^2P\ (P-1)/d^2$ (Daniel, 1999):

Where n = sample size.

Z = Z statistic for a level of confidence.

P = expected prevalence or proportion

d = precision (in proportion of one; if 5%, d = 0.05

We used the prevalence rate of 53 stillbirths per thousand as recoded in the Sub-Saharan Africa region (Aminu *et al.*, 2019). Using the above formulae and the calculations below, the sample size was found to be **77** although only **60 samples were collected.**

Sample Calculation:

$$n = \frac{(Z^2) \times p(1-p)}{d2}$$

$$n = \frac{(1.96^2) \times 0.053(1-0.053)}{0.05^2}$$

$$n = \frac{(3.8416) \times 0.053(0.947)}{0.0025}$$

n= 77

For the subgroup analysis:

A subgroup analysis was performed to determine difference in outcomes between FMH positive and negative groups. The sample size was calculated using G*Power software (Version 3.1.9.2). The effect size was based on a primary outcome reported in a previous study ((Scholz *et al.*, 2012) . The following assumptions were used in determining the minimum number of required participants: a medium effect size (d) = 0.898071, αerr prob = 0.05, power (1-βerr prob) = 0.80 and an allocation ratio. The software suggested 35 samples be used per group but however only 15 were used per as this was the number of positive determined samples observed in the study.

3.1.4 Data and Sample Collection

Samples were collected by a registered nurse recruited by the researcher. A consent form and an information slip were attached to each sample. Only patients who signed consent were allowed to be part of the study. The information slip was completed by the nurse for demographic information such as age, date of stillbirth, collection date and gravida. Venous blood (4ml) samples were collected in purple top tubes containing Ethylenediaminetetraacetic acid (EDTA) anticoagulant. Ethylenediaminetetraacetic acid prevents clotting by chelating calcium which is necessary for the coagulation process to take place. Samples were then transported from the hospital to the Namibia University of Science and Technology (NUST) laboratories and slides immediately made before aging of specimen. As previously mentioned, sample collection took place between July 2019 to February 2020.

Laboratory measurements of full blood count (FBC), urea and electrolytes (U&E), liver function tests (LFT) and c-reactive protein (CRP) were retrieved from laboratory data from patients' files upon admission. These tests were performed by the local laboratory service Namibia Institute of Pathology (NIP) which is accredited to carry out these tests using suitable techniques. Rhesus and antibody screen of the participants were retrieved from the NAMBTS as they handle antenatal blood group testing. All antibody positive samples are subjected to antibody identification using different serological methods to identify antibody specificities. Furthermore, clinical, and demographic data was retrieved from the patient files.

3.2 Sample Analysis for FMH

3.2.1 Preparation of blood smears and staining

The FMH was analysed using the Sigma Aldrich Foetal Haemoglobin kit as per the manufacture's details (Appendix 5). The kit is designed to detect foetal cells by the acid elution principle which retains foetal haemoglobin after being immersed in acid buffer while eluting adult haemoglobin (Kim and Makar, 2012). The slides were prepared from freshly collected maternal EDTA tubed whole blood sample. Controls were run with every batch processed whereby; cord blood diluted with adult EDTA male blood was used a positive control and male blood as a negative control. The prepared blood films were then fixed in ethanol and left to airdry prior to staining. Films were then incubated in citric-acid buffer for 20 minutes, stained with haemotoxylin for 3 minutes and then counter stained with eosin.

The foetal cells take up the eosin and stain bright red/pinkish while the adult cells do not take up the eosin and stain as ghost cells.

3.2.2 Examination of stained slides and determination of the degree of FMH

The study slides were examined using light microscope at 40x and 100x power of magnification. Foetal red cells appeared bright pink with adult cells appearing as faintly stained red cells in the background as in Figure 3.1 below. A total of 2000 cells were counted, and the percentage of foetal red cells determined. The volume of FMH was determined using the Mollison's formula as indicated below (Delaney, Svensson and Lieberman, 2017)

Mollison's Formula:
Foetal red cells volume= % Foetal cells x maternal blood volume (5000mL)

3.3 Statistical analysis

The data was subjected to normality testing using D'Agostino & Pearson and Shapiro Wilk test for the subgroups as the data was fewer. Descriptive statistics were performed on clinical and demographic data available to obtain mean SD values and median interquartile ranges based on their distribution. The unpaired t-test was used to compare means of continuous data which was corrected using the Welch's test of correction in cases of unequal variance. Pearson correlation analysis was performed to determine association between significant laboratory values within FMH positive individual and r and p values were obtained. Graph Pad Prism 8 version 8.0.2 Software, (Graph Pad Software Inc, San Diego, CA, USA) was used to perform all statistical analysis.

CHAPTER FOUR

RESULTS

4.1 General Characteristics of participants

A total of 60 participants who delivered stillborn babies were included in this study. The mean age of the included participants was 31.75 years, and the mode age range was 25-29 years (Table 4.1). Most of the included participants (75%, n=45) were multigravida whilst 13.3% (n = 8) and 11.7% (n = 7) were grand multigravida and primigravida, respectively (Table 4.1). The overall prevalence of FMH was 25% (Table 4.1). The degree of FMH was determined and only 6.7% (n = 4) of FMH positive cases had an estimated FMH volume of more than 30mL while the remaining 73.3% (n=11) had FMH less than 30mL (Table 4.1).

