KELL BLOOD GROUP DISTRIBUTION AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE IN CENTRAL HOSPITAL, BENIN CITY, EDO STATE, NIGERIA

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CERTIFICATION

This is to certify that this project was carried out by **(MISS) JESSICA OSARUGUE IGBINOSA** with matriculation number BMS2001164 and submitted to the Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City, in partial fulfilment of the requirement for the award of Bachelor of Medical Laboratory Science (BMLS) degree.

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External Examiner DATE

DEDICATION

This project is dedicated to God, for His wisdom, grace and strength to accomplish this. I would also like to dedicate this piece to pioneer undergraduate researchers in Edo State. Long live the heartbeat of Nigeria!

ACKNOWLEDGMENT

Dear Abba, I just want to say thank you. The one who calls me His beloved has kept to His promises. Thanks be to God Almighty for taking me through every season in this phase of life. His enormous mercies, grace, strength and divine provisions has brought me this far.

My immense gratitude goes to my parents - Mr ANDREW and MRS FAITH IGBINOSA. Daddy, Thank you for supporting me through school. You've been a great pillar of support through every season.

Mummy, we did it. Thank you for listening to my academic rants and worries. For always cheering me up and constantly supplying me with good food. You went the extra mile and I'm super grateful.

To my siblings— Dominica and Eric. You both are one of the reasons why I never settle for less. The fact you are both watching me is a great inspiration for me to keep pushing. I love you both.

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ABSTRACT

The Kell blood group system is one of the most clinically significant after ABO and Rhesus. Antibodies to Kell antigens are highly immunogenic and can cause haemolytic transfusion reactions and haemolytic disease of the fetus and newborn (HDFN). Despite its clinical importance, data on the distribution of Kell antigen among Nigerian pregnant women remain limited. This study aimed to determine the prevalence and distribution of the Kell blood group among pregnant women attending antenatal care at Central Hospital, Benin City, Edo State. A descriptive cross-sectional study was conducted among 100 pregnant women aged 18–43 years attending antenatal clinic at Central Hospital, Benin City. Blood samples were collected and tested for Kell antigen using the conventional tube method with commercially prepared anti-Kell reagents. Socio-demographic and obstetric data were obtained through structured questionnaires.The gestational age of the women in their first trimester were fourty-two(42), second trimester, fifty (50) and third trimester, eight(8).Of the 100 women studied, 2 (2.0%) were positive for the Kell antigen who were in their first and second trimester respectively. While 98 (98.0%) were negative.The Kell-positive phenotype was observed across both primigravidae and multigravidae with no statistically significant difference. The overall prevalence of Kell antigen in this cohort was consistent with reports from other populations in Nigeria and across Africa but considerably lower than values reported among Caucasians.

In conclusion, the study demonstrated a low prevalence (2.0%) of Kell antigen among pregnant women in Central Hospital. Given the clinical significance of Kell antibodies in HDFN and transfusion reactions, routine screening of pregnant women for Kell antigen and the provision of Kell-negative blood for women of childbearing potential is strongly recommended to enhance safe obstetric and transfusion practices.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Blood group antigens are markers of the external surface of the red blood cell (RBC) membrane. They are proteins or carbohydrates attached to lipids or protein. The Kell blood group is numbered 006 according to the International Society of Blood Transfusion. It is also ranked as the 3rd most immunogenic antigen after the ABO and Rhesus blood groups. There are 30 antigens in the Kell blood group with K and k being the most clinically encountered in the laboratory (Armstrong, 2008).

1.2 OVERVIEW OF THE KELL BLOOD GROUP SYSTEM

1.2.1 History

In 1946, Coombe, Mourant and Race discovered a strange antibody in Mrs Kelleher who had been delivered of a child suffering from haemolytic disease. This antibody was of the incomplete type and observed to have reacted with the red cells of Mr Kell, their other two children and 7% of some random blood groups. The corresponding antigen was named K (KEL1) which is denoted as the Kell factor and symbol of the blood group. Further studies were performed and this made Mourant to conclude that the inheritance of the Kell factor follows the Mendelian dominance pattern. Kell is determined by two allelomorphic genes K and k. The K antigen is demonstrated with anti-K in homozygotes (KK) and heterozygotes (Kk) (Dean, 2005). Levine *et al.*,(1949) discovered another antibody which took 2 years of studies to decipher its synthesis for existence. This antibody was present in the serum of a Mrs Cellano who had been delivered to an infant who suffered from a mild haemolytic disease in 1947.The corresponding antigen was named k/KEL 2

1.2.2 Clinical significance

- Haemolytic Disease of the Foetus and Newborn
- Transfusion Reaction

The Kell blood group has been investigated to be one of the leading causes of Haemolytic Disease of the Foetus and Newborn (HDFN). Studies revealed that anti-K is capable of crossing the transplacental barrier causing transplacental haemorrhage (TPH) associated with Childbirth (Serekara *et al.,* 2025*)*. It is known to be a cause of transfusion reaction due to Kell alloantibodies from a Kell positive individual.

1.3 STATEMENT OF PROBLEM

Despite its clinical relevance, limited data exist on the distribution of Kell blood group antigens in various populations, especially among pregnant women in Edo State. Establishing baseline data in Central Hospital, Benin City, will provide insight into Kell antigen prevalence and assist in improving maternal and neonatal care for the prevention of death-in-uterine cases as well as HDFN in Edo State.

1.4 JUSTIFICATION OF STUDY

The Kell blood group system is clinically significant in transfusion medicine and obstetrics due to its association with haemolytic disease of the fetus and newborn (HDFN) and transfusion reactions. Despite its importance, routine blood group testing in Nigeria and many other developing countries often focuses only on the ABO and Rh systems, leaving out the Kell system. This oversight can result in preventable complications during pregnancy and blood transfusion. However, there is a lack of local data on the prevalence and distribution of the Kell blood group among pregnant women in Nigeria, particularly in

Central Hospital, Benin City. This study will fill that gap by generating baseline data that can inform hospital policies, transfusion protocols, and antenatal screening programs. Therefore, the study is justified as it addresses a critical knowledge gap, promotes safe motherhood, and supports the integration of extended blood group screening in antenatal care practices.

1.5 AIM OF STUDY

The aim of this study is to determine the distribution of the Kell Blood Group System amongst pregnant women attending antenatal care in Central Hospital.

1.6 SPECIFIC OBJECTIVES

1. To determine the frequency of Kell-positive and Kell-negative phenotypes among the study population.
2. To evaluate the association between Kell status and demographic variables such as age, parity, and gravidity.
3. To raise awareness of the Kell blood group system in maternal health management.

1.7 RESEARCH QUESTIONS

1. What is the prevalence of Kell-positive blood group among pregnant women in Central Hospital, Benin City?
2. Is there any significant association between Kell antigen presence and demographic factors?
3. What is the statistical awareness of pregnant women about the Kell blood group?

1.8 RESEARCH HYPOTHESIS

Null Hypothesis: There is no significant prevalence of Kell blood group among pregnant women attending antenatal care in Central Hospital.

Alternate Hypothesis: There is a significant prevalence of Kell blood group among pregnant women attending antenatal care in Central Hospital

1.9 SIGNIFICANCE OF STUDY

1. Supports improved antenatal screening protocols.
2. Contributes to the prevention of HDFN.
3. Enhances the database of blood group antigen frequencies in Nigeria.
4. Guides blood bank practices in prenatal and transfusion medicine.

CHAPTER TWO

LITERATURE REVIEW

2.1 BLOOD GROUP SYSTEMS

The term “blood group” refers to the entire blood group system comprising red blood cell (RBC) antigens whose specificity is controlled by a series of genes which can be allelic or linked very closely on the same chromosome. “Blood type” refers to a specific pattern of reaction to testing antisera within a given system.(Rath *et al.,* 2014)

2.1.1 ABO Blood Group System

In 1900, Karl Landsteiner discovered the ABO blood group system. His extensive research on serology based on simple but strong scientific reasoning led to identification of major blood groups such as O, A, and B types, compatibility testing, and subsequent transfusion practices. There are about thirty- three blood group systems representing over 300 antigens listed by the International Society of Blood Transfusion.The genes of these blood group systems are autosomal, except XG and XK which are X-borne, and MIC2 which is present on both X and Y chromosomes. The antigens can be integral proteins where polymorphisms lie in the variation of amino acid sequence (e.g. Rhesus, Kell), glycoproteins or glycolipids (e.g. ABO. (Rath *et al.,* 2014*).*

2.1.2 Rhesus Blood Group System

The Rhesus blood group system is the most polymorphic of the human blood groups, consisting of at least 45 independent antigens. It is next to ABO and is the most clinically significant in transfusion medicine. The common Rh antigens: D, C or c, and E or E controlled by RHD gene for D allele and RHCc, RHEe gene for allele C, c and E, e respectively (Avent and Reid, 2000).

Name	Symbol	Number of antigens	Gene name	Chromosome
ABO	ABO	4	ABO	9
MNS	MNS	43	GYPA, GYPB, GYPE	4
P	P1	1	P1	22
Rhesus	Rh	49	RhD, RhCE	1
Lutheran	LU	20	LU	19
Kell	KEL	25	KEL	7
Lewis	LE	6	FUT3	19
Duffy	FY	6	FY	1
Kidd	Jk	3	SLC14A1	18

Fig 1: Showing Blood Group system (Rath *et al.,* 2025).

2.2 KELL BLOOD GROUP SYSTEM

2.2.1 Genetics and Molecular Basis

The Kell system is highly polymorphic, consisting of 38 different blood group antigens. The Kell antigens are found on erythroid cells and progenitor myeloid cells and are also present in skeletal muscles and testes.The Kell antigens are located on a single red cell transmembrane glycoprotein, encoded by the 19 exons of the KEL gene..The KEL gene encodes the Kell antigens and is located at chromosome 7q34, comprising 20 exons spanning 21.25 kb of genomic DNA. This gene is also known as Kell metallo-endopeptidase, ECE3, or CD238. Single nucleotide polymorphisms are responsible for multiple Kell antigens. Another important XK gene required for the expression of Kell antigen is present on the short arm of chromosome X (Xp21.1). This XK gene is also responsible for the expression of the Kx antigen. (Yu *et al.,* 2001) The XK gene responsible for the expression of Kell glycoprotein was cloned in 1991. Lee and coworkers used a short oligonucleotide probe deduced from a tryptic peptide of the proposed glycoprotein to screen a λgt cDNA library. Later in 1995, Lee reported that KEL was organized into 19 exons and spanned approximately 21.5 kilobasepair(Zelinski *et al*.,

1991),(Lee *et al*., 1995). The distinguishing feature of KEL is that it is predicted to be a type II single transmembrane spanning protein; the N-terminal is on the cytoplasmic side of the plasma membrane. The metalloendopeptide studies were performed on the basis of sequence homology with neutral endopeptides and the fact that the positions of many of the cysteines are conserved.(Lee *et al*., 1991),(Lee *et al.,* 2003). Lee determined the molecular basis of KEL1/KEL2 and, with that publication, the ability to predict fetal inheritance of KEL1 and haemolytic disease using amniotic fluid–derived DNA.

Structure of The Kell Glycoprotein

The KEL gene encodes the polymorphic Kell and para-Kell glycoproteins, structurally single-pass RBC membrane proteins, or type II glycoproteins. The N terminal is intracytoplasmic, and the C terminal is multi-folded, bound by disulfide bonds, and extracytoplasmic. They constitute around 732 amino acids; mutations in these lead to the formation of a multitude of Kell antigens (Mohandas and Narla, 2005). The Kell glycoprotein is covalently linked to the Kx protein via a single disulfide bond. This Kx antigen protein traverses the RBC membrane 10 times. The absence of Kx protein leads to McLeod syndrome. The Kell glycoproteins have been found to have a similar sequence as the neprilysin (M13) family of zinc endopeptidases and, hence, act like proteolytic enzymes. They share a pentameric sequence HEXXH, which is needed to add zinc and proteolytic activity. Kell preferentially cleaves big endothelin-3, converting it to the bioactive peptide endothelin-3. This potent vasoconstrictor peptide leads to vascular endothelial growth factor formation (Bland *et al.,* 2008),(Lee *et al.,* 2003).

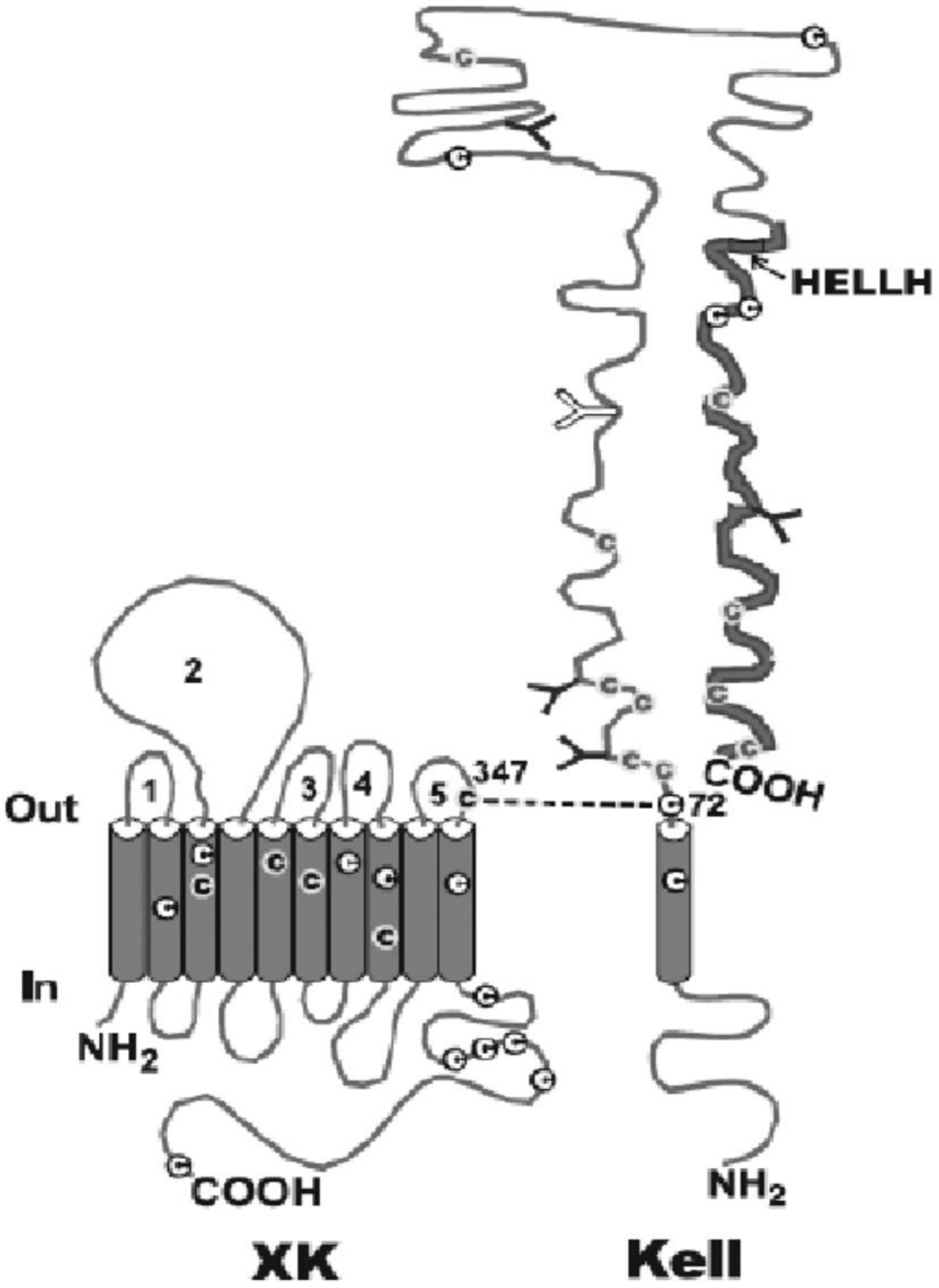


Fig 2.2:Schematic diagram showing the Kell–XK protein complex(Denomme,2015).

2.2.2 Kell Antigens

There are two major codominant allelic genes that produce K and k antigens (known as Kell and Cellano, respectively) that are different by a single amino acid. The k antigen is more common than the K antigen in most populations, as the K-k+ phenotype is 98% for blacks but 91% for Caucasians.However, the K antigen is the most significant in transfusion medicine and hemolytic disease of the newborn. (Imoru et al., 2025)

KEL 1

The K antigen appears on fetal red blood cells by the 10th week of gestation and is fully developed at birth. It is highly immunogenic, second only to the Rh system, with about 10% of Kell-negative individuals developing anti-K antibodies after exposure to Kell-positive blood.

KEL 2

k is formerly termed as Cellano. It is the antithetical antigen of KEL1 with a high prevalence. KEL 2 is expressed in cord RBCs and can be detected as early as seven weeks of gestation. Resistance and sensitivity to enzymes and chemicals are the same as those of KEL1

KEL 3

Kpa antigen with ISBT number: 006.003

It is found at low frequency.

Antithetical antigens: Kpb (KEL4), Kpc (KEL21)

Present on cord red blood cells and unaffected by enzyme treatment (ficin, papain, chymotrypsin)

KEL 4

Kpb antigen with ISBT number: 006.004.

It occurs at high frequency.

Antithetical antigens: Kpa (KEL3), Kpc (KEL21)

Present on cord red blood cells and enzyme resistant (ficin, papain, chymotrypsin)

KEL 6

Jsa antigen with ISBT number: 006.006

It is found at low frequency

Antithetical antigen: Jsb (KEL7)

Present on cord red blood cells and unaffected by enzyme treatment (ficin, papain, chymotrypsin)

KEL 7

Jsb antigen with ISBT number: 006.007

It occurs at high frequency.

Antithetical antigen: Jsa (KEL6).

Present on cord red blood cells and enzyme resistant (ficin, papain, chymotrypsin)

These antigens are stable under proteolysis by ficin and papain but are sensitive to reducing agents such as dithiothreitol (DTT),which disrupt disulfide bonds critical for epitope conformation. This susceptibility has important laboratory implications, as DTT treatment not only destroys Kell antigens but also impacts multiple other antigen systems (e.g., Lutheran, Yt, JMH), influencing antibody detection strategies.(Maheshwari et al., 2024).

Other Rare Kell Antigens

High prevalence antigens: Ku (KEL5), KEL11, KEL12, KEL13, KEL14, KEL16, KEL18, KEL19, Km (KEL20), KEL22, TOU (KEL26), RAZ (KEL27), KALT (KEL29), KTIM (KEL30), KUCI (KEL32), KANT (KEL33), KASH (KEL34), KELP (KEL35), KETI (KEL36), KHUL(KEL37), KYOR(KEL38), KEL40

Low prevalence antigens: Ula (KEL10), Wka (KEL17), KEL21, KEL23, KEL24, VLAN (KEL25), VONG (KEL28), KYO (KEL31), KEAL (KEL39), KEL41

2.2.3 Kell Antibody

Anti-K antibodies are primarily IgG, react optimally at 37 °C in the AHG phase, and generally do not bind complement. They are most often formed after transfusion or pregnancy, though naturally occurring IgM anti-K has been reported, including in Escherichia coli infections, and may react at room temperature in saline phase. Detection can be challenging due to depressed reactivity in some low–ionic-strength solution reagents, making the AHG phase essential (Maheshwari *et al.,* 2024). Anti-K is a major cause of Haemolytic Transfusion Reactions and Hemolytic Disease of the Foetus and Newborn (HDFN) (Koumoutsea *et al.,* 2019). A titer ≥4 is clinically significant and requires monitoring (Slootweg *et al.,* 2018). Antibodies against high-prevalence Kell antigens (e.g., k) are rare but complicate finding compatible donors. Antibodies to low-prevalence Kell antigens are difficult to identify as they are often absent from antibody panels. Anti-K reacts with K+k+ and K+k- cells without dosage effect. Kell antibodies include anti-K1 (causing severe anemia in ~10% of cases), anti-K2, anti-K3, and anti-K7, which have been linked to HDFN (Kausar *et al.,* 2022). The splenic marginal zone B cells play a key role in initiating Kell antibody formation after sensitization (Patel *et al.,*2018).

2.2.4 Kell Phenotype

K Phenotype

The K_o (Kell-null) phenotype, first described by Chown *et al.* in 1957, is extremely rare (~0.001% prevalence, more frequent in Finland and Japan) and results from homozygous inheritance of two recessive K_o genes. Individuals lack all Kell antigens but have normal RBC function. After exposure, they can form clinically significant anti-Ku (anti-KEL5) antibodies, requiring K_o-compatible transfusions.

A variant with reduced Kell expression, Kmod, arises from missense mutations. Both K_o and Kmod show increased Kx protein, and Kmod may also produce anti-Ku–like antibodies (Maheshwari *et al.,*2024). Individuals with K_o produce anti-Ku when they encounter red blood cells that express Kell antigens. Anti-Ku has been responsible for mild to severe transfusion reactions(Imoru *et al.,* 2025).

McLeod Phenotype

The McLeod phenotype is a rare X-linked recessive condition caused by the absence of the Kx protein due to XK gene mutations (90%) or Xp21.1 deletions (10%). It results in weak expression of Kell antigens, complete absence of the Km (KEL20) antigen, and is associated with McLeod syndrome, a progressive neuromuscular disorder (Maheshwari *et al.,* 2024).

2.3 CLINICAL SIGNIFICANCE

The Kell blood group system extends beyond its significance in transfusion medicine and Haemolytic Disease of the Foetus and Newborn (HDFN). The Kell blood group is seen to demonstrate its involvement in red cell physiology, hematopoiesis, and even pathological processes.

2.3.1 Red Blood Cell (RBC) Functions

RBC Adhesion: Kell antigens participate in RBC adhesion to vascular endothelium through their enzymatic activity in cleaving endothelin-3, a potent vasoconstrictor (Bland *et al.,* 2008).

RBC Signaling and Immune Regulation: Studies indicate that type-1 interferon production in recipients influences alloimmunization against the Kell (KEL1) antigen, highlighting the role of Kell in immune signaling pathways (Liu *et al.,* 2019).

Structural Integrity of RBCs: Kell antigens, along with the Cartwright (Yta) antigen, are completely denatured upon treatment with dithiothreitol (DTT), suggesting their dependence on disulfide bond integrity for structural stability of the RBC membrane (Branch *et al.,*1983).

2.3.2 Erythropoiesis and Hematopoietic Regulation

Suppression of Erythroid Lineage: Kell antigens are expressed on erythroid precursors. Anti-Kell antibodies can suppress erythropoiesis by inhibiting colony-forming units, leading to reduced red cell production. This mechanism contributes to the severe anemia observed in HDFN due to Kell alloimmunization (Vaughan *et al.,*1998).

Role in Platelet Production: Kell antigens are also present on megakaryocytic precursors. Formation of anti-Kell antibodies may suppress these cells, resulting in thrombocytopenia in affected individuals (Wagner *et al.,* 2000).

2.3.3 Associations with Infections

Gram-negative Bacterial Infections: IgM-type anti-Kell antibodies have been identified in patients with Escherichia coli O125:B15 enterocolitis, suggesting a possible immunological link between Kell antigens and bacterial infections (Savalonis *et al.,* 1988).

Mycobacterial Infections: Naturally occurring IgM-type anti-Kell antibodies have also been reported in non-sensitized individuals with pulmonary tuberculosis, indicating a potential role in host–pathogen interactions (Marsh *et al.,*1978).

2.4 HAEMOLYTIC DISEASE OF THE FOETUS AND NEWBORN (HDFN)

Hemolytic disease of the fetus and newborn (HDFN) is an immune-mediated condition caused by the transplacental passage of maternal red blood cell (RBC) antibodies, leading to hemolysis of fetal or neonatal red cells. These antibodies may be naturally occurring (e.g., anti-A, anti-B) or immune antibodies acquired following sensitizing events such as transfusion or pregnancy. For HDFN to occur, the fetus must inherit the antigen from the father while the mother lacks the corresponding antigen, resulting in maternal alloimmunization (Basu *et al.,* 2011).

The clinical severity of HDFN ranges from mild to life-threatening. In less severe cases, hemolysis causes neonatal anemia and hyperbilirubinemia, sometimes persisting after birth due to continued red cell destruction and erythropoietic suppression. Severe anemia in utero can lead to complications such as edema, ascites, hepatosplenomegaly, hydrops fetalis, cardiac failure, and intrauterine death.

“Erythroblastosis fetalis” describes the compensatory increase in erythropoiesis, with extramedullary hematopoiesis causing organ enlargement, while hydrops fetalis represents the most severe outcome, characterized by generalized edema and effusions due to low oncotic pressure and high-output cardiac failure (Dennery *et al.,*2001).

Before the introduction of Rhesus immunoglobulin (RhIG) prophylaxis in 1968, HDFN due to anti-D antibodies was a leading cause of perinatal morbidity and mortality. Prophylactic administration of RhIG to Rh(D)-negative women during pregnancy and after delivery of Rh(D)-positive infants significantly reduced the incidence of RhD-associated

HDFN. Consequently, other red cell alloantibodies have emerged as important causes of HDFN. The prevalence of non-RhD alloantibodies in pregnancy ranges from 0.15% to 1.1%, with clinically significant antibodies including anti-c, anti-E, anti-K, and others. ABO incompatibility, though often milder, has become the most common cause of HDFN in many parts of the world.

The immunopathology of HDFN is primarily mediated by IgG1 and IgG3 antibodies, which are efficiently transported across the placenta from the second trimester via Fc receptors (Firian *et al.,* 2001).

Once in the fetal circulation, these antibodies bind antigen-positive fetal RBCs, leading to their destruction in the spleen. The breakdown of hemoglobin produces bilirubin, which is cleared by the maternal liver during pregnancy but accumulates in the neonate after birth, leading to jaundice.(Delaney and Matthews, 2015)

Epidemiological data highlight its clinical significance. In the United States, HDFN is estimated to occur in 3–8 per 100,000 births annually (Geaghan,2011).

2.4.1 Alloimmunization

Maternal alloimmunization occurs when a mother is exposed to foreign red blood cells, usually through pregnancy, transfusion, or organ transplant. During pregnancy, fetal–maternal hemorrhage (FMH) causes mixing of fetal and maternal blood, increasing from about 3% in the first trimester to 45% in the third. The volumes are usually small, which is why hemolytic disease of the fetus and newborn (HDFN) is rare in first pregnancies, with higher risk at delivery when exposure is greatest. FMH risk also rises with trauma, abortion, ectopic pregnancy, invasive procedures, or multiple gestations (Sebring *et al.,*1990).

Not all exposures result in antibody production, but when they do, the immune response is significant. The RhD antigen is the most

immunogenic; about 85% of RhD-negative individuals develop anti-D after transfusion of 200 mL Rh D-positive blood, though smaller volumes (0.1–1 mL) can trigger sensitization. Before Rh immunoprophylaxis (introduced in 1968), around 16% of D-negative mothers with D-positive infants developed anti-D, whereas ABO incompatibility reduced the rate to ≤2% due to rapid clearance of fetal cells.(Ayache and German, 2008), (Bowman, 1985).

While anti-D remains the most important cause of HDFN, other clinically significant antibodies include anti-E, anti-k, anti-Kpa, anti-Kpb, anti-Ku, anti-M, anti-Jsa, anti-Jsb, anti-Jka, anti-Fya, anti-Fyb, anti-S, anti-s, and anti-U. (Delaney and Matthews, 2015)

	Early Onset Anaemia	**Late Haemolytic Anaemia**	**Late Hyporegenerative Anaemia**
Onset	Within 7 days of birth	>/= 2 weeks of age	
Mechanisms	Antibody mediated haemolysis	1.Antibody mediated haemolysis 2.Natural decline of Hb levels	1.Antibody destruction of RBC precursors and RBCs. 2. Marrow suppression by IUT and transfusions.

		3. Expanding intravascular volume of growing infant	3. Erythropoietin deficiency 4. Expanding intravascular volume of growing infants.
Bilirubin	Elevated	Usually elevated	Normal
Reticulocyte count	Normal or high	Normal or high	Low or absent

Table 2.1; Showing the neonatal Manifestations of HDFN anaemia by time of onset(Rath *et al.,* 2014)

2.4.2 HDFN Due to Kell Factor

In Kell-related disease, the severity of anemia does not correlate with maternal antibody titers or amniotic fluid bilirubin levels. Affected fetuses typically present with inappropriately low reticulocyte counts relative to the degree of anemia, in contrast to the robust reticulocytosis seen in Rh alloimmunization.

Experimental studies have shown that anti-Kell IgG and IgM antibodies suppress the growth of Kell-positive erythroid burst-forming units and colony-forming units in vitro. Similarly, serum from women with anti-Kell antibodies exerts the same inhibitory effect. This suggests that the anemia in Kell-HDN is due not only to hemolysis of fetal red cells but also to direct suppression of erythropoiesis. (Basu *et al.,* 2011).

2.4.3 Diagnosis

Routine Screening

All pregnant women should undergo routine screening that includes blood grouping (ABO, RhD) and antibody detection to identify clinically significant alloantibodies,detailed

maternal history—particularly outcomes of prior pregnancies complicated by stillbirth, hydrops fetalis, or severe neonatal jaundice—and ultrasound evaluation to confirm gestational age and exclude ascites (Kamphuis *et al.,* 2008) (Moise and Argoti, 2012).

Paternal Typing

Paternal red cell antigen typing is performed to predict fetal risk. In most blood group systems, serological testing is sufficient to determine homozygosity or heterozygosity. When paternal heterozygosity is identified, the fetus has a 50% risk of inheriting the antigen (Wagner and Flegel, 2000).

Fetal Genotyping

Direct fetal genotyping provides the most accurate method of determining antigen inheritance. This can be achieved through invasive sampling (amniocentesis or chorionic villus sampling) or, more recently, noninvasive analysis of cell-free fetal DNA from maternal plasma, which has become the preferred approach due to its safety (Finning *et al.,* 2007)(Daniels *et al.,* 2009).

Indirect Antiglobulin Testing (IAT)

The IAT (indirect Coombs test) detects unbound IgG antibodies circulating in plasma. Postnatally, it is performed on the neonate's serum to check if free maternal IgG antibodies are still present in the newborn's circulation. It can also be used on maternal serum after delivery to characterize antibodies responsible for neonatal hemolysis (Moise, 2008).

Antibody Titration

Antibody titration is also used to monitor immune activity. Serial titers are typically followed, with critical thresholds (commonly 1:16–1:32) used as indicators of potential risk for fetal anemia. However, in Kell alloimmunization, severe hypoproliferative anemia can occur at titers as low as 1:8, due to suppression of erythropoiesis in addition to hemolysis.

This highlights the limitations of titers in predicting disease severity. More advanced methods, such as flow cytometry for quantification of antibody binding, may offer greater precision than traditional titration techniques(AuBuchon *et al.,*2008),(Hildén, 1997).

Direct Antiglobulin Test

After delivery, the Direct Antiglobulin Test (DAT) is a central diagnostic tool for confirming immune-mediated hemolysis. The DAT detects maternal IgG bound to neonatal red blood cells and is performed on cord blood or neonatal samples. A positive DAT supports the diagnosis of HDFN when the neonate inherits the antigen corresponding to the maternal antibody.

In the case of Kell-mediated HDFN, interpretation of DAT requires caution. Because anti-K antibodies not only cause hemolysis but also suppress fetal erythropoiesis, infants may present with severe anemia even when the DAT is weakly positive or negative. Therefore, the absence of a strongly positive DAT does not exclude significant disease (Makarovska *et al.,* 2009).

2.4.4 Neonatal Care

Initial management of neonates with hemolytic disease of the fetus and newborn (HDFN) should focus on treating hyperbilirubinemia. Obtaining cord blood hemoglobin, total bilirubin, and a direct Coombs test at delivery provides valuable information for the neonatologist. Intensive phototherapy remains the primary treatment, with intravenous immunoglobulin (IVIG) and, if necessary, exchange transfusion used in cases of rising bilirubin levels. Neonates with anti-Kell–associated HDFN typically require less phototherapy and fewer exchange transfusions due to lower bilirubin levels, as anti-Kell is more often associated with hypoproliferative anemia rather than hemolytic anemia.

In cases of HDFN requiring serial intrauterine transfusions, suppression of fetal erythropoiesis is common. These neonates are usually born with a marked absence of

reticulocytes, and their red cell mass consists almost entirely of donor erythrocytes. Due to this profound erythropoietic suppression, they are at increased risk of requiring additional transfusions and developing “late anemia.”

It is therefore advisable that these infants be followed closely by a pediatric hematologist, with weekly monitoring of reticulocyte counts and hematocrit levels for 1 to 3 months, until a sustained rise in reticulocyte count is observed for at least two consecutive weeks (Rath *et al.,* 2011).

2.4.5 Treatment

Erythropoietin (EPO) therapy has been successfully employed in cases of late anemia following Rh-HDN, where suppression of erythropoiesis often results from multiple intrauterine transfusions. These infants typically demonstrate low EPO levels. Although reports of EPO levels in Kell-HDN are scarce, emerging evidence suggests that affected infants may also exhibit inappropriately low endogenous EPO. Our findings indicate that the use of recombinant erythropoietin (rEPO) can stimulate erythropoiesis, correct anemia, and reduce the need for blood transfusion in these patients.

Thus, rEPO therapy should be considered as a supportive treatment option for infants with severe anemia due to Kell alloimmunization, potentially minimizing exposure to blood products and associated risks.

HDFN remains a critical concern in obstetric and transfusion medicine. Although RhIG prophylaxis has dramatically reduced the burden of RhD-mediated disease, the emergence of non-RhD alloantibodies as significant contributors underscores the ongoing importance of antenatal antibody screening, maternal-fetal monitoring, and transfusion support. Early detection and timely intervention are essential to improving neonatal survival and reducing long-term complications, reinforcing the role of transfusion services

as a cornerstone in the management of maternal alloimmunization and HDFN (Dhodapkar *et al.,*2001).

2.5 TRANSFUSION REACTIONS

Transfusion reactions are undesirable responses or effects in a patient temporarily associated with the administration of blood or blood components.Reactions can occur during the transfusion (acute transfusion reactions) or days to weeks later (delayed transfusion reactions) and may be immunologic or non-immunologic. World Health Organization [WHO], 2020).

Types of transfusion reactions include the following:(Roback *et al.,* 2011)

- Haemolytic
- Febrile non-haemolytic
- Anaphylactic
- Allergic
- Septic (bacterial contamination)
- Transfusion-related acute lung injury (TRALI)
- Transfusion-associated circulatory overload (Taco)

Clinical signs and symptoms of a complication of transfusion may be associated with more than one type of reaction, and early recognition and evaluation is important for the

best outcome.Signs of a transfusion reaction may include the following:(Callum *et al.,* 2016)

- Fever ≥1° C increase or >38° C
- Chills/rigors

- Respiratory distress—wheezing, coughing, dyspnea, cyanosis
- Hypertension or hypotension
- Pain—abdominal, chest, flank or back, infusion site
- Skin manifestations—urticaria, rash, flushing, edema
- Jaundice, hemoglobinuria
- Nausea/vomiting
- Abnormal bleeding
- Oliguria/anuria

2.5.1 Haemolytic Transfusion Reaction(HTR)

A haemolytic transfusion reaction is the destruction of transfused red cells that results in intravascular or extravascular hemolysis or a combination of both. Hemolytic reactions are classified as acute or delayed. Both types may stem from immune or non-immune causes (Yazer and Fung, 2018).

2.5.2 Acute Hemolytic Transfusion Reaction (AHTR)

An acute hemolytic transfusion reaction (AHTR) is the rapid destruction of red blood cells occurring during, immediately after, or within 24 hours of a transfusion (Roback *et al.,*2011). Clinical manifestations range from mild fever to life-threatening complications, including death. Common signs and symptoms include fever, chills, pain or oozing at the infusion site, back or flank pain, hypotension, epistaxis, hemoglobinuria, disseminated intravascular coagulation (DIC), oliguria, anuria, and renal failure. (American Association of Blood Banks [AABB], 2021).

The severity of an AHTR results from multiple simultaneous clinical events triggered by the interaction of preformed antibodies with red cell antigens, most often due to ABO incompatibility(Yazer andFung, 2018). The immunologic basis of AHTR involves:

Antibody binding to red cells, complement activation, stimulation of mononuclear phagocytes and cytokine release, coagulation system activation, progression to shock and renal failure.

Pathophysiology of AHTR

The initiating event in an immune-mediated AHTR is the binding of recipient antibodies to transfused red cell antigens. This antigen–antibody complex activates complement and sets off a cascade of clinical effects. Both antibody characteristics (class, titer) and antigen properties (density, distribution) determine the severity of hemolysis(Shanbhag and Spivak, 2015).

Complement contributes through three main mechanisms:

1. Opsonization: Membrane-bound complement components are cleared by phagocytes.
2. Anaphylatoxin generation: C3a and C5a trigger inflammation, mast cell degranulation, histamine and serotonin release, vascular dilation, bronchospasm, and increased vascular permeability. Bradykinin further contributes to vasodilation and hypotension. Immune complex deposition and microthrombus formation impair renal vasculature (Zimring and Spitalnik, 2013).
3. Red cell lysis: Completion of the membrane attack complex leads to hemolysis. Released hemoglobin is bound by haptoglobin until its capacity is exceeded, resulting in hemoglobinemia and hemoglobinuria(Roback *et al.,* 2011).

Complement activation may also initiate the coagulation and fibrinolytic systems, contributing to Disseminated Intravascular Coagulation (DIC). This is characterized by consumption of clotting factors (fibrinogen, factor V, factor VIII), thrombocytopenia, and diffuse microvascular bleeding. Thrombus promotes tissue ischemia, while lab profiles show low platelets, decreased fibrinogen, and fibrin degradation products (AABB, 2021).

The systemic release of inflammatory mediators (anaphylatoxins, vasoactive amines, kinins, cytokines) produces shock, defined as inadequate tissue perfusion leading to life-threatening cellular dysfunction.Renal failure in severe, untreated AHTR is multifactorial. Contributing mechanisms include systemic hypotension, renal vasoconstriction, and intravascular thrombus, all of which reduce renal cortical blood flow. Norepinephrine release in response to shock further intensifies renal and pulmonary vasoconstriction. This may lead to renal ischemia, acute tubular necrosis, and eventual kidney failure Heddle, 2019).

2.5.3 Delayed Hemolytic Transfusion Reaction (DHTR)

Delayed hemolytic transfusion reactions (DHTRs) occur more than 24 hours after transfusion. They are usually less severe, often self-limiting, and rarely require treatment.(Roback *et al.,*2011). The underlying cause of a DHTR is an IgG alloantibody formed from prior exposure to red cell antigens through transfusion or pregnancy (Hillyer *et al.,*2007). In most cases, pretransfusion antibody screening detects clinically significant alloantibodies. However, a DHTR may still occur if the antibody titer was too low to be detected at the time of testing, and occurred during antibody screening, or antibody was directed against a low-frequency antigen not represented on the screening cells. (Yazer *et al.,*2008).

Clinical indicators of DHTR include:

- Inadequate post-transfusion rise in hemoglobin
- Rapid decline of hemoglobin back to pre-transfusion levels
- Unexplained appearance of spherocytes on the blood film

Definitive diagnosis is made when a new red cell alloantibody is identified between 24 hours and 28 days post-transfusion.(Roback *et al.,*2011) The antibodies most frequently

associated with DHTRs, in order of occurrence, are those in the Kidd, Duffy, Kell, and MNS blood group systems (Dean, 2005).

Pathophysiology of DHTR

In DHTR, transfused red cells sensitized with immunoglobulin or complement are removed from circulation primarily by the mononuclear phagocyte system (MPS). Macrophages in the spleen are the main mediators of this clearance, though Kupffer cells in the liver also contribute (Hillyer *et al.*,2007).

Additionally, activated mononuclear phagocytes release cytokines, which are protein mediators of cell-to-cell communication. These cytokines are responsible for some systemic features of DHTR, including fever, hypotension, and immune activation. They can also stimulate endothelial cells to express procoagulant activity, linking the immune response to the coagulation system(Yazer and Fung, 2018).

2.5.4 Transfusion Reaction Due to Kell Factor

Anti-K may be formed following previous red cell exposure through blood transfusion or pregnancy (Yates & Howell, 2020). When transfused red cells expressing the K antigen enter a sensitized recipient, anti-K antibodies bind to these cells. Unlike ABO antibodies, anti-K does not usually cause acute intravascular hemolysis but rather induces extravascular hemolysis through the mononuclear phagocyte system (Win et al., 2021).

Because anti-K antibodies can have significant adverse transfusion and obstetric consequences, proper pre-transfusion antibody screening and identification is essential. Standard pre-transfusion testing may miss low-titer or low-frequency alloantibodies, leading to potential mismatches. Therefore, Extended phenotyping or Kell antigen typing should be performed in multi-transfused patients, women of childbearing age, and individuals with prior alloimmunization (Win et al., 2021). Blood banks should maintain

strict adherence to antibody screening and cross-matching protocols to reduce the risk of alloimmunization and hemolytic reactions (Yates & Howell, 2020).

2.5.5 Prevention

The majority of HTRs are preventable and are most often caused by clerical or identification errors, particularly ABO-incompatible transfusions. These errors include patient misidentification, improper sample collection, and incorrect or missed entry of test results. Adherence to strict standard operating procedures in patient identification, sample handling, testing, and transfusion is essential to prevent these reactions. (Yazer and Fung, 2018);AABB, 2021).In relation to Kell, only Kell negative typed blood should be transfused to the recipient.

2.6 LABORATORY DETECTION OF KELL ANTIGEN

The determination of Kell blood group antigens can be performed using serological and molecular methods, each with distinct applications, strengths, and limitations.

2.6.1 Serological Methods

Serological testing remains the most widely used approach in routine blood banking and transfusion practice.

Conventional Hemagglutination (Forward Typing)

This technique uses specific anti-K antibodies to detect Kell antigens on red blood cells (RBCs). Agglutination indicates the presence of the K antigen, while absence of agglutination denotes K-negative cells. The method is simple, rapid, and cost-effective but may fail to detect weakly expressed or variant Kell(Murphy and Fraser,1997).

Reverse Typing (Antibody Screening)

Patient serum is tested for the presence of anti-K antibodies, serving as a confirmatory step to forward typing. This approach helps identify unexpected

alloantibodies, which are clinically important in preventing transfusion reactions and haemolytic disease of the fetus and newborn (HDFN).(Li and Guo, 2022)

Gel Centrifugation Technique

This method uses gel cards to separate agglutinated from non-agglutinated RBCs, improving both sensitivity and specificity compared with conventional hemagglutination. It reduces false-negative results and has become increasingly common in blood banks for routine Kell antigen testing. (Lapierre *et al.,* 1990)

2.6.2 Molecular Methods

Molecular approaches are particularly valuable when serological methods are inconclusive, such as in patients with recent transfusions, autoimmune hemolytic anemia, or weak/variant antigen expression.

Polymerase Chain Reaction (PCR)-based Methods: PCR with Sequence-Specific Primers (PCR-SSP): Detects specific Kell alleles with high accuracy. It is cost-effective, reliable, and suitable for routine use, while also identifying rare Kell phenotypes undetectable by serology.(Gorakshakar *et al.,* 2017)

PCR with Restriction Fragment Length Polymorphism (PCR-RFLP): Identifies polymorphisms in Kell genes by analyzing restriction enzyme digestion patterns. This provides detailed genetic information, useful in transfusion compatibility testing and population studies.(Quirino *et al.,* 2019).

Microarray Technology: DNA microarrays allow simultaneous genotyping of multiple blood group antigens, including Kell, in a single test. This method is efficient for large-scale population screening and donor registries, providing comprehensive genetic data for precision transfusion medicine. (Arnoni *et al., 2013).*

2.7 PREVALENCE OF KELL ANTIGEN IN NIGERIA

A multi-ethnic cohort of 302 healthy Nigerian individuals was created to study RBC antigen prevalence. The antigen status of these individuals for Rh and K antigens was determined using commercially prepared antisera and conventional tube agglutination methods. The prevalence of K was 0 percent (Adewoyin *et al.,* 2018).

Sokoto state

The prevalence of Kell Antigen among pregnant women in Sokoto state was conducted with a sample size of one hundred and fifty participants attending antenatal clinics in UDUTH, 3 (2.0%) of subjects were positive and 147 (98.0) were negative for K antigen. Kell phenotype was more prevalent among primigravidae (3.1%) compared to multigravidae (1.7%) women. The distribution of Kell phenotype among the pregnant subjects was compared based on ethnicity. The prevalence of Kell antigen was significantly higher among the Hausa ethnic group (3.2%) compared to other ethnic groups which indicated zero prevalence (p=0.001). Kell negative phenotype was ≥ 96.8% among all the ethnic groups(Osaro *et al.,* 2015).

Awka and Asaba

Onyenekwe *et al.,* (2023) conducted research on the occurrence of the kell blood group among individuals with ABO and Rh (D) blood groups in Awka and Asaba. The

result showed a 2.1% prevalence. Two individuals with expression of kell antigen on their red cell were of O Rh (D) positive blood while the third individual was of A Rh (D) positive.

Kebbi

Amongst Lelna ethnic group in Kebbi state, from a population size of one hundred and ninety-six pregnant women, the prevalence of Kell blood group was seen to be 6.6%(Imoru *et al.,* 2025).

Igbo descents in Rivers State

Christian *et al.* (2025) conducted a research aimed at determining the presence of Kell blood group antigen(s) amongst some Igbos residing in Port Harcourt, Nigeria. With a sample size of two hundred and four (204) subjects, (seventy-four (74) males and one hundred and thirty (130) females) within the ages of 15 to 47years. The result showed zero frequency occurrence and percentage distribution(0%) of Kell blood group antigen in the studied population.

In the same year, another study was carried out on the prevalence of Kell blood group amongst indigenes of Ikwerre. A total number of two hundred and six (206) subjects consisting of one-hundred males (100) and one-hundred and six females (106) within the age bracket of 18 - 40 years were recruited for the study. The result showed a total of four (4) positive cases of Kell antigen in only Females and none in males, as well as a percentage distribution of 1.9 % in Females.

University College Hospital, Ibadan

A Study aimed at investigating the prevalence of ABO and Kell blood group antigens in 287 donated blood units at the University College Hospital in Ibadan, Nigeria showed a Kell prevalence of 6.0%(Nurudeen *et al.,* 2024b). These findings highlight the

clinical importance of Kell antigen screening, which underpins the rationale for investigating its prevalence among pregnant women attending antenatal care in Central Hospital, Benin City.

CHAPTER THREE

MATERIALS AND METHODS

3.1 AREA OF STUDY

The study was carried out at Central Hospital, Benin City, Edo State. Edo State was created on August 27, 1991, during the administration of President Ibrahim Badamosi Babangida. The state was carved out of the former Bendel State in response to the people's agitation for a distinct state of their own. Geographically, Edo State lies approximately between longitudes 6°04′E and 6°43′E of the Greenwich Meridian and latitudes 5°44′N and 7°34′N of the equator. It is bounded to the south by Delta State, to the north by Kogi State, to the east by the River Niger and Anambra State, and to the west by Ondo State. The relief features of the state include swamps and creeks, the Esan Plateau, and the dissected uplands of Akoko-Edo Local Government Area. The predominant soil type is the red-yellow ferrous variety. Edo State experiences a typical tropical climate with two distinct seasons: the wet (rainy) season, which spans from April to November, and the dry season, which lasts from December to March. The natural vegetation is characterized by rainforest in the Benin lowlands and Esan Plateau, and savanna in the Orle Valley and Akoko-Edo uplands. Human activities, however, have led to the development of rubber and oil palm plantations as well as forest reserves. (Ogunleye and Amen, 2010)

There are three senatorial districts in Edo State: North, Central and South Senatorial Districts. Edo-South Senatorial District is made up of seven local government areas ~ are: Oredo, Egor, Ikpoba-Okha, Orhionmwon, Uhunmwode, Ovia Southwest and Ovia North

The major urban centres in the state include Benin City, Auchi, and Uromi with Central Hospital located within Benin Metropolis.

Benin City has grown remarkably;.Currently, Benin is a city encompassing five Local Government Areas namely: Egor, Ikpoba Okha and Oredo with its surrounding Local Government Areas of Uhunmwode and Ovia North east. The Benin region had a natural population growth rate of 3.0 per cent during the early years of British colonization which later decreased to about 2.5 per cent in the 1960s. Due to the high rate of migration in Benin City, the estimated population was 314,219, derived from the annual growth rate of 8.5 per cent between 1963 and 1976. According to the 1991 census,the total population of Edo State was 2,172,005 persons with Benin City having 762,717 of the total population. A total of 3,233,366 persons were recorded in 2006 census and Benin City has 1,086,882 with a population growth rate of 2.78 per cent.The region's surrounding communities (Uhunmwode and Ovia North east) have a population of 461,087. The population of Benin City varies in terms of age and sex structure, marital status, literacy level, household size and ethnic group. This accounts remarkably for the aerial variations in the cultural landscape and development pattern. (National Population Census, 2006), (John *et.,*2021).

The social economic activities of the people in the region includes the formal sector mainly federal and state public/civil servants as well as the informal sector which comprises private workers, traders and farmers (John *et al* 2021). Samples were collected in four batches (every Monday) which corresponded to the antenatal clinic day of the hospital. Also, blood group analysis of samples collected were determined after each day of collection.

3.2 STUDY DESIGN

This study adopted a **descriptive cross-sectional design,** which is considered most suitable for the research objective. A cross-sectional design involves the collection of data from a defined population at a single point in time or over a short period. It is particularly

useful for estimating the prevalence of characteristics, conditions, or exposures within a target group.

In this research, the design will allow the determination of the distribution of the Kell blood group system among pregnant women attending antenatal care at Central Hospital, Benin City. By recruiting participants as they present for routine antenatal visits, information and biological samples will be obtained to provide a "snapshot" of the occurrence of Kell antigens and antibodies in the study population.

The descriptive approach is appropriate because the primary aim of the study is not to establish causality but to describe the frequency and pattern of Kell blood group antigens among pregnant women. This design is time-efficient, cost-effective, and feasible within the hospital setting, while also ensuring that data collected can serve as baseline information for future analytical or interventional studies in the region.

3.3 STUDY POPULATION

The study subject was targeted at pregnant women within the age range 18-60 years from Central Hospital, Benin City, Edo State. During the period of the study, all pregnant women who visited the laboratory for antenatal screening tests, met inclusion criteria and gave informed consent were included for the study. The women were given questionnaires to fill after their samples were obtained.

3.3.1 Inclusion Criteria

Pregnant women who give informed consent.

Pregnant women attending antenatal care at Central Hospital during the study period.

3.3.2 Exclusion Criteria

Women who refuse to give consent.

Women in parturition

3.4 SAMPLE SIZE

The sample size was calculated using the formula for descriptive cross-sectional using the formula (Pourhoseingholi, 2013).

$$n = \frac{Z^2(1-P)}{d^2}$$

Where:

n = required sample size

Z = standard normal deviation (1.96 at 95% confidence level)

p = estimated prevalence of Kell blood group in the population (5%)

d = margin of error (0.05)

Calculation

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

n= 72.96 ~73

A total of one hundred samples was collected

3.5 ETHICAL APPROVAL

Ethical Approval was sought and obtained (Appendix IV) from the Edo State Ministry of Health, Health Research and Ethics Committee with approval number HA/737/25/D/09180750.

3.6 SAMPLE COLLECTION AND PREPARATION

Under an aseptic environment, blood specimens were collected from the ante-cubital vein using a dry sterile disposable needle and syringe. Two millimeters of blood was

collected and put in an Ethylene Diamine Tetracetic Acid (EDTA) container and thoroughly mixed.

Precautions taken during the course of the experiment

- The use of complete personal protective equipment was utilized.
- Care was taken during venal puncture to avoid hematoma.
- Proper glove removal technique was used to avoid skin contact with any chemical.

3.6.1 Laboratory Analysis

Principle

Blood group is based on agglutination reactions. When red blood cells carrying the K antigen are exposed to the corresponding antibodies (Anti-K), they interact with each other to form visible agglutination or clumping.

Materials

Anti-K Reagent, 100 blood samples, clean test tubes, water bath, bucket centrifuge, pasteur pipette, normal saline and test tube rack.

Reagent

Anti Kell with Lot number K0724-22 was purchased commercially. The reagent contains monoclonal human IgM antibodies in a buffer solution with macro molecular chemical potentiators, sodium azide(0.1% w/v) and bovine material. It was stored between 2-8°C.

Procedure (Tube technique)

- Each sample was washed thrice with adequate volume of normal saline.
- A 5% red cell suspension was made from the washed cells —1 drop of washed cells to 19 drops of normal saline.

- One drop of 5% cell suspension of the red cells was dispensed into a labelled clean test tube.
- One drop of Anti - K reagent was added and mixed thoroughly and observed for agglutination macroscopically.
- All tubes showing a negative or weakly positive reaction were incubated for 5 minutes at 37°C
- After incubation, the tubes were centrifuged at 1000rpm for 20 seconds.
- The tubes were gently agitated to dislodge the red cells and examined macroscopically for agglutination.

3.7 STATISTICAL ANALYSIS

Data obtained from the laboratory analysis were analysed using Statistical Package for Social Sciences(IBM SPSS) Statistics version 27 (IBM Corp., Armonk, NY, USA).

CHAPTER FOUR

RESULT

As presented in Figure 4.1, the study revealed that the prevalence of Kell-phenotypes among pregnant women attending antenatal care in Central Hospital, Benin City, was very low, accounting for only 2% of the population studied, while 98% were Kell-negative. The distribution of Kell status across age groups, shown in Table 4.1 and further illustrated in Figure 4.2, indicates that Kell positivity was observed only among women aged 26–33 years (50% of Kell-positive cases) and 34–43 years (50%), with none identified in the 18–25 years category. The chi-square test, however, showed no significant association between Kell status and age group ($\chi^2 = 0.646$, $p = 0.724$).

With regard to gravidity, Table 4.1 shows that Kell-positive cases were recorded only in women with a gravidity of two and four (5.0% and 5.6% respectively), while no positive cases were observed in other gravidity groups. Parity distribution followed a similar trend, with Kell positivity observed only in women with parity zero (3.1%) and parity three (5.6%). These variations were not statistically significant, as indicated by chi-square results for gravidity ($\chi^2 = 3.291$, $p = 0.771$) and parity ($\chi^2 = 2.300$, $p = 0.890$).

Other sociodemographic variables also showed no significant association with Kell status. As indicated in Table 4.1, marital status revealed that 2.2% of married women were Kell positive, while all single women were Kell negative ($\chi^2 = 0.132$, $p = 0.717$). With respect to religion, Kell positivity occurred only among Christians (2.1%), while none was found among Muslims or participants classified under “others” ($\chi^2 = 0.086$, $p = 0.958$). Similarly, Kell positivity was detected only among women of Bini ethnicity (4.5%), while all other ethnic groups recorded no positive cases, with no significant association observed ($\chi^2 = 2.552$, $p = 0.959$). Educational level also showed a comparable pattern: Kell positivity occurred in 2.0% of women with secondary education and 2.9% with tertiary education, but

none with primary education, and this relationship was not statistically significant (χ^2 = 0.203, p = 0.903).

Table 4.1 Distribution of Kell Status by Sociodemographic Characteristics of Pregnant Women Attending Antenatal Care in Central Hospital, Benin City.

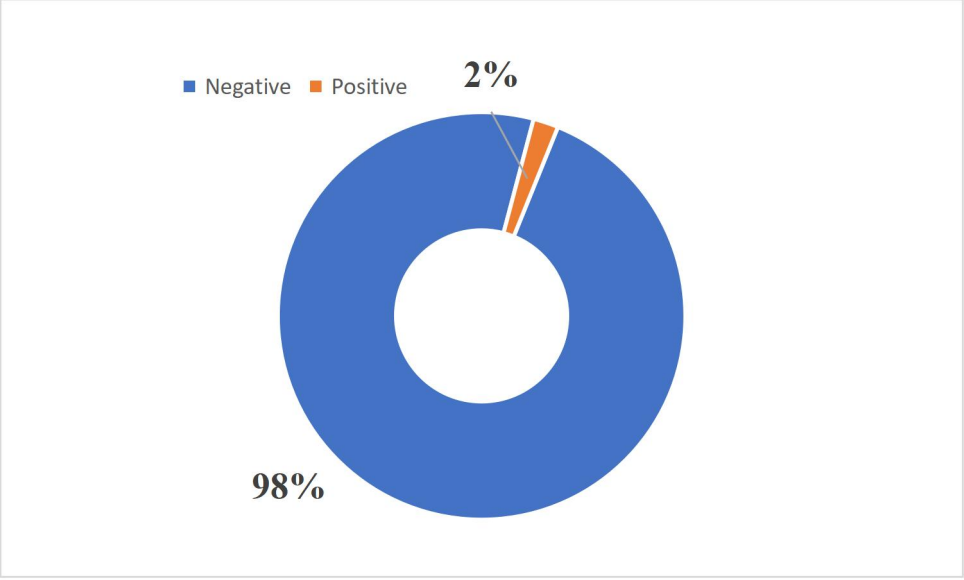
Variable	Category	Kell Positive n (%)	Kell Negative n (%)	Total n	χ^2	p-value
Age Group (years)	18–25	0 (0.0%)	20 (20.4%)	20 (20.0%)	0.646	0.724
	26–33	1 (50.0%)	48 (49.0%)	49 (49.0%)		
	34–43	1 (50.0%)	30 (30.6%)	31 (31.0%)		
Gravidity	1	0 (0.0)	31 (100.0)	31	3.291	0.771
	2	1 (5.0)	19 (95.0)	20		
	3	0 (0.0)	17 (100.0)	17		
	4	1 (5.6)	17 (94.4)	18		
	5	0 (0.0)	10 (100.0)	10		
	6	0 (0.0)	2 (100.0)	2		
	8	0 (0.0)	1 (100.0)	1		
Parity	0	1 (3.1)	31 (96.9)	32	2.3	0.89
	1	0 (0.0)	18 (100.0)	18		
	2	0 (0.0)	19 (100.0)	19		
	3	1 (5.6)	17 (94.4)	18		
	4	0 (0.0)	9 (100.0)	9		
	5	0 (0.0)	1 (100.0)	1		
	7	0 (0.0)	1 (100.0)	1		
Marital Status	Single	0 (0.0)	6 (100.0)	6	0.132	0.717
	Married	2 (2.2)	91 (97.8)	93		
Religion	Christianity	2 (2.1)	93 (97.9)	95	0.086	0.958
	Islam	0 (0.0)	3 (100.0)	3		
	Others	0 (0.0)	1 (100.0)	1		
Ethnicity	Bini	2 (4.5)	42 (95.5)	44	2.552	0.959
	Esan	0 (0.0)	16 (100.0)	16		
	Urhobo	0 (0.0)	11 (100.0)	11		
	Igbo	0 (0.0)	14 (100.0)	14		
	Yoruba	0 (0.0)	1 (100.0)	1		
	Hausa	0 (0.0)	5 (100.0)	5		
	Others	0 (0.0)	6 (100.0)	6		
Educational Level	Primary	0 (0.0)	5 (100.0)	5	0.203	0.903
	Secondary	1 (2.0)	49 (98.0)	50		
	Tertiary	1 (2.9)	33 (97.1)	34		

Overall, chi-square analyses across all sociodemographic variables consistently indicated p-values greater than 0.05, highlighting the absence of statistically significant

associations between Kell status and age, gravidity, parity, marital status, religion, ethnicity, or educational level.

Table 4.2 reports on awareness of the Kell blood group system among the participants. None of the pregnant women in the study population had prior knowledge of the Kell blood group system. All 100 respondents (100%) reported being unaware of it.Chi-square tests show no significant association between Kell status and age, gravidity, or parity ($p>0.05$). Chi-square analysis indicates no significant association between Kell status and any of the sociodemographic characteristics ($p>0.05$). Percentages are calculated within each category.

Figure 4.1 Prevalence of Kell Phenotypes among Pregnant Women Attending Antenatal Care in Central Hospital, Benin City (N=100).



This figure shows the overall prevalence of Kell-positive and Kell-negative blood groups among the study population, 98% negative, 2% positive.$n = \frac{Z^2(1-P)}{d^2}$

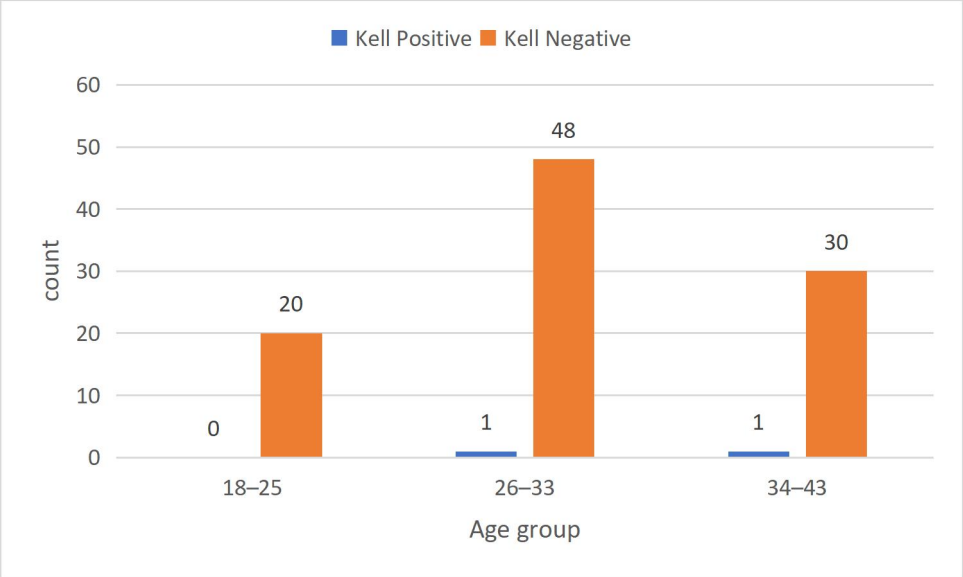


Figure 4.2 Distribution of Kell phenotypes by age group among pregnant women attending antenatal care at Central hospital, Benin city.

Table 4.2: Awareness of the Kell Blood Group System among Pregnant Women

Kell Awareness	Frequency (n)	Percentage (%)
Aware	0	0
Not Aware	100	100
Total	100	100

CHAPTER FIVE

5.1 DISCUSSION

The Kell antigen is highly immunogenic and remains a leading cause of alloimmunization in mismatched blood transfusions, haemolytic transfusion reactions (HTRs), and maternal alloimmunization, which can result in severe anaemia in neonates. This study was aimed at determining the distribution of the Kell blood group among pregnant women attending antenatal care in Central Hospital in Benin City, southern region of Edo State, South South Nigeria.

A total of 100 pregnant women aged 18–43years attending the antenatal clinic at Central Hospital were screened for Kell antigen using the conventional tube method with anti-Kell reagent. Among the participants, 2 (2.0%) were positive for the Kell antigen, while 98 (98.0%) were negative. Distribution by gravidity reveals no significance in Kell distribution. Parity distribution mirrored the gravidity pattern with no significant association between Kell positive cases and number of deliveries. Marital status, religion and ethnicity also showed no meaningful association in Kell distribution. Educational analysis revealed that one participant with secondary education (2%) and another with tertiary education (2.9%) were Kell positive but still no significant association using Pearson Chi-square test. The result shows that none of the participants were aware of the existence of the Kell blood group. The 2.0% Kell prevalence observed in this study aligns with findings from previous studies. A prevalence of 2% reported from a study in Port Harcourt (Ugboma and Nwauche, 2009) and Sokoto(Osaro *et al.,* 2015). However, ethnic variations have been observed in the prevalence of Kell blood group in Nigeria (Adedoyin et al., 2018) with a higher prevalence of 21.7% kell blood has been reported in northern Nigeria among blood donors in Kano (Gwaram and Yusuf, 2020).

The result from this study remains considerably lower than values reported among Caucasians. Lamba *et al.,* (2013) reported a prevalence of 2.8% among 1,000 Indian blood donors. Chaudhary *et al.,* (2003) observed 1.92% of North Indian donors.Thakral *et al.,* (2010) reported a higher prevalence of 5.56% in North India. Makroo *et al.,* (2013) documented 3.5% in a cohort of over 3,000 Indian donors.

Among Caucasians, reported prevalence is around 9%, highlighting racial differences in Kell distribution (Nathalang *et al.,* 2001). Other global studies further illustrate variation: 5.7% among Maldivians (Chan *et al.,*1996), 0% in South Gujarat (India)(Kahar and Patel, 2013) and Taiwan(Chinese population) (Lin-Chu *et al.,* 1988), 0.5% in Senegalese(Blaby *et al,* 1987). 0.8% of the Bengalee population (Rahman, 1975), and 0.25% of the Macedonians (Makarovska, 2009). These findings emphasize ethnic and geographical variability in Kell antigen distribution.

5.2 CONTRIBUTION TO KNOWLEDGE

This study contributes to the existing body of knowledge by providing baseline data on the prevalence of Kell blood group among pregnant women in Benin City, Edo State. The findings highlight the presence, albeit low, of the Kell antigen in this population and underscore its clinical significance in transfusion medicine and maternal health. It also adds to the limited data available on Kell antigen distribution in Edo State providing a foundation for future studies and policy development

5. 3 CONCLUSION

This study observed a 2.0% prevalence of Kell antigen among pregnant women in Central. Hospital in Benin City. The finding is consistent with reports from other African and Asian populations but significantly lower than that among Caucasians. The clinical

implications of Kell antigen in transfusion medicine and maternal-fetal health underscore the urgent need for routine screening and provision of Kell-negative blood, particularly for women of reproductive age. Establishing such preventive measures will reduce the risk of haemolytic transfusion reactions and Kell-associated HDFN, contributing to safer obstetric and transfusion practices in Nigeria.

5.4 RECOMMENDATIONS

Based on the findings of this study and the clinical relevance of Kell antigen:

1. Routine Screening: Pregnant women should be screened for clinically significant red cell antigens, including Kell, during their first antenatal visit.
2. Provision of Kell-Negative Blood: Blood banks should prioritize providing Kell-negative red cells to pregnant women and women of childbearing age to prevent alloimmunization.
3. Health Education: Pregnant women should receive sustained health education to encourage early antenatal booking, enabling timely detection and management of alloantibodies.
4. Phenotype-Matched Transfusions: For transfusion-dependent patients, particularly women of reproductive age, phenotype-matched RBCs for C, E, and K antigens should be routinely considered to reduce alloimmunization risk.
5. Further Research: Larger, multi-ethnic studies in Nigeria are recommended to better define Kell antigen distribution across regions and its implications for transfusion practice and maternal health.
6. Future studies in Nigeria should adopt these more advanced techniques to enhance accuracy, efficiency, and reliability of Kell antigen screening.

7. A nationwide study involving larger cohorts is needed to provide a more representative estimate of the prevalence of Kell antigens in Edo State.

5.5 LIMITATIONS OF THE STUDY

Firstly, the sample size was relatively small compared to the large population of the Edo State and the spatial distribution of Hospitals in it. Therefore, the prevalence obtained here only provides an estimate of the frequency of the Kell phenotype among pregnant women in Central Hospital. Cost constraints and limited access to reagents were major factors that restricted the number of subjects enrolled.

Secondly, this study employed the conventional manual tube method for Kell antigen screening. While widely used, this technique has certain drawbacks when compared to more advanced and sensitive methods such as automated platforms, gel card systems, and glass bead-based card methods.

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APPENDIX I

QUESTIONNAIRE

SECTION A: Socio-Demographic Information

1. Age: _____

2. Marital Status
☐ Single ☐ Married ☐ Divorced ☐ Widowed

3. Religion:
☐ Christianity ☐ Islam ☐ Traditional ☐ Others (Specify): _____

4. Ethnicity:
☐ Bini ☐ Esan ☐ Yoruba ☐ Hausa ☐ Others (Specify): _____

5. Educational Level:

☐ No formal education ☐ Primary ☐ Secondary ☐ Tertiary

6. Occupation: _____

SECTION B: Obstetric History

7. Gravida (Number of pregnancies): _____

8. Parity (Number of deliveries): _____

9. Gestational age (weeks): _____

10. Have you had any blood transfusions before?
☐ Yes ☐ No

11. Any history of complications in previous pregnancies (e.g., miscarriage, stillbirth, HDN)?
☐ Yes ☐ No
If yes, please specify: _____

12. Any previous knowledge about Kell blood group?
☐ Yes ☐ No

13. Have you ever been told you are Kell-positive or Kell-negative?
☐ Yes ☐ No ☐ Not sure

APPENDIX II

INFORMED CONSENT FORM

Project Title: Distribution of Kell Blood Group Among Pregnant women Attending Antenatal care in Central Hospital
Principal Investigator:Jessica Osarugue Igbinosa.
Department of Medical Laboratory Science, University of Benin

Purpose of the Study
This information will help improve knowledge of blood group prevalence and may be useful in preventing complications such as hemolytic disease of the foetus and newborn.

Procedures
About two millilitres of venous blood will be collected from you under aseptic conditions.
The blood sample will be used to determine your Kell blood group.
No extra cost will be incurred by you for participating.

Risks and Discomforts
Minimal discomfort may be felt during blood collection (such as slight pain, bruising, or swelling at the puncture site).
There are no major risks anticipated from participating in this study.

Benefits
Your participation will contribute to medical knowledge and may guide future health interventions.
You may not directly benefit from this study, but the findings could help improve maternal and child health care in the future.

Voluntary Participation and Confidentiality
Your participation in this study is entirely voluntary. You are free to decline or withdraw at any time without giving any reason and without affecting the care you receive at the hospital. Also, all information obtained is strictly confidential. Your name will not appear in any publication. Data will be used for only research purposes

Contact Information
If you have any questions or concerns about this study, please contact:
Principal Investigator: Jessica Osarugue Igbinosa, 08155723556 jessicaigbinosa83@gmail.com]
Statement of Consent
I have read and understood the information provided above(or it has been read and explained to me in a language I understand). I have had the opportunity to ask questions, and they have been answered satisfactorily. I voluntarily agree to participate in this research study.
Participant's Name: .....
Signature: ....
Date: .....
I do not consent to participate in this study

APPENDIX III