THE DISTRIBUTION OF RHESUS E AND e BLOOD GROUP ANTIGEN AMONG PREGNANT WOMEN ATTENDING ANTE NATAL CARE AT CENTRAL HOSPITAL BENIN CITY

BY

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DEPARTMENT OF MEDICAL LABORATORY SCIENCE,

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BEING A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELORS DEGREE IN MEDICAL LABORATORY SCIENCE (BMLS) UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

SUPERVISED BY

PROF. (MRS) E. O. OSIME

SEPTEMBER,2025.

CERTIFICATION

This is to certify that this research work reported in this project work was carried out by OBASOHAN PEACE with Matriculation Number: BMS2001184 in the Department of Medical Laboratory science, School of Basic Medical Sciences, University of Benin 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

I dedicate this project work to God Almighty who has been my origin of strength, wisdom, knowledge and also my beloved family for their support towards the success of this project work.

ACKNOWLEDGEMENT

First and foremost, I give all glory and honor to the Almighty God for His divine guidance, wisdom, strength, and grace that have enabled me to successfully complete this project work. Without His blessings, this achievement would not have been possible.

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Abstract

The Rhesus (Rh) blood group system is one of the most clinically significant after the ABO system, with the E and e antigens playing important roles in transfusion medicine and maternal-fetal health. This study assessed the distribution of Rhesus E and e antigens among pregnant women attending antenatal care at Central Hospital, Benin City, Nigeria. A cross-sectional study design was employed, and a total of 100 pregnant women were recruited. Data on sociodemographic and clinical characteristics were collected through structured proformas, and blood samples were analyzed for the presence of Rh E and e antigens using standard serological techniques. Statistical analysis was performed using SPSS version 27, with descriptive statistics and Chi-square tests applied; significance was set at $p < 0.05$.

The majority of participants were aged 26–35 years (62.0%), of Bini ethnicity (40.0%), and engaged in business (63.0%). Clinically, 32.0% were nulliparous, 53.0% were in their second trimester, and 31.0% were in their first pregnancy. The prevalence of Rhesus E antigen was 15.0%, while Rhesus e antigen was observed in 91.0% of participants. Analysis revealed no statistically significant associations between sociodemographic variables and the prevalence of either antigen ($p > 0.05$). However, a significant association was found between parity and Rhesus e antigen distribution ($X^2 = 11.35$, $df = 5$, $p = 0.045$), with higher positivity observed among women with parity zero and one.

The findings indicate that while Rhesus e antigen is highly prevalent among pregnant women in Benin City, Rhesus E antigen remains relatively low. The significant link between parity and Rhesus e antigen suggests that parity may influence antigen distribution in this population. These results underscore the need for continued monitoring of Rh antigen patterns in pregnant women to inform transfusion services and obstetric care.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

The Rhesus (Rh) blood group system stands as one of the most clinically significant and genetically complex human blood group systems, second only to the ABO system in its importance in transfusion medicine and obstetrics. Discovered in 1940 by Landsteiner and Wiener, the Rh system is defined by a series of antigens, or proteins, located on the surface of red blood cells. The clinical relevance of this system is primarily centered around five major antigens: D, C, c, E, and e (Walker, 2014). The presence or absence of the D antigen, a highly potent immunogen, is the basis for classifying individuals as either Rh positive (D positive) or Rh negative (D negative). The other antigens, C, c, E, and e, are also integral components of the system, and their expression, in various combinations, creates a diverse range of Rh phenotypes (Reid and Mohandas, 2018).The distribution of these Rh antigens is far from uniform across the global population, exhibiting substantial ethnic and geographical variations. These differences are a result of complex evolutionary and genetic factors (Liumbruno and Bennardello, 2017). For instance, the prevalence of Rh-negativity is markedly higher in populations of Caucasian descent, where it affects approximately 15% of individuals. In stark contrast, Rh negativity is a rare occurrence in individuals of Asian ancestry, with a prevalence of less than 1%. Populations of African descent, including those in Nigeria, generally show an intermediate prevalence, typically ranging from 3% to 8% (Daniels, 2013). This geographical heterogeneity is not limited to the D antigen alone; the frequencies of the other major antigens (C, c, E, e) also vary significantly among different ethnic groups. Understanding these population-specific frequencies is not merely an academic exercise; it is a critical component of public health and clinical practice, influencing blood donor recruitment strategies, blood inventory management, and the risk assessment for potential alloimmunization in patients requiring transfusions or during pregnancy (Olaiya *et al.,* 2019).The profound obstetric significance of the Rhesus system lies in the potential for Hemolytic Disease of the Fetus and Newborn (HDFN), a condition that can arise from Rh incompatibility between a mother and her fetus. HDFN occurs when a Rh negative mother is exposed to and becomes sensitized by the Rh-positive red blood cells of her fetus, typically during the first Rh incompatible pregnancy. This sensitization leads to the production of maternal antibodies against the fetal Rh antigen. While the first pregnancy is often unaffected, these maternal antibodies can cross the placenta in subsequent Rh-incompatible pregnancies, targeting and destroying the fetal red blood cells. This destruction leads to fetal anemia, which can range from mild to severe, and in the most severe cases, can result in hydrops fetalis, stillbirth, or significant neonatal morbidity and mortality (Gottstein and Wahn, 2017). The D antigen is the most frequent cause of severe HDFN, but antibodies against the C, c, E, and e antigens can also cause HDFN, albeit usually in a less severe form. The potential for such complications underscores the absolute necessity for routine antenatal screening of pregnant women to determine their Rh status (Walker, 2014).Preventative measures against Rh sensitization have been one of the most significant advances in modern obstetrics. The administration of Rh immune globulin (RhIG) to Rh negative mothers at specific intervals during pregnancy and after delivery of a Rh POSITIVE baby has been highly successful in preventing maternal sensitization and, consequently, subsequent HDFN (Wylie, 2016). This preventative strategy, however, is contingent on accurate and timely Rh antigen typing of pregnant women. Beyond the D antigen, the identification of other Rh antigens (C, c, E, e) is also gaining importance. Alloimmunization to these other antigens can occur in Rh positive women who are exposed to a fetus with a different Rh phenotype. For example, a Rh positive mother who lacks the E antigen can become sensitized to a E-positive fetus (Sorensen and Nørgaard, 2018). Such cases, though less common than D-related HDFN, require careful monitoring and management, further emphasizing