Table 4.1 General characteristics of included participants (n = 60)

Variable	Number of participants (n=60)	Percentage (%)
Age		
20-24	3	5.0
25-29	20	33.3
30-34	16	26.7
35-39	9	15
40-44	6	10
Not recorded	6	10
Gravity		
Primigravida (1st pregnancy)	7	11.7
Multigravida (> 1 <5 pregnancies)	45	75
Grand multigravida (>5 pregnancies)	8	13.3
Parity		
Nulliparous (no birth)	11	18.4
Primiparous (1 live birth)	19	31.6
Multiparous (>1 live births)	24	40
Grandparous (>5 live births)	6	10
Rh Factor		
Rh Negative	3	5
Rh Positives	50	83.3
Not recorded	7	11.7
Screens		
Positive	2	3.3
Negative	36	60
Not done	22	36.7
Antibodies Identified		

Anti-E	2	3.3
Foetal maternal haemorrhage (FMH)		
Positive	15	25
Negative	45	75

4.2 Laboratory Profiles of participants

Routine laboratory results were obtained from patient files and mean was calculated. The median interquartile range was used in cases were values failed normality tests. The most common test profiles ordered for each woman upon admission where the Full Blood Count (FBC), the Urea and Electrolytes, (U&E) for renal function, the Liver Function Tests (LFT) and C-reactive Proteins (CRP) to assess inflammation. The mean white cell count (WCC) for the entire population was 10.71 ± 3.50, with a mean neutrophil absolute value of 8.3 ± 3.21 and 1.72 ± 0.58 for lymphocyte counts. The overall red cell count (RCC) mean was 3.56 ± 0.69 and an overall mean of 10.49 ± 1.96 was reported for haemoglobin amongst participants. The C-reactive protein (CRP) had a median interquartile range of 23.8 [6.9 - 52.6] indicating inflammation amongst the overall population. From the renal function tests, urea and creatinine had median interquartile ranges of 3.1 [2.2 - 4] and 58 [48 - 67], while the Glomerular Filtration Rate (eGFR) had a mean of 725.1 ± 394.3. The mean and median interquartile range values for liver enzymes Alkaline Phosphate (ALP), Gamma-glutamyl Transferase (GGT), Alanine Transferase (ALT) and Aspartate Transaminase (AST) where 9 [4.5 - 16.5], 153.1 ± 71.4, 53.5 ± 32.25 and 35 [13 - 51.75] respectively. The values are as reported in Table 4.3 where the rest of the laboratory values are also reported.

Table 4.2 Laboratory values of participants (n=60)

Parameter	Mean SD/Interquartile range values	Reference Ranges (Namibia Institute of Pathology)	Reference Ranges: 3rd Trimester Pregnancy (Cummings, 2010)
Absolute White Cell Counts ($10^3/\mu L$)			
White Cell count	10.71 ± 3.50	4 - 11	5.6 - 16.9
Neutrophils	8.3 ± 3.21	2.0 – 7.5	3.9 - 13.1
Lymphocyte	1.72 ± 0.58	1 - 4	1 - 3.6
Monocytes	0.70 [0.46 - 0.85]	0.18 – 0.8	0.1 1.4
Eosinophils	0.07 [0.02 - 0.15]	0 – 0.40	0 - 0.6
Basophils	0.02 [0.01 - 0.03]	0 – 0.2	0 - 0.1
Differential WCC %			
Neutrophil %	74.78 ± 9.70		
Lymphocyte %	16.95 ± 7.57		
Monocyte %	6.15 [5.20 - 8.05]	N/A	**N/A**
Eosinophils %	0.6 [0.1 - 14]		
Basophils %	0.2 [0.1 - 0.3]		
Red Cell Indices			
Red Cell Count ($10^6/\mu L$)	3.56 ± 0.69	3.8 - 5.4	2.7 - 4.43
Haemoglobin (g/dL)	10.49 ± 1.96	12 -16.9	9.5 - 15
Haematocrit (%)	31.90 [25.85 - 35.38]	37 - 44	28 - 40
MCV (fl)	87.75 ± 6.09	80 - 100	81 - 99
MCH (pg)	29.41 ± 2.33	27 - 32	29 - 32
MCHC (g/dL)	33.48 ± 1.14	32 - 36	31.9 - 35.5
Platelet Indices			
Platelet Count	169 [94 - 259]	150 - 400	146 - 429
C-Reactive Protein mg/l	23.8 [6.9 - 52.6]	0 - 10	0.4 - 8.1
Renal Function Tests			
Potassium mmol/l	3.85 ± 0.51	3.6 - 5.1	3.3 - 5.1
Sodium mmol/l	136 [133 - 144]	136 - 144	130 -148
Urea mmol/l	3.1 [2.2 - 4]	2.1 – 7.1	3 - 11
Creatinine umol/l	58 [48 - 67]	35 - 88	35 - 80
eGFR (mL/min/1.73 m^2)	725.1 ± 394.3	117-182	117 - 182
Liver Function Tests			
Protein g/l	52.08 ± 6.78	61 - 79	56 - 67
Albumin g/l	20.93 ± 5.62	36 - 51	2 - 25
Globulin g/l	30.50 [28 - 33.5]	N/A	N/A
A/G ratio	0.68 ± 0.21	N/A	N/A
Total Bilirubin umol/l	9 [4.5 - 16.5]	0 – 20.5	8.8 - 97
ALP IU/l	153.1 ± 71.4	32 - 91	38 - 299
GGT IU/l	53.5 ± 32.25	7 - 50	3 - 26
ALT IU/l	35 [13 - 51.75]	7 - 35	2 - 25
AST IU/l	46 [17.5 - 51.5]	15 - 41	4 - 32
LD IU/l	475.1 ± 227	98 - 192	82 – 524

MCV: Mean Cell Volume**; MCH:** Mean Cell Haemoglobin**; MCHC:** Mean Cell Haemoglobin Concentration**; RDW:** Red cell distribution width**; eGFR:** Modification of Diet in Renal Disease Glomerular filtration rate**; A/G ratio:** Albumin/Globulin ratio**; ALP:** Alkaline Phosphate**; GGT:** Gamma-glutamyl Transferase**; ALT:** Alanine Transferase**; AST:** Aspartate Transaminase**; LD:** Lactate Dehydrogenase**;** Results expressed as mean ± standard deviation and median interquartile range.

4.3 Haematological indices and inflammatory markers in FMH positive participants

Foetal during FMH, the foetal blood cells infiltrate the maternal circulation therefore it was imperative to assess haematological indices of FMH positive cases. Subgroup analysis was performed to compare the haematological indices in FMH positives to those in FMH negative. Red cell counts and haemoglobin levels where comparable between the two groups ($p > 0.05$) (Table 4.3, Figure 4.1) while both mean cell volume (MCV) and mean cell haemoglobin (MCH) were lower in the FMH positive group (86.10 ± 3.53, 29.00 ± 1.34) in comparison to lower MCV and MCH levels in the FMH negative groups (94.03 ± 5.56, 31.76 ± 2.07) with significant $p = 0.0043$ and $p = 0068$ respectively (Table 4.5). Haematological profiles such as WCC and platelets are also a good indicators of inflammation (Sproston and Ashworth, 2018). Therefore inflammation in FMH positive and negative groups was assesed and compared using WBC, platelet and CRP values. White cell count levels were significantly elevated in patients with FMH positive (11.66 ± 3.99) when compared to FMH negative (10.11 ± 2.74) (Table 4.3, Figure 4.1). The differences in WCC between the two groups were found to be significant ($p = 0.0143$) (Table 4.3, Figure 4.2). Similarly, lymphocyte levels were also different between the two groups ($p = 0.0237$) (Table 4.3). Neutrophils and CRP had comparable values in both groups with a p value exceeding 0.05 in each (Table 4.3, Figure 4.1).

Table 4.3 Haematological indices of FHM negative and FMH positive patients (n=30)

Parameter	FMH Negative (n=15)	FMH Positive (n=15)	P Value
Absolute WCC ($x10^9$/l)			
White Cell count	10.11 ± 2.74	11.66 ± 3.99	**0.0143**
Neutrophil	7.02 ±3.92	7.49 ± 2.58	0.8876
Lymphocyte	1.19 ± 0.73	1.88 ± 0.50	**0.0237**
Monocyte	0.69 ± 0.26	0.69 ± 0.15	0.9590
Eosinophils	0.09 ± 0.07	0.18 ± 0.15	0.6420
Basophils	0.10 ± 0.69	0.18 ± 0.15	0.1409
Differential White Cell Counts (%)			
Neutrophils	73.60 ± 8.40	73.35 ± 8.84	0.9545
Lymphocyte	16.81 ± 3.10	16.79 ± 7.92	0.9935
Monocytes	6.64 ± 1.42	6.88 ± 1.38	0.6206
Eosinophils	0.25 [0.10-1.00]	1.10 [0.03-2.60]	0.2231
Basophils	0.21 ± 0.14	0.21 ± 0.17	0.9999
Red Cell Indices			
Red Cell Count (10^6/µL)			
Haemoglobin (g/dl)	3.50 ± 0.53	3.38 ± 0.64	0.6780
Haematocrit (%)	10.37 ± 1.33	9.93 ± 1.80	0.5702
Mean cell Volume (fl)	30.06 ± 494	27.24 ± 4.13	0.2699
MCH (pg)	94.03 ± 5.56	86.10 ± 3.53	**0.0043**
MCHC (g/dl)	31.76 ± 2.07	29.00 ± 1.34	**0.0068**
RDW (%)	33.66 ± 1.31	33.73 ± 1.50	0.9266
	13.61 ± 1.060	14.06 ± 1.18	0.4357
Platelet Indices			
Platelet Count			
	160 ± 72.18	136 ± 69.69	0.4872
Inflammatory profiles			
C-Reactive Protein (mg/l)			
	24.68 ± 20.03	44.50 ± 30.22	0.2102

MCV: Mean Cell Volume**; MCH:** Mean Cell Haemoglobin**; MCHC:** Mean Cell Haemoglobin Concentration**; RDW:** Red cell distribution width

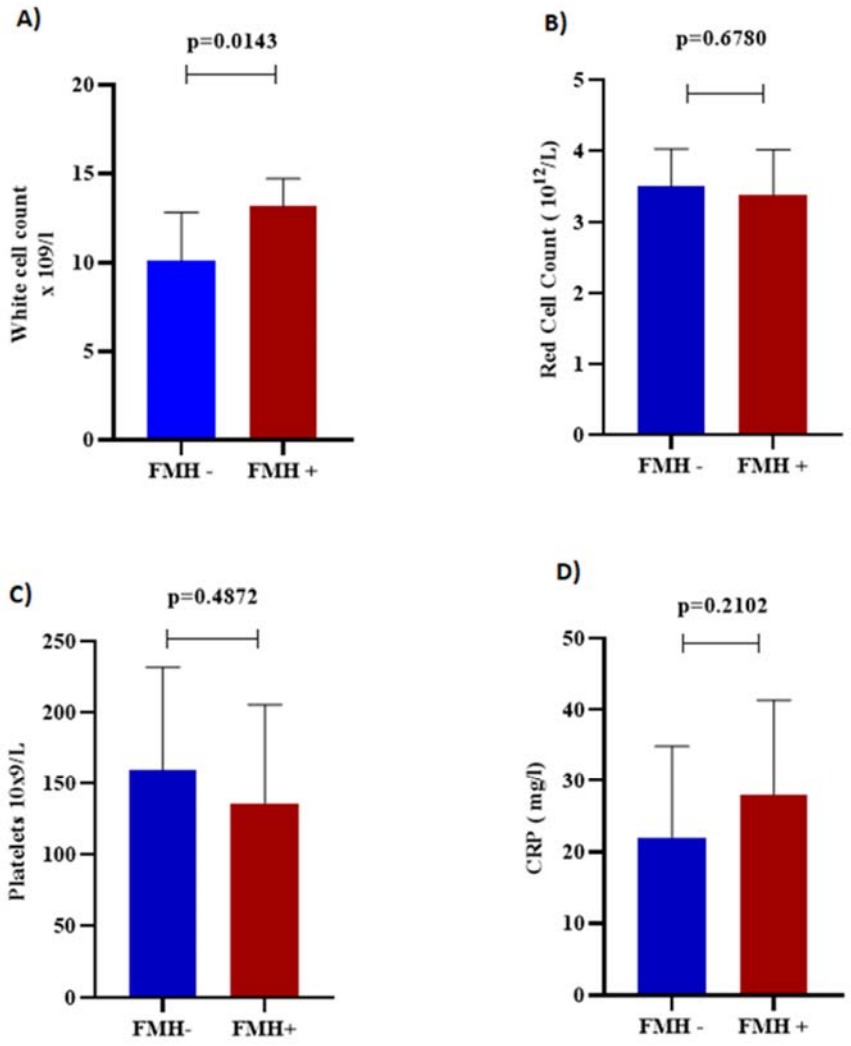

Figure 4.1 A comparison of haematological indices and inflammatory markers between FMH positive and negative patients. Figure (A) highlights significant differences in WBC values, while (B), (C), (D) Show comparable values of Red Cell Count, Platelets and C-Reactive Protein (CRP), All results are expressed as mean ± standard deviation.

4.4 Renal function in FMH

Pregnancy alters renal function due to changes in hormal releases (Hussein and Lafayette, 2014) and the compuding effect of FMH on renal function is unknown. Therefore, renal function test were compared between FMH postive and negative patients. Notably, Potassium levels were shown to be comparable inbetween the two groups (p = 0.4919) (Table 4.4, Figure 4.2) while a slight significance was demonstrated in sodium levels between the two groups (p = 0.0488) (Table 4.4, Figure 4.2). Urea

and creatinine levels where significantly more elevated in the FMH positive group with mean values of (4.99 ± 1.86, 70.33 ± 17.10) as compared to the (2.711 ± 1.07, 51.78 ± 8.41) in the FMH negative group (Table 4.4, Figure 4.2). As expected, the Glomerular Filtration Rate in the FMH group was lower (112.4 ± 37.91) than the rate of the FMH negative group (151.9 ± 24.90) (Table 4.4, Figure 4.2). The rates in the two-group proved to be significantly different when statistically compared (p = 0.0477) (Table 4.4, Figure 4.2).

Table 4.4 Renal and liver function profiles of FMH negative and positive patients (n=30)

Parameter	FMH Negative (n=15)	FMH Positive (n=15)	P Value
Renal Function			
Potassium (mmol/l)	3.67 ± 0.41	3.87 ± 0.75	0.4919
Sodium (mmol/l)	137.4 ± 3.59	133.3 ± 4.03	**0.0488**
Urea (mmol/l)	2.711 ± 1.07	4.99 ± 1.86	**0.0058**
Creatinine (umol/l)	51.78 ± 8.41	70.33 ± 17.10	**0.0100**
eGFR (mL/min/1.73 m^2)	151.9 ± 24.90	112.4 ± 37.91	**0.0401**
Liver Function (IU/l)			
ALT			
AST	20.57 ± 13.84	35.57 ± 13.69	0.0641
	25.33 ± 10.98	53.17 ± 29.04	**0.0477**

eGFR: Glomerular Filtration Rate **ALT:** Alanine Transferase**; AST:** Aspartate Transaminase

4.5 Hepatic function in FMH

No significant differences were found in ALT levels (p = 0.067) however, AST levels where much higher in FMH positive (53.17 ± 29.04) participants as when compared to FMH negative participants (25.33 ± 10.98) (Table 4.4, Figure 4.2).

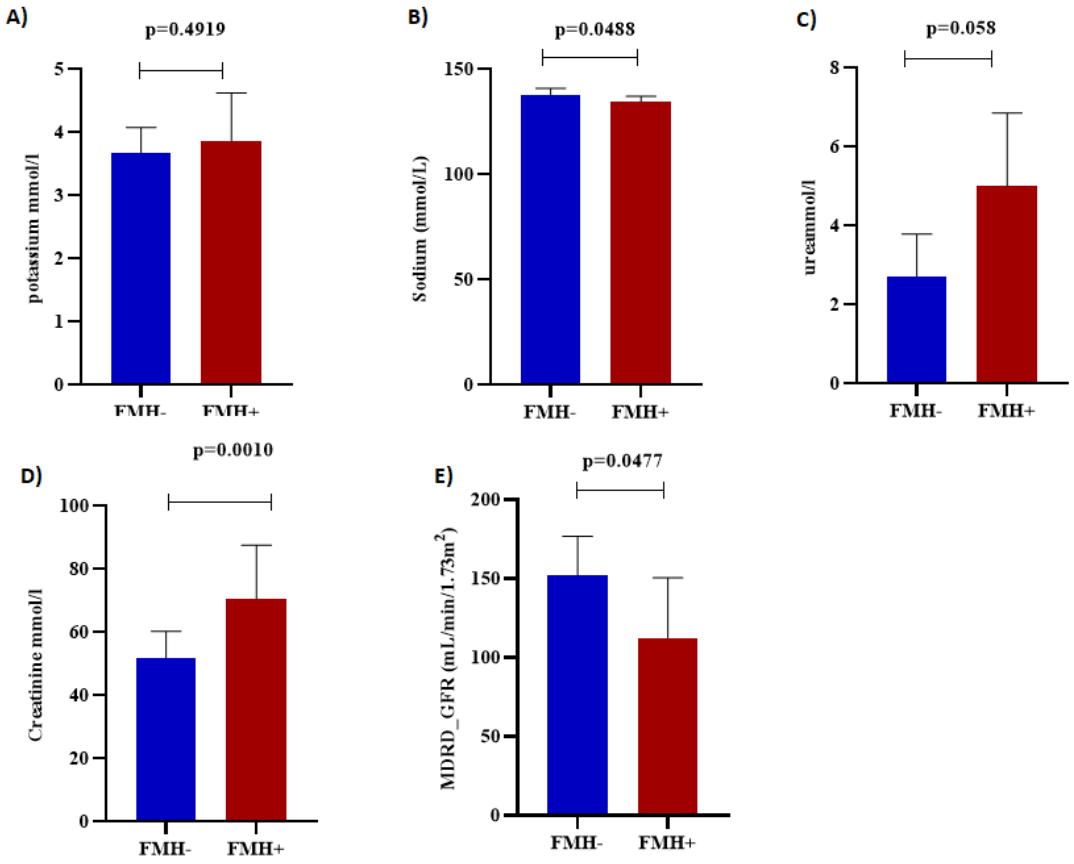

Figure 4.2 A comparison of renal function between FMH positive and negative patients. Figure (A) shows comparable potassium levels whilst (B), (C), (D) and E highlight significant differences in the levels of sodium, urea, creatinine and eGFR levels, respectively. All results are expressed as mean ± standard deviation.

4.6 Correlations haematological, renal, and hepatic profiles in FMH positive

Multiple variable correlation analysis was done to determine any notable associations between the measured parameters. Notably MCV levels were associated to eGFR (r=0.78, p = 0.037). A similar association was also noted between MCH and eGFR levels (r=0.80, p=0.027). Urea levels also correlated with eGFR levels however negatively (r=-0.87, p=0.01). A significant association of both urea and eGFR to AST was noted (<0.05) however urea correlated positively (r=0.97) to AST while eGFR correlated negatively (r=-0.82). No associations were demonstrated in levels of WCC, lymphocytes and sodium to the other parameters (p<0.05).

CHAPTER FIVE

DISCUSSION

Stillbirth rates in Sub-Saharan Africa make up more than two thirds of global stillbirth but remains poorly understood (Bhutta *et al.*, 2020). This was study aimed at investigating foetal maternal haemorrhage (FMH) in stillbirths and to determine whether it is associated with alloimmunisation, maternal inflammation and altered maternal hepatorenal function. Notably, FMH was detected in approximately a quarter of the included participants. This prevalence is higher than the 10-22% detected in other developing African countries (Adeniji *et al.*, 2008; Akorsu *et al.*, 2019). The study further quantified the FMH to determine whether it was massive according to published thresholds. A total volume of at least thirty millilitres (ml) is characterised as a massive haemorrhage and is associated with serious perinatal and neonatal mortality and morbidity outcomes (Stefanovic, 2016). In this study, only a total of 6.7 % of the positive cases were categorised as massive FMH. The prevalence of alloimmunisation in stillbirth mothers in Windhoek is 3.3 %, this is higher than the 1.15% prevalence of alloimmunisation reported in women of childbearing ages (Webb and Delaney, 2018). The antibody specificity for the alloimmunised mothers found in this study (3.3%), was detected as an anti-E.

Alloimmunisation is one of the major consequences of FMH with only as little as 0.1ml bleed is necessary to evoke maternal immune system to build up red cell antibodies (Delaney, Svensson and Lieberman, 2017). A previous study demonstrated the increased odds (1.53-3.98) of stillbirth and preterm birth in red cell immunised mothers (Fan *et al.*, 2014). The anti-E antibody identified amongst participants in this study, is immunoglobulin (Ig) G in nature and can cross the placenta causing severe haemolytic disease of the foetus and new-born (HDFN) leading to severe foetal and neonatal outcomes (Fan *et al.*, 2014). The HDFN is characterised by haemolysis of foetal red cells by IgG antibodies that leads to foetal anaemia, high levels of bilirubin and hydrops foetalis (Haas *et al.*, 2015). Although anti-D is the most prevalent antibody globally (Pegoraro *et al.*, 2020), anti-E is the most prevalent antibody amongst pregnant women in Sub-Saharan Africa (Ngoma *et al.*, 2016). The Rh negative status of some participants in this study predisposes them to build up anti-D should they be exposed to paternal Rh D antigens by the foetal cells during even undetectable levels of FMH following a potentially sensitising events (McEwan, 2019).

Trauma to the abdomen such as abruptio, blunt trauma, cordocentesis, amniocentesis and inflammatory conditions pre-eclampsia, chorioamnionitis and placental tumours have been identified as potentially sensitising to FMH (McEwan, 2019). These events can worsen the inflammatory state in pregnancy which is on its own already physiologically considered as an inflammatory condition(Maguire *et al.*, 2015; Raio *et al.*, 2019). C-reactive protein (CRP) and white cell count (WCC) are reliable markers in systemic inflammation (Sproston and Ashworth, 2018) (Farhangi *et al.*, 2013). Notably, high levels of CRP and WCC were associated with FMH. This means inflammation is associated with the occurrence of FMH. Previous studies have associated placental inflammation to FMH after finding elevated serum mucin 1 (MUC1) levels in FMH positive patients (Scholz *et al.*, 2012). This should be expected as the placenta is central in the pathophysiology of FMH. This explains the higher levels of lymphocytes and in turn WCC found in the FMH positive group. Inflammation modulates the synthesis of hepcidin which inhibits release of iron from intestines and macrophages, iron absorption and subsequent transportation of iron to the bone marrow for formation of red blood cells (Ndevahoma). The latter explains the pathogenesis of anaemia of inflammation.

Anaemia of inflammation is characterised by microcytic hypochromic red cells (Ganz, 2019).The overall population in this study had slightly a lower haemoglobin and red blood cell count (RBC) than expected normal values indicating general anaemia which is expected in pregnancy due to increased demand (Stephen *et al.*, 2018). The levels of haemoglobin and red cell counts were comparable between the FMH positive and negative groups although the MCV and MCH levels were much lower in the FMH positive group indicating exacerbated state of anaemia. The worsening anaemia in this group could be attributed the inflammatory nature FMH sensitising events. In addition to altered iron metabolism, Inflammation inhibits the synthesis of erythropoietin (EPO) and ultimately erythropoiesis (Nielsen *et al.*, 2018). Another important function of EPO together with the erythroid transcriptional factor (GATA1), is to regulate the synthesis of haem which is an important component of haemoglobin (Chung *et al.*, 2017). Erythropoietin is mainly synthesised in the kidneys; this explains the strong association between the MCV and MCH to the renal markers of women who tested positive for FMH in this study.

Apart from an indication of anaemia, the correlation of red cell indices to renal markers in the FMH positive group, confirms conclusions of previous studies that linked red cell indices such as MCV and haemoglobin as a predictor of renal dysfunction (Solak *et al.*, no date; Elsayed and Azab, 2017). Renal function is generally abnormal in pregnancy but can be severe in some instances (Hussein and Lafayette, 2014). The association between FMH and maternal renal function is however unknown. This study found altered renal function in women with FMH. Although the renal function tests (glomerular filtration rate (eGFR) sodium, urea, creatinine) were within normal expected limits, they

were significantly different in women with positive FMH in comparison to those that tested negative for FMH. The eGFR measures how well the kidneys filters waste and excess fluids and is a reliable marker for assessing renal function (Jamshidi *et al.*, 2020). Sodium on the other hand is an electrolyte and abnormal levels could indicate renal impairment (Cole *et al.*, 2019). Both sodium and the eGFR were lower in the FMH positive group while urea and creatine were higher. Urea and creatinine are waste products of the kidneys, and both have an inverse relationship to eGFR (Higgins, 2016). These analytes are increased with a low eGFR and was similarly so in this study as indicated by the negative correlation detected between urea and eGFR. Both urea and creatinine are by products of protein metabolism (Salazar, 2014) and elevation in FMH indicating altered renal function in this group.

Amongst the liver enzymes, AST proved to be significantly higher in the FMH positive group and had a strong positive correlation with urea levels. Conclusion whether this is attributed to hepatic function could not be made as evaluation of liver function based on AST alone is not advised, furthermore this enzyme is also released from other organs such as muscles, heart, kidneys, pancreases making it non-specific to hepatic function at times (Xu, Higgins and Cembrowski, 2015). An association between AST and low haemoglobin levels was however determined in a study that found higher AST levels in anaemic patients (Nsiah *et al.*, 2011). The general population in this study manifested with elevated liver enzymes suggesting possible cholestasis which is common pregnancy (Guarino, Cossiga and Morisco, 2020). Intrahepatic cholestatic of pregnancy (ICP), is a common manifestation of late (2^{nd}/3^{rd} trimester) pregnancy, characterized by itchy skin and elevated liver enzyme but resolve well and fast after delivery (Ozkan *et al.*, 2015). Normal values of albumin and globulins suggest normal synthetic hepatic function (Guarino, Cossiga and Morisco, 2020)

As with any other study, this study had a few limitations. Firstly, a major limitation was that we could not meet sample size due to interruptions in sample collection. Secondly, although the world health organization (WHO) recommendations for Rh and antibody screening as part of antenatal management of pregnant women (WHO, 2016), the majority of antibody screens were either not done or recorded. These challenges contribute to the findings of poor data management and poor-quality antenatal care as a challenge to reducing the occurrence of stillbirths in Sub-Saharan Africa (Joy E. Lawn *et al.*, 2016; Aminu, Bar-zeev and Broek, 2017). Future studies should include a bigger sample size and consider investigating possible causes and risk factors of FMH such as abdominal trauma.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

The occurrence of foetal maternal haemorrhage in pregnancy is a reality with volumes of FMH in this study exceeding 30ml having previously been associated to increased morbidity and mortality of foetus and neonates which could not be established in this study due to sampling limitations, while bleeds as low as 0.1ml have the potential of immunising the mother in building up allo-antibodies which could be detrimental to subsequent pregnancies. There is a gap Systemic maternal response to FMH with majority of previous studies only focusing on foetal and neonatal outcomes. This study positively contributes to that gap by associating FMH to maternal inflammation and altered renal function. Inflammation contributes to the deficiency of iron which may worsen pre-existing gestational anaemia in pregnant women. Availing simple FMH screening and diagnostic methods such as the Keilhauer Bekte (KB) test used in this study, will be a game changer in antenatal care and management.

This can lead lifesaving interventions such as intrauterine transfusions to alleviate foetal anaemia and administration of anti-D immunoglobulin (RhIG) to prevent anti-D alloimmunisation. Antibody screening and identification during antenatal care should be taken seriously to prevent and manage the occurrence of Haemolytic Disease of Foetus and New-born (HDFN) amongst alloimmunised mothers. After a potentially sensitising event, mother can be given ant-inflammatory drugs to alleviate inflammation that in turns also prevents development of anaemia of inflammation in these mothers. In addition, renal function should be closely monitored.

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Appendices

Appendix 1: Namibia University of Science and Technology ethical clearance

NAMIBIA UNIVERSITY OF SCIENCE AND TECHNOLOGY
13 Storch Street
Private Bag 13388
Windhoek
NAMIBIA
T: +264 61 207 911
F: +264 61 207 2444
W: www.nust.na

FACULTY OF HEALTH AND APPLIED SCIENCES RESEARCH ETHICS COMMITTEE (FHAS-REC)

DECISION/FEEDBACK ON RESEARCH PROPOSAL ETHICAL CLEARANCE

Dear: Prof/Dr/Mr/Ms/Other	Edwig Hauwanga Student Number: 200867491
Research Topic:	Assessing foetal maternal haemorrhage in Stillbirths at Windhoek Central Hospital, Namibia
Supervisor (if applicable):	Dr Yapo Aboua
Co-supervisor(s): if applicable	Dr Martin Gonzo
Qualification registered for (if applicable):	Master of Health Sciences

Re: Ethical Screening Application No: FHAS 19/2018

The Faculty of Health and Applied Sciences Research Ethics Screening Committee has reviewed your application for the above-mentioned research project. Based on the recommendation of the expert reviewer, the research as set out in the application is hereby:

(Indicate with an X)

Approved: i.e. may proceed with the project, subject to Ministry of Health and Social Service Clearance.	X
Approved provisionally: i.e. may proceed but subject to compliance with recommendation(s) listed below	
Not approved: Not to proceed with the project until compliance with recommendation listed below and resubmit ethics application for consideration	

It is important to note that as a researcher, you are expected to maintain ethical integrity of your research. You are encouraged to strictly adhere to the research ethics policy of NUST. You should remain within the scope of your research proposal and support evidence as submitted to the FHAS-REC. Should any aspect of your research change from the information as presented, which could have an impact or effect on any research participants/subjects/environment, you are to report this immediately to your supervisor and to the FHAS-REC as applicable in writing. Failure to do so may result in withdrawal of approval.

Kindly consult the committee if you need further clarification in this regard. We wish you success in your research endeavour and are of the belief that it will have positive impact on your career as well as the development of NUST and the society in general.

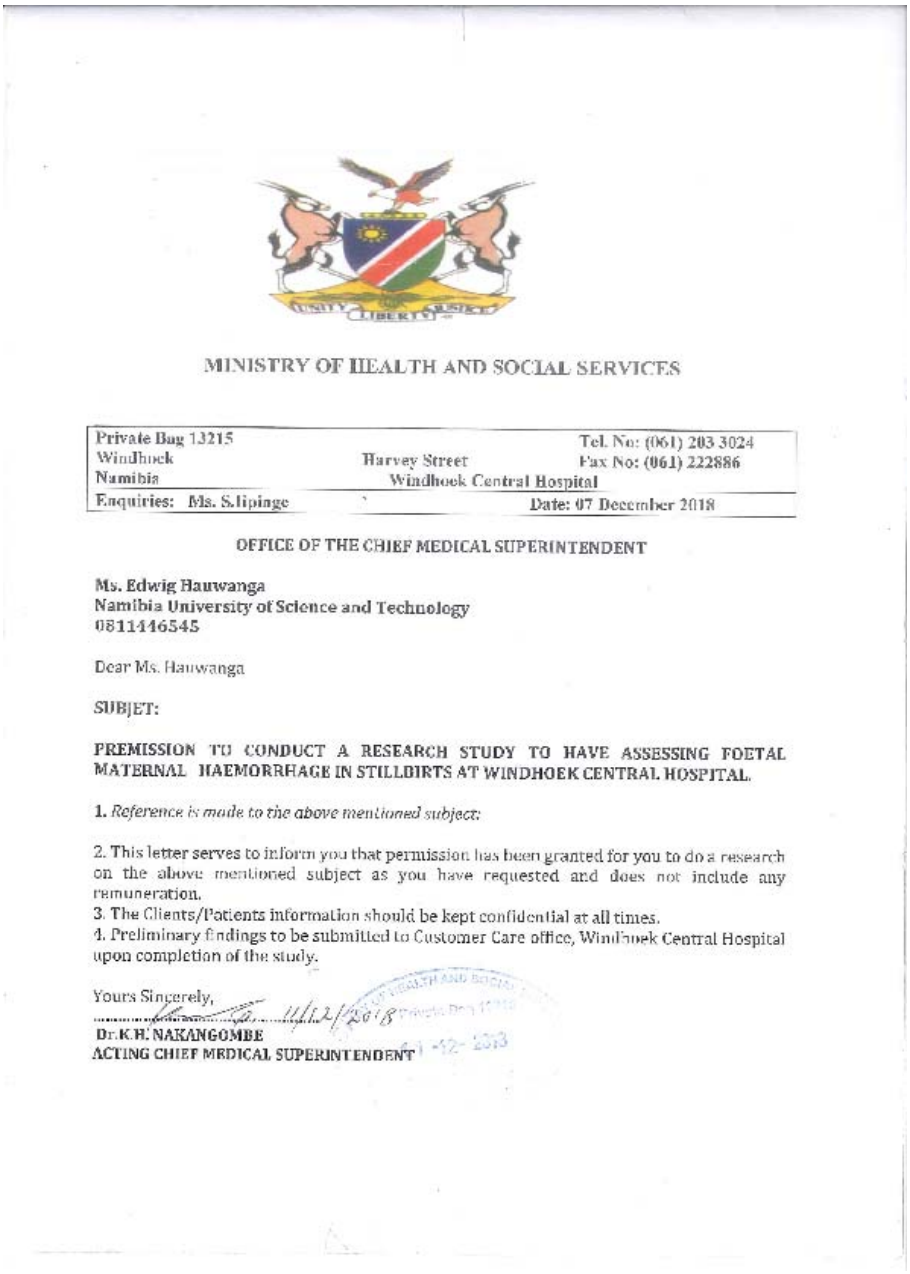
Ethical issues that require compliance/ must be addressed : None		
No.	**Ethical issues**	**Comment/recommendation**
		No ethical issues noted in this proposal.

NB: May attach additional page as required

Sincerely Yours,

Name: Prof Sylvester R Moyo **Signature:** **Date:** 4th September 2018
Chairperson: FHAS Ethics Screening Committee.

Appendix 2: Ethics clearance Ministry of Health and Social Services



MINISTRY OF HEALTH AND SOCIAL SERVICES

Private Bag 13215		Tel. No: (061) 203 3024
Windhoek	Harvey Street	Fax No: (061) 222886
Namibia	Windhoek Central Hospital	
Enquiries: Ms. S.Iipinge		Date: 07 December 2018

OFFICE OF THE CHIEF MEDICAL SUPERINTENDENT

Ms. Edwig Hauwanga
Namibia University of Science and Technology
0811446545

Dear Ms. Hauwanga

SUBJET:

PREMISSION TO CONDUCT A RESEARCH STUDY TO HAVE ASSESSING FOETAL MATERNAL HAEMORRHAGE IN STILLDIRTS AT WINDHOEK CENTRAL HOSPITAL.

1. *Reference is made to the above mentioned subject:*

2. This letter serves to inform you that permission has been granted for you to do a research on the above mentioned subject as you have requested and does not include any remuneration.
3. The Clients/Patients information should be kept confidential at all times.
4. Preliminary findings to be submitted to Customer Care office, Windhoek Central Hospital upon completion of the study.

Yours Sincerely,
11/12/2018
Dr.K.H. NAKANGOMBE
ACTING CHIEF MEDICAL SUPERINTENDENT

Appendix 3: Namibia Blood Transfusion Service Ethical Clearance

THE BLOOD TRANSFUSION SERVICE OF NAMIBIA AN AFFILIATION OF VOLUNTARY BLOOD DONORS NAMIBIA LTD) FORMERLY
Tel: +264 (0)61 236300 Fax: +264 (0)61 220834 / 366301 PO Box 3434 Windhoek 55 Tal Street, Windhoek

www.bts.com.na

29 July 2021

Dr Yapo Aboua
Senior Lecturer
Health Science, NUST
Namibia

Cc. Ms Edwig Hauwanga

REF: Request to access antenatal test results data stored in NAMBTS database.

The above subject refers:

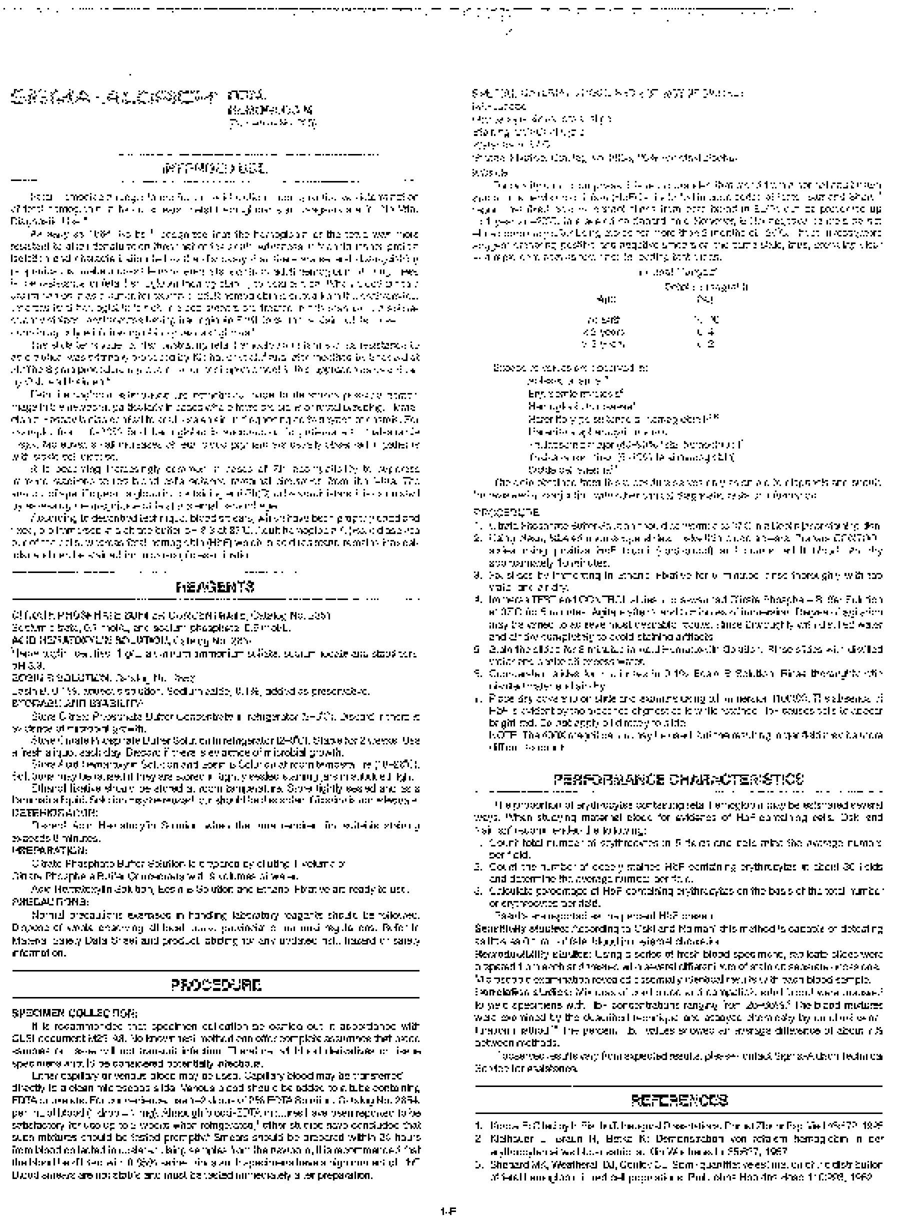
Permission is hereby granted to Ms Edwig Hauwanga and NUST lecturers to access antenatal results data from NAMBTS electronic database and to the data in addressing the objectives of a research study titled **"Assessing foetal maternal haemorrhage in stillbirths at the Windhoek state hospitals, Namibia"**. Please note that the data is to be used for this research project only, a new approval will be required for a different research.

Hoping this is in order.

Kind regards,

Mr. Israel Chipare
Chief Operations Officer
Blood Transfusion Service of Namibia

Appendix 4: Sigma Adlrich Foetal Haemoglobin kit


REAGENTS

PROCEDURE

PERFORMANCE CHARACTERISTICS

REFERENCES