the need for comprehensive Rh phenotyping (Olaiya *et al.,* 2019).In Nigeria, a country characterized by its immense ethnic diversity and a large population, the demographic distribution of blood groups, including the Rh system, is a matter of critical public health importance. A number of studies conducted in different regions of the country have consistently reported a low prevalence of Rh negativity compared to Western populations (Egesie *et al.,* 2018; Olaiya *et al.,* 2019). However, a significant gap remains in the detailed knowledge of the distribution of the other major Rhesus antigens, E and e, within specific local populations. The Central Hospital in Benin City, a major urban center and a key referral hospital for Edo State and its surrounding regions, serves a highly diverse population of pregnant women. The absence of specific, localized data on the distribution of E, and e antigens in this population presents a substantial challenge to effective clinical practice. Clinicians may be working with incomplete information, which can compromise the ability to accurately assess HDFN risk, manage blood product requirements, and implement targeted preventative interventions. Therefore, a comprehensive study of the prevalence and distribution of these specific antigens in pregnant women attending antenatal care at Central Hospital, Benin City, is essential to provide an evidence-based foundation for improving obstetric care and public health outcomes in the region. This study aims to fill this critical void by providing a detailed profile of the Rhesus antigen distribution, which will be invaluable for informing clinical decisions, optimizing blood bank services, and developing local guidelines for the management of pregnant women.

1.2 Statement of the Problem

The Rhesus blood group system, particularly the D antigen, is a major cause of Hemolytic Disease of the Fetus and Newborn (HDFN). While Rh negativity is relatively low in Nigeria compared to Western countries, the sheer volume of births and the ethnic diversity of the population mean that a significant number of Rh incompatible pregnancies still occur. The lack of comprehensive data on the distribution of E and e antigens among pregnant women in Benin City makes it difficult to accurately assess the local risk of HDFN and to develop a robust, evidence-based strategy for its prevention and management. This knowledge gap can lead to inadequate screening protocols, delayed or incorrect diagnoses, and a suboptimal use of resources, including Rh immune globulin (RhIG). Consequently, this can result in preventable maternal and fetal morbidity and mortality. Therefore, there is a critical need to determine the prevalence of these specific Rhesus antigens in the pregnant population of Benin City to inform clinical practice and public health policy.

women?

1.3 Aim of study

The aim of this study is to determine the distribution of the Rhesus E and e blood group antigens among pregnant women attending antenatal care at Central Hospital, Benin City.

1.4 Specific Objectives

1. To determine the prevalence of Rhesus E and e antigens among pregnant women among the study population

2. To assess the distribution pattern of Rhesus E and e blood groups in relation to maternal age, parity, and gestational age.

3.To raise awareness of the rhesus E and e blood group antigen in maternal health management.

1.5 Research Question

1. What is the prevalence of Rhesus E and e antigens among pregnant women in the study population?

2. How are the Rhesus E and e blood groups distributed in relation to maternal age, parity, and gestational age?

3. What is the level of awareness of the Rhesus E and e blood group antigens in maternal health management among the study population?

1.6 Research hypothesis

Null Hypothesis (H_o):There is no significant difference in the distribution of Rhesus E and e blood group antigens among pregnant women attending ante natal care.

Alternate Hypothesis (H_1):There is a significant difference in the distribution of Rhesus E and e blood group antigens among pregnant women attending antenatal care .

1.7 Significance of the Study

The findings of this study will be of significant value to several stakeholders. For clinicians and healthcare providers at Central Hospital, Benin City, the data will provide a clear picture of the local risk of Rh incompatibility, enabling them to implement more targeted and effective screening and management protocols. This will lead to better patient outcomes and a reduction in the incidence of

HDFN. For blood bank managers, the study's findings on the distribution of these antigens will be crucial for optimizing blood inventory management and ensuring the availability of compatible blood for transfusions, particularly in cases of massive hemorrhage or other emergencies. The study will also be of value to public health policymakers, who can use the data to design and implement evidence based antenatal care guidelines and RhIG distribution strategies. Finally, this research will contribute to the global and regional body of knowledge on Rhesus antigen distribution, providing a reference for future studies and comparative analyses.

1.8 Scope of the Study

This study is focused on the distribution of E and e Rhesus antigens among pregnant women attending antenatal care at Central Hospital, Benin City, Nigeria. The study will involve the collection of blood samples from consenting pregnant women, followed by laboratory analysis to determine their Rh antigen status. The study's scope will focus on the determination of the prevalence of these specific antigens and their association with demographic factors.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Rhesus blood group system

The Rhesus (Rh) blood group system is a classification system for human blood based on the presence or absence of specific antigens found on the surface of red blood cells (RBCs). It is the second most important blood group system after the ABO system in terms of clinical significance, particularly in transfusion medicine and maternal-fetal medicine (Rosenkrans, Zubair, and Doyal, 2023). The system includes over 50 distinct antigens, but five of these—D, C, c, E, and e—are most clinically relevant because of their high immunogenic potential, meaning they can easily stimulate an immune response in a person who lacks them (Ramsey, 2025).

Immunogenicity is the ability of a substance (usually a protein) to provoke an immune response. Among the Rh antigens, D antigen is the most immunogenic. Exposure of a D-negative individual to D-positive red blood cells, whether through blood transfusion or pregnancy, can lead to the formation of anti-D antibodies, which may attack and destroy D-positive red blood cells in future exposures (Rosenkrans *et al.,* 2023). This makes Rh typing especially critical in blood transfusion practices and in pregnancy care, where mismatched Rh status between mother and fetus can lead to serious complications like hemolytic disease of the fetus and newborn (HDFN) (Ramsey, 2025).

Historically, the Rh system was discovered after clinicians observed severe hemolytic reactions in patients who had received transfusions that were ABO-compatible but still caused complications. These unexplained reactions pointed to the existence of another antigen system—eventually identified as the Rh system (Levine and Stetson, 1939). Since then, understanding and managing Rh incompatibilities has become a cornerstone of safe blood transfusion and maternal care (Rosenkrans *et al.,* 2023).

2.1.1 Rh-Positive and Rh-Negative

In everyday clinical language, people are often classified as either "Rh-positive" or "Rh-negative." This binary classification refers specifically to the presence or absence of the D antigen, which is the most immunogenic component of the Rh system (Ramsey, 2025). A person is considered Rh-positive if their red blood cells express the D antigen, and Rh-negative if their red blood cells lack it. This classification is crucial when determining blood donor compatibility, and also in assessing the risk of maternal-fetal Rh incompatibility.

However, this simplified terminology tends to overlook the complexity of the Rh system. In reality, an individual's Rh phenotype is determined by the expression of not just D, but also the other major antigens: C, c, E, and e. For example, someone may be D-positive but have different combinations of the other antigens, such as Cc or Ee. These antigen combinations can be relevant in transfusion reactions and in identifying specific alloantibodies in a sensitized patient (Rosenkrans *et al.*, 2023).

Even more importantly, an individual's Rh genotype—the underlying genetic makeup—may reveal variant forms of the Rh antigens that are not detectable by standard serological tests. For instance, some people carry "weak D" or "partial D" variants, where the D antigen is either expressed at lower density or is missing specific antigenic components (Ramsey, 2025). These variants can lead to diagnostic confusion: a person might be classified as Rh-negative using traditional serology but could actually express a weak form of the D antigen and thus might not need anti-D prophylaxis (in pregnancy) or might serve as a Rh-positive donor in some contexts (Castilho, 2022).

Therefore, clinicians and laboratory scientists must go beyond the Rh-positive/negative terminology and consider full Rh phenotyping and even molecular genotyping where necessary, particularly in complex cases such as blood transfusion for multi-transfused patients or management of pregnancies in alloimmunized mothers (Rosenkrans *et al.,* 2023; Ramsey, 2025).

2.2 Importance of the Rhesus Blood Group System in Transfusion and Maternal-Fetal Medicine

2.2.1 Importance in Transfusion Medicine

a. Prevention of Hemolytic Transfusion Reactions (HTRs)

If RhD-negative individuals receive RhD-positive red blood cells, especially more than once, their immune system may develop anti-D antibodies. On subsequent exposure, these antibodies cause

extravascular hemolysis, leading to potentially life-threatening transfusion reactions (Rosenkrans *et al.,* 2023).

b. Role in Crossmatching and Alloimmunization

Alloimmunization to Rh antigens, especially in multi-transfused patients (e.g., those with sickle cell disease or thalassemia), complicates transfusion management by limiting compatible blood availability. Strict Rh antigen matching—particularly for D, C, E—is vital to reduce alloimmunization (Sanusi Nurul 'Adani *et al.,* 2024).

c. Blood Typing and Identification of Weak D Phenotypes

Some individuals possess partial or weak D variants, which may be serologically misclassified as RhD-negative. Molecular genotyping helps prevent inappropriate administration of RhD-positive blood (Clausen and van der Schoot, 2025).

2.2.2 Importance in Maternal-Fetal Medicine

a. Hemolytic Disease of the Fetus and Newborn (HDFN)

In Rh incompatibility, an Rh-negative mother carrying an Rh-positive fetus may become sensitized during pregnancy or delivery. In subsequent pregnancies, maternal anti-D IgG antibodies can cross the placenta and destroy fetal red blood cells, causing fetal anemia, hydrops fetalis, or even intrauterine death (Sanusi Nurul 'Adani *et al*., 2024).

c. Antenatal Screening and Prophylaxis

Standard antenatal protocols include:

RhD typing at first visit,

Antibody screening during early and late pregnancy,

Administration of anti-D immunoglobulin at 28 weeks and post-delivery if the newborn is Rh-positive.

The use of anti-D immunoglobulin has dramatically reduced Rh sensitization rates in countries with well-implemented antenatal care programs (Sugrue *et al.*, 2024).

d. Noninvasive Fetal RhD Genotyping

Recent advances in cell-free fetal DNA testing allow non-invasive prenatal prediction of fetal RhD status from maternal blood. This helps in targeted administration of RhIG, avoiding unnecessary prophylaxis in

Rh-negative women carrying Rh-negative fetuses (Clausen and van der Schoot, 2025; van ’t Oever *et al.,* 2025).

2.3 Genetic Basis of Rh Antigens

The RH blood group system consists principally of two homologous genes: RHD (which encodes the D antigen) and RHCE (which encodes the C, c, E, e antigen combinations). (Rouillac and Colin, 1995;Owen, 2004)

These genes are located on the short arm of human chromosome 1, in region 1p34‑ p36. . Both genes have 10 exons each.

RHD and RHCE are in tail‑ to‑ tail orientation (their 3’ ends face one another), with a small intervening gene called SMP1 (also called “SMP‑ 1” or “RH55”) located interspersed near the 3’ end of RHCE. (Moran *et al.,* 2008)

RHD is considered to have arisen from a duplication of an ancestral RHCE‑ like gene. Thus RHCE is regarded as ancestral, and RHD the “duplicate” after an evolutionary event. (Moran *et al.,* 2008)

There are flanking repeats, called Rhesus boxes, approximately 9 kilobases (kb) each, upstream (5’) and downstream (3’) of the RHD gene; these are highly homologous. These Rhesus boxes play a major role in causing deletions of RHD via unequal homologous recombination. (Moran *et al.,* 2008; Karafin *et al.,* 2022)

The physical distance separating RHD and RHCE is of the order of ~ 30 kb, including the Rhesus boxes and SMP1 and intergenic sequences. (Wagner and Flegel, 2000).

The sequence similarity between RHD and RHCE is high — often quoted ~ 90‑ 95% in many exons and introns. This homology is the basis for crossovers, gene conversions, hybrid gene formation, etc. (Zhang *et al.,* 2022).

2.3.1. Mechanisms of Variation: How Different Alleles Arise

Because of the structure and homology, multiple mechanisms produce variation in the RH locus. These affect D vs d phenotypes, partial or weak D, variants in RHCE (C, c, E, e), hybrid genes, etc.

Some key mechanisms:

1. Gene deletion

The most straightforward cause of the D- negative phenotype is complete deletion of the RHD gene, typically via unequal homologous recombination between the upstream and downstream Rhesus boxes. In effect, the two boxes recombine and excise the RHD coding region. (Wagner and Flegel 2000).

Studies (e.g. in many European populations) show that a large proportion of RhD- negative individuals are homozygous for this deletion. (North India study, 2022) .

2. Pseudogenes and non- functional alleles

Some RHD alleles are present but have mutations (e.g. premature stop codons, frameshifts, insertions, deletions) that render the encoded protein nonfunctional or severely reduced in expression. (India study, 2022)

3. Hybrid / Recombinant Genes

Because of high homology, recombination between RHD and RHCE leads to hybrid genes: parts of RHD replaced by RHCE sequence or vice versa. Sometimes exons 3- 10 of RHD replaced by RHCE or the reverse. (Li *et al.*, 2024)

For example, in the study "Integrated analyses reveal unexpected complex inversion and recombination in RH genes" (Li et al., 2024), the authors identified novel RHCE recombinant haplotypes such as RHCE*Ce(1- 2)- D(3- 10) and a more complex one
RHCE*Ce(1- 2)- D(3- 10)- Ce(10- 8)- Ce(3- 10), which affect expression of RhCE protein. (Li, Wang *et al*., 2024)

4. Point mutations / missense / nonsense

Single nucleotide polymorphisms (SNPs) in exons that encode extracellular loops change amino acids; these can alter antigenic epitopes (partial D / weak D) or change the stability or folding of the protein. (Zhang *et al*., 2022)

5. Deletion of exons or partial gene segments

Some alleles have deletion of individual exons or parts thereof (for example exclusion of exon 9, etc.), or insertions / deletions in introns affecting splicing. (India study, 2022)

6. Structural rearrangements: Inversions, Copy Number Variation (CNV)

Recent studies using long- read sequencing and optical mapping have uncovered more complex rearrangements: inversions between introns, CNV (loss or gain), translocations, etc. (Li *et al.,* 2024; Zhang *et al*., 2022)

2.3.2 Mendelian Principles of E/e Inheritance

1.Autosomal Inheritance of RHCE

The inheritance of E and e antigens follows classical Mendelian autosomal inheritance. The gene RHCE located on chromosome 1 encodes the RhCE protein, which carries both C/c and E/e antigens (Flegel, 2007). Unlike sex-linked traits, where genes are located on sex chromosomes and show patterns such as X-linked or Y-linked inheritance, the RHCE gene is autosomal, meaning individuals inherit one copy from each parent regardless of sex.

Because of this, both males and females have an equal probability of inheriting any allele of RHCE, including those encoding E or e antigens.

2.Codominant Expression of E and e Alleles

The E and e antigens are encoded by two alleles at a single genetic locus—the RHCE gene. These alleles are described as codominant because if an individual inherits both an E allele and an e allele (heterozygous), both antigens will be expressed simultaneously on the surface of their red blood cells (Fisher-Race model). This is distinct from classic dominant-recessive relationships, where one allele's expression masks the other.

Homozygous E/E genotype: Only the E antigen is expressed; no e antigen appears on RBC surfaces.

Homozygous e/e genotype: Only the e antigen is expressed; no E antigen is present.

Heterozygous E/e genotype: Both E and e antigens are co-expressed in approximately equal amounts on RBCs.

The codominance reflects the molecular mechanism whereby the RHCE alleles produce distinct versions of the RhCE protein differing by a single amino acid substitution, allowing simultaneous display of both antigenic determinants in heterozygotes (Flegel, 2007).

Mendelian Punnett Squares and Predicting Offspring Phenotypes

The simple inheritance of E and e alleles fits well within Mendelian genetics. Since each parent contributes one allele of the RHCE gene, we can use Punnett squares to predict the genotypes and phenotypes of offspring based on parental genotypes.

Example:

If both parents are heterozygous E/e, the potential offspring genotypes and phenotypes are:

Parent 1 \ Parent 2	E	e

E	E/E (25%)	E/e (25%)
e	E/e (25%)	e/e (25%)

Genotype frequencies: 25% E/E, 50% E/e, 25% e/e.

Phenotype frequencies: 25% express only E antigen, 50% express both E and e, and 25% express only e antigen.

If one parent is E/E and the other e/e, all offspring will be E/e heterozygotes, expressing both antigens.

2.3.3 Molecular Genetics of E/e Alleles

RHCE Gene and Protein Structure

The RHCE gene encodes a 12-transmembrane domain protein embedded in the red blood cell membrane. The E and e antigen difference arises from a single nucleotide polymorphism (SNP) in exon 5 of the RHCE gene.

The E allele encodes Proline (Pro) at amino acid position 226.

The e allele encodes Alanine (Ala) at position 226 (Flegel, 2007).

This single amino acid substitution results in a conformational difference on the RhCE protein's extracellular loops, giving rise to distinct antigenic epitopes recognized serologically as E or e.

2.3.4 Allelic Variants and Their Expression

Because the difference between E and e antigens is so small molecularly, variant alleles of RHCE can influence antigen expression levels and serological detection.

Some alleles result in weak or partial expression of E or e antigens.

Other variants may cause complete loss (null phenotype) of antigen expression.

This is especially important for individuals with variant RHCE alleles, who may develop antibodies against E or e antigens if exposed to normal antigen through transfusion or pregnancy (Mouro *et al.,* 2021).

Variant alleles may also cause discrepancies between genotype and phenotype, complicating genetic prediction and clinical management.

2.3.5 Haplotypes and Linkage of RHCE with RHD

Chromosomal Location and Linkage

The genes RHCE and RHD reside in close proximity on chromosome 1p36.11, separated by only about 30 kb of DNA (Flegel, 2007). RHD encodes the D antigen, the most immunogenic antigen in the Rh system.

Because of this close physical proximity, RHCE and RHD genes tend to be inherited together as haplotypes. The most common haplotypes combine particular alleles of RHD with RHCE alleles encoding specific C/c and E/e antigens.

Common Haplotypes

A haplotype is a set of alleles inherited together from a single parent on the same chromosome. In the Rh system, typical haplotypes include combinations of:

RHD gene (D antigen present or absent)

RHCE gene variants encoding C/c and E/e antigens

For example, common haplotypes include:

DCe (RHD with RHCE*ce allele encoding c and e antigens)

DCE (RHD with RHCE*cE allele encoding C and E antigens)

dce (absent RHD gene with RHCE*ce allele encoding c and e)

dCe (absent RHD with RHCE*cE)

The frequencies of these haplotypes vary widely among populations due to evolutionary, demographic, and selective pressures (Flegel, 2007).

2.3.6 Major Rh Antigens E and e

Molecular and Genetic Basis

The E and e antigens are encoded by the RHCE gene. The RHCE gene is one of two closely linked Rh genes, the other being RHD. Both genes each have 10 exons and encode multi-pass transmembrane proteins (~416 amino acids) expressed on RBC membranes. The RHCE gene product carries C, c, E, e antigen specificities. (Daniels, 2009)

The E/e polymorphism arises from single nucleotide changes (single nucleotide polymorphisms, SNPs) in the RHCE gene. A key SNP is 676 G→C, which causes an amino acid substitution Alanine → Proline at position 226 (A226P) in the RHCE protein; this determines whether E or e is expressed. (NCBI Bookshelf, "Blood Group Antigens," as in The Rh Blood Group)

The E antigen has multiple epitopes (regions recognized by different antibodies). Variants ("E variants") show heterogeneity in how monoclonal anti-E antibodies react, because of mutations affecting specific epitopes. For example, studies have defined categories of E variants (e.g. Cat EI, EII, EIII, EIV) associated with different mutations/exon exchanges in RHCE or hybrid structures with RHD. (Kashiwase *et al*., 2000)

A rare antigen E^w (E-w) has been molecularly characterized: the T500A nucleotide change in exon 4 of RHCE (causing Met167→Lys) underlies expression of Ew (which is related to—but distinct from—ordinary E) and causes alloimmunization against the wild-type E. (Scharberg *et al.,*2004)

2.3.7 Protein Structure and Key Functional Regions

The RHCE protein spans the membrane 12 times, with extracellular loops (particularly loops 3 & 4) involved in E/e antigen expression. Mutations in those loops (or in exons encoding them) can weaken or alter antigen expression. (Kashiwase *et al.,* 2000)

For example, in certain E variants, replacement of exons 1-3 (or 2-3) of RHCE by corresponding exons of RHD, or exchanges within exon 5 have been implicated. These can alter the antigen's epitope profile, affecting which monoclonal antibodies recognize the aged RBCs. (Kashiwase *et al.,* 2000)

2.3.8 Fisher-Race vs Wiener Nomenclature: Description of E and e

These two nomenclatures reflect different models of how Rh antigens are inherited and expressed. Below, how E and e are represented in each, with examples.

Aspect.	Fisher-Race Nomenclature	Wiener Nomenclature
Basic model	Three closely linked loci (genes): D/d, C/c, E/e. Each locus has two alleles. Under this model, E (capital) means the allele coding for E antigen is present; e means allele coding for e antigen present. So a haplotype might include E (e.g. DcE) or e (e.g. Dce). A single gene locus (per chromosome) with multiple alleles (agglutinogens) each encoding combinations of D/d, C/c, E/e (and possibly other antigens).	Wiener uses symbols/subscripts/primes to represent these combinations.
Representation of E and e	If the RHCE allele carries E, then "E" appears in the haplotype (e.g., DcE, DCE, cDE, etc.). If the e allele is present, "e" appears (e.g., Dce, dce, etc.). Individuals have two haplotypes	In Wiener, the presence of E is indicated by the "double prime" factor (") in some symbols; the presence of e is indicated by the hr" (or hr prime etc.) depending on which allele. E.g. R_2 in Wiener corresponds to DcE (i.e. E present, e absent on that haplotype). r" corresponds to dcE. E.g. R_2

	(one from each chromosome). So someone might be DcE/Dce etc.	has the Wiener factor indicating E, whereas hr" or similar indicates e. (Medscape "Rh Typing: Overview…" etc.)
How haplotypes including E/e are named	Examples: DcE, DCE, cDE, cDe, etc. Each haplotype shows which of C/c and E/e (plus D/d) are on that chromosome. Fisher- Race is more explicit in labeling E vs e. Wiener notation assigns shorthand names: R_2 = DcE, r" = dcE, etc. The primes or double primes denote the presence of C or E respectively.	For example, in Wiener, "2" or double prime (") factor corresponds to E (presence), while absence or lowercases etc correspond to e (or ce) etc.

(Fisher *et al.*,1944).

Suppose a person has the haplotype DcE / Dce.

Under Fisher- Race: one haplotype has D, c, E; the other has D, c, e. So this person is E- positive (because there is at least one E) and e also present.

Under Wiener: that might correspond to R_2 / R_o (depending on conventions) because DcE is R_2; Dce is R_o. The R_2 allele carries the factor for E; the R_o allele carries e (or ce) factor.

If someone is dce/dce (both haplotypes have e, no E), in Fisher- Race they are E- negative (only e), and in Wiener they might be 'r' or some such allele that indicates absence of E (and e present).

2.4 Antigenicity and Immunogenicity

2.4.1 Antigenicity of RhE and Rhe Antigens

Antigenicity refers to the ability of a molecule, such as the RhE or Rhe antigen, to bind specifically to antibodies or T-cell receptors. The RhE and Rhe antigens are located on the extracellular domains of the RhCE protein, which spans the red blood cell membrane multiple times. Structural variations in the RHCE gene determine whether the E or e antigen is expressed (Malone and Dunsford,1951).

The E antigen differs from the e antigen by specific amino acid substitutions, which alter the antigenic determinants and thus influence the immune recognition (Scharberg *et al.* 2004). This subtle structural difference is sufficient to elicit an immune response when a person lacking the E antigen (E-negative) is exposed to E-positive red cells. The same principle applies to the e antigen, though it is less commonly immunogenic due to its higher prevalence in the population (Daniels, 2013).

2.4.2 Immunogenicity of RhE and Rhe Antigens

Immunogenicity is the ability of an antigen to provoke a host immune response, leading to antibody production. The RhE antigen is highly immunogenic, second only to the RhD antigen in its ability to induce alloantibodies. This means individuals negative for the E antigen who are transfused with E-positive blood or carry a fetus expressing the E antigen can produce anti-E antibodies (Gwaram *et al.* 2023).

In contrast, the e antigen is less immunogenic, partly because it is nearly ubiquitous in many populations, resulting in fewer individuals lacking it and thus less frequent exposure to foreign e antigens (Issitt 1991). However, anti-e antibodies can still develop, especially in multi-transfused patients or during pregnancy.

The development of alloantibodies like anti-E or anti-e involves the recognition of these antigens as foreign by the recipient's immune system, activation of helper T-cells, and subsequent B-cell mediated antibody production. These antibodies are typically IgG, which can cross the placenta, making them clinically significant in HDFN (Daniels 2013).

2.4.3 Mechanisms of Immune Response to RhE and Rhe Antigens

The immune response to RhE and Rhe antigens follows classical alloimmunization pathways:

1. Exposure: Sensitization occurs when an RhE or Rhe antigen-negative individual is exposed to incompatible red blood cells, either via transfusion or fetomaternal hemorrhage during pregnancy (Malone and Dunsford 1951).

2. Recognition: The foreign antigen is processed and presented by antigen-presenting cells (APCs) to helper T lymphocytes.

3. Activation: Helper T cells activate B cells specific for the RhE or Rhe antigen, triggering clonal expansion.

4. Antibody Production: Activated B cells differentiate into plasma cells producing IgG anti-E or anti-e antibodies, which bind to the corresponding antigen on red cells (Daniels,2013).

5. Hemolysis: Upon re-exposure to the antigen, these antibodies mediate hemolysis through complement activation or opsonization, leading to destruction of red blood cells (Daniels 2013; Gwaram et al. 2023).

2.4.4 Antibody formation (Anti-E and Anti-e)

When a person who lacks the E or e antigen (encoded by the RHCE gene) is exposed to that antigen through transfusion or pregnancy, an immune response can be triggered. This process, known as alloimmunization, leads to the formation of IgG antibodies against the foreign antigen. These antibodies are clinically significant, particularly during pregnancy or blood transfusions.

1. Sensitization Events

The primary sensitization events involve:

Red cell transfusion: Individuals who are E-negative or e-negative may develop anti-E or anti-e antibodies after receiving blood that expresses the E or e antigen (Fridey and Westhoff, 2022).

Pregnancy: Alloimmunization may also occur when a pregnant woman lacks the E or e antigen and carries a fetus who inherits the antigen from the father. Fetal red blood cells may enter the maternal circulation through fetomaternal hemorrhage during childbirth, miscarriage, trauma, or invasive procedures (Chao *et al.,* 2009; Joy *et al.,* 2020). This introduces the antigen into the maternal system, potentially triggering immune recognition.

2. Immune Recognition and Antibody Formation

Once exposed to the antigen, the following immune processes are initiated:

Antigen presentation: Maternal antigen-presenting cells (APCs), such as dendritic cells, phagocytose fetal red blood cells or their fragments, processing the antigen and presenting peptides on MHC class II molecules to CD4+ T helper cells (Daniels, 2022).

B-cell activation and class switching: Naïve B cells that recognize the antigen receive T-cell help and undergo proliferation, class switch recombination (mostly to IgG), affinity maturation, and eventually form plasma cells that secrete IgG antibodies (Hassall *et al.,* 2023).

IgG antibodies specific to the E or e antigen can cross the placenta in future pregnancies and cause hemolytic disease of the fetus and newborn (HDFN) by targeting fetal RBCs (Soler-Noda *et al*., 2021).

3. Memory Response and Anamnestic Reaction

Once a person is alloimmunized:Memory B cells remain primed to respond to the same antigen. Upon re-exposure, either through another pregnancy or transfusion, the immune system mounts a stronger and faster response, producing a higher titer of anti-E or anti-e antibodies (Turbil *et al.,* 2023).

This secondary immune response explains why subsequent pregnancies can be more dangerous, even if the initial antibody levels were low or undetectable (Joy *et al.,* 2020).

4. Antigen Density, Dosage Effect, and Variant Expression

The degree to which the immune system reacts also depends on:

Antigen density: The E/e antigen density on red cells influences immunogenicity. Individuals who are homozygous for the antigen (e.g., E/E or e/e) express more antigen and may provoke stronger alloimmune responses in an antigen-negative recipient (Fridey and Westhoff, 2022).

Dosage effect: Stronger immune reactions are often observed when the fetus or donor is homozygous for the antigen. The “dosage effect” refers to this variation in antigen density based on zygosity (Daniels, 2022).

RHCE variant alleles: Some individuals may carry partial or weak forms of the E or e antigen, which may not be detectable with routine serology. These variant alleles can still induce alloimmunization or lead to misclassification of antigen status, especially in multiethnic populations (Westhoff and Denomme, 2022).

Silent exposure risk: Variant antigens may evade detection in standard typing but still stimulate antibody formation if transfused into a negative recipient (Hassall *et al.,* 2023).

2.5 Clinical Significance of Rh E and e Antigens in Pregnancy

2.5.1 Role in Alloimmunization – Immune Response due to E/e Incompatibility

Mechanism of Alloimmunization to E/e Antigens

Alloimmunization in pregnancy is initiated when fetal red blood cells carrying paternal antigens—E or e in this context—enter the maternal circulation. This can happen due to small fetomaternal hemorrhages during pregnancy, miscarriage, trauma, delivery, or invasive procedures such as chorionic villus sampling (CVS) or amniocentesis (Adesina *et al.,* 2024). If the mother lacks the E or e antigen, her immune system may recognize these antigens as non-self and initiate an immune response.

The primary immune response involves antigen presentation by maternal antigen-presenting cells to T-helper cells, which activate B-cells to produce IgM antibodies. However, these cannot cross the placenta. With continued or subsequent exposure, a class switch occurs to IgG antibodies, which are capable of

crossing the placenta and binding fetal RBCs that express the antigen, leading to hemolysis (Ssenyonga *et al.,* 2023).

The secondary immune response is faster and more robust. Maternal IgG antibodies bind to the fetal red cells expressing the E or e antigen and facilitate their clearance by fetal macrophages in the spleen and liver. The severity of hemolysis depends on several factors, including the antibody titer, the subclass (IgG1 vs. IgG3), and the density of antigen expression on fetal cells (Wang *et al.,* 2022).

2.5.2 Hemolytic Disease of the Fetus and Newborn (HDFN)

1.Pathophysiology of HDFN Due to Anti-E or Anti-e

Hemolytic Disease of the Fetus and Newborn (HDFN) arises from an immunological incompatibility between the mother and fetus regarding red blood cell (RBC) antigens. The Rh blood group system is among the most clinically significant in this context, and while the RhD antigen is the most commonly implicated, other antigens like E and e can also cause HDFN (Chuang & Chang, 2022). Anti-E and anti-e antibodies develop when a mother lacking these antigens becomes sensitized by exposure to fetal red cells expressing them, often during a previous pregnancy or blood transfusion (Drozdowska-Szymczak *et al.,* 2024).

2.Immune Sensitization and Antibody Production

The sensitization process begins with the transplacental passage of fetal RBCs into maternal circulation. If the mother's immune system recognizes these RBCs as foreign due to the presence of E or e antigens that the mother lacks, B cells in the maternal immune system produce specific IgG antibodies against these antigens (Chuang and Chang, 2022). These IgG antibodies are capable of crossing the placenta due to their ability to bind to the neonatal Fc receptor (FcRn) in placental syncytiotrophoblasts.

The production of anti-E or anti-e antibodies often remains subclinical in initial pregnancies but may have increasingly severe consequences in subsequent pregnancies. The primary sensitization event primes the immune system, and subsequent exposures elicit a robust secondary immune response with higher antibody titers (Drozdowska-Szymczak *et al.,* 2024).

3.Mechanisms of Fetal RBC Destruction

Once maternal IgG antibodies reach the fetal circulation, they bind to the specific antigens on fetal RBC membranes. This antigen-antibody complex then activates the fetal mononuclear phagocyte system, primarily in the spleen and liver, leading to extravascular hemolysis (Yousuf *et al.,* 2012). The destruction of RBCs results in fetal anemia, which can be mild or severe depending on the extent of hemolysis.

Concurrently, the degradation of hemoglobin from lysed RBCs produces bilirubin. The fetus has a limited ability to conjugate and eliminate bilirubin due to immature hepatic enzyme systems, leading to hyperbilirubinemia. If uncorrected, elevated bilirubin levels may cause kernicterus, a neurotoxic condition with potential lifelong neurological sequelae (Chuang and Chang, 2022).

4.Differences Between Anti-E and Anti-e Antibodies

Although both anti-E and anti-e belong to the Rh system, their immunogenicity and the clinical course of HDFN they cause differ. Anti-E antibodies tend to have moderate immunogenicity but can cause severe HDFN, especially if antibody titers are high (Drozdowska-Szymczak *et al.,* 2024). Anti-e antibodies are generally less immunogenic, and HDFN cases due to anti-e are often milder; however, severe cases have been reported (Chuang and Chang, 2022).

The antigen density and the expression pattern on fetal RBCs affect the severity of hemolysis. E antigen expression is variable but usually more consistent than e, which is highly prevalent in most populations, reducing the likelihood of maternal anti-e formation unless rare antigen-negative status occurs (Yousuf *et al*., 2012).

5.Severity Compared to RhD-Related HDFN

RhD incompatibility remains the most common cause of severe HDFN, largely due to the immunogenic nature of the RhD antigen and the frequency of RhD-negative mothers (Chuang and Chang, 2022). However, HDFN due to anti-E or anti-e antibodies, while less common, can sometimes present with comparable clinical severity.

6. Epidemiology and Incidence

RhD-related HDFN incidence has decreased due to widespread use of anti-D immunoglobulin prophylaxis, which prevents maternal sensitization. In contrast, non-RhD antibodies such as anti-E or anti-e are not prevented by this prophylaxis and remain important causes of alloimmunization in pregnancy (Drozdowska-Szymczak *et al.,* 2024). Studies report that non-RhD alloimmunization accounts for up to 20-25% of all alloimmune HDFN cases (Yousuf *et al., 2012).*

7. Clinical Severity and Outcomes

While many anti-E or anti-e-mediated cases are mild or moderate, severe disease can occur, particularly with high antibody titers or in cases of multiple alloantibodies. For example, Chuang and Chang (2022) reported a fatal case of anti-E alloimmunization with very high antibody titers, leading to severe fetal anemia and neonatal death.

Comparatively, RhD-related HDFN tends to have a more predictable clinical course, with established protocols for prevention and treatment (Drozdowska-Szymczak *et al.,* 2024). Non-RhD antibodies can be unpredictable, with some cases rapidly progressing to severe anemia and hydrops fetalis even with moderate titers, necessitating vigilant antenatal surveillance.

Pathophysiological Differences Influencing Severity

The RhD antigen is highly immunogenic, eliciting a strong maternal immune response. Anti-E and anti-e antibodies generally demonstrate weaker immunogenicity; however, the clinical impact depends on individual immune responses, antigen density, and fetal susceptibility (Yousuf *et al.,* 2012).

Moreover, anti-E is often associated with milder anemia but can cause severe hemolysis in rare cases. Anti-e, due to its high prevalence in most populations, is less likely to cause sensitization but can contribute to hemolysis when present (Chuang and Chang, 2022).

2.6 Importance of Rhesus Typing in Antenatal Care

Rhesus (Rh) typing is a crucial component of antenatal care, especially in identifying and managing Rh incompatibility between the mother and fetus. Early detection of Rh-negative status enables healthcare providers to implement preventive strategies to protect maternal and fetal health. The following are five key reasons why Rh typing is essential during pregnancy:

1.Prevention of Hemolytic Disease of the Fetus and Newborn (HDFN). When an Rh-negative mother carries an Rh-positive fetus, there is a risk of maternal sensitization, where the mother's immune system produces antibodies against the Rh antigen. These antibodies can cross the placenta in future pregnancies and attack fetal red blood cells, leading to anemia, jaundice, hydrops fetalis, or even intrauterine death. Early detection allows for appropriate intervention, such as administration of anti-D immunoglobulin, to prevent sensitization (Aliyo *et al.,* 2023).

2.Timely Rh typing enables the appropriate and timely administration of anti-D immunoglobulin. Anti-D prophylaxis is effective when administered after sensitizing events, during routine antenatal care, or immediately after delivery if the baby is Rh-positive. This intervention significantly reduces the risk of alloimmunization. However, studies show that awareness of this protocol remains low in some regions. For instance, a study in Saudi Arabia revealed that only 41.7% of pregnant women understood the importance of anti-D administration (Yahia *et al.,* 2020).

3.Rh typing facilitates identification of other red cell antibodies beyond anti-D, allowing for better monitoring of alloimmunization risk. Alloantibodies against other Rh antigens (e.g., C, E, c, e) or other blood group systems can also lead to fetal hemolysis. A study in South-Western Nigeria emphasized that many Rh-negative women also possessed clinically significant alloantibodies, which could complicate pregnancy if not detected early (Ibrahim *et al.,* 2024). Therefore, antibody screening should accompany Rh typing to manage these risks effectively.

2.7 Global and Regional Prevalence of RhE and Rhe Antigens

South India (Blood Donors)

A study conducted among 1,200 voluntary blood donors in South India (2017–2019) reported that the E antigen was present in 19.3%, while the e antigen was highly prevalent at 98.8%. This study highlights the low frequency of E and near-universal presence of e in the South Indian donor population (Asian Journal of Transfusion Science, 2022) .

Assam, India

More recent data from a 2024 donor study in Assam indicated that the e antigen remained the most prevalent at 97%, while E antigen was the least common at 17.3%. These frequencies align with findings across India and globally (Dr. D.Y. Patil Vidyapeeth, 2024) .

Nigeria (Multi-ethnic Cohort)

A 2018 study in Nigeria, investigating 302 multi-ethnic healthy adults, found the E antigen prevalence at 19.5% and e antigen at 97.4%. The most common Rh phenotype was Dce, and importantly, no K antigen was detected (Jeremiah et al., 2018) .

Nigeria – Sokoto Region (Pregnant Women)

Among pregnant women in Sokoto, Nigeria, e antigen prevalence was 98.5%, though c antigen data were more prominent; details for E were not the main focus in this source (Ommega Online, year not specified) .

Uganda (Pregnant Women)

A cross-sectional study conducted from August 2020 to July 2021 among 1,369 pregnant women in southwestern Uganda found that the e antigen was almost universal at 99.8%, while the E antigen was present in only 19.66% of samples (Mbalibulha *et al., 2022) .*

Saudi Arabia (Riyadh, Blood Donors 2019)

In a 2022 retrospective study analyzing 4,675 blood donor samples from Riyadh (collected in early 2019), the prevalence of E antigen was 26% and e antigen 97%, demonstrating a slightly higher E frequency in this population (Hematology, Transfusion and Cell Therapy, 2022) .

Saudi Arabia – Samtah Region (Multiple Ethnicities)

A study (January 2019–August 2020) of 3,863 blood donors in the Samtah (Jazan) region revealed ethnic differences: E antigen was 19.5% among Yemeni donors, with e antigen extremely high at 99.2%. Variations among other ethnic groups were also noted (PubMed, 2022) .

Nigeria – Calabar (Blood Donors)

In 2020, a cross-sectional study at the University of Calabar Teaching Hospital analyzed 130 blood donors, finding that while “E” and “e” were not the most highlighted, the "c" antigen was most prevalent (98.5%), and "C" was least (30.7%)—with “E” notably less common though exact percentages were not specified *(*Etura *et al., 2020) .*

Summary Table

Region / Population.	E Antigen (%)	e Antigen (%)
South India (donors, 2017–19)	19.3	98.8
Assam, India (donors, 2024)	17.3	97.0
Nigeria (multi-ethnic cohort, 2018)	19.5	97.4
Sokoto, Nigeria (pregnant women)	—	98.5
Uganda (pregnant women, 2022)	19.66a	99.8
Riyadh, Saudi Arabia (donors, 2019 samples)	26.0	97.0
Samtah, Saudi Arabia (Yemeni donors)	19.5	99.2
Calabar, Nigeria (donors, 2020)	Low/not specified	—

Insights and Clinical Implications

1. Consistent Pattern

Across these studies—from South Asia to Africa and the Middle East—the e antigen is nearly universal, usually exceeding 97–99%, while the E antigen is consistently less common, typically in the 17–26% range.

2. Regional Variation

The somewhat elevated E antigen (26%) in Riyadh suggests regional differences possibly due to genetic admixture or sampling characteristics (Alalshaikh *et al.*,2022)

3. Transfusion Strategy and Alloimmunization

Given the low prevalence of E antigen, E-negative blood is widely available, but E-positive recipients may still develop anti-E alloantibodies—which are clinically significant. Conversely, given the near-universal presence of the e antigen, e-negative units are exceedingly rare, raising challenges in cases of anti- e antibodies.

4. Need for Local Antigen Databases

The observed regional differences underscore the importance of population-specific antigen frequency data, vital for transfusion safety, alloimmunization prevention, and pregnancy management (e.g., choosing Rh prophylaxis) in diverse settings.

2.8 Identification of Gaps: Prevalence of Rhesus E and e Antigens Among Pregnant Women Attending Antenatal Clinics at Central Hospital, Benin City

The Rh blood group system is clinically significant in obstetrics due to its role in alloimmunization and hemolytic disease of the fetus and newborn (HDFN). While extensive research has been conducted on the distribution of Rh antigens, particularly the D antigen, across various populations in Nigeria, studies focusing specifically on the prevalence of Rh E and e antigens among pregnant women remain limited. This paucity of data is particularly evident concerning pregnant women attending antenatal clinics at Central Hospital, Benin City, creating a critical knowledge gap in this geographic and demographic context *(*Buhari *et al., 2023).*

Research conducted in different regions of Nigeria has shed some light on the prevalence of these antigens. For example, Buhari et al. (2023) reported a high prevalence of Rh e antigen (90.8%) among pregnant women in Port Harcourt, emphasizing its clinical importance in transfusion medicine and the management of HDFN. Similarly, studies in Sokoto identified the prevalence of Rh c and e antigens as 92% and 98.5%, respectively *(*Abubakar *et al., 2022).* These findings underscore the widespread distribution of these antigens in Nigerian populations and highlight the necessity of routine antigen typing to prevent alloimmunization during pregnancy. Despite these insights, the applicability of such data to Benin City remains unclear due to possible ethnic, genetic, and environmental variations that influence antigen prevalence (Ibrahim and Musa*, 2021).*

The absence of localized data from Central Hospital, Benin City, represents a significant limitation in both clinical practice and research. Without precise knowledge of the prevalence of Rh E and e antigens in this population, healthcare providers may face challenges in adequately screening and managing pregnant women at risk of alloimmunization. This lack of information may contribute to missed opportunities for timely interventions, such as immunoprophylaxis, which could prevent adverse pregnancy outcomes related to Rh incompatibility (Okonkwo and Eze, 2020).

Moreover, the inconsistency and scarcity of data across different Nigerian regions suggest the need for a comprehensive study targeting Central Hospital in Benin City. Filling this gap is crucial not only for improving prenatal care but also for establishing regional blood bank policies and transfusion protocols that reflect the antigenic profile of the local population. Understanding the distribution of Rh E and e antigens will aid in developing strategies to minimize transfusion-related complications and improve maternal and neonatal health outcomes (Chukwu *et al., 2024).*

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in the university of Benin teaching hospital (ubth) with samples collected from Central hospital Benin city, Edo State.

Edo State is one of the 36 states of Nigeria, located in the South-South geopolitical zone of the country. It was created in 1991 from the former Bendel State, with Benin City serving as the capital. The state is

bounded in the north and east by Kogi and Anambra States, to the south-east by Delta State, and to the west by Ondo State. Edo State also has international boundaries with the Republic of Benin to the west.

Geographically, the state covers a landmass of about 17,802 square kilometers and lies largely within the tropical rainforest belt of southern Nigeria. It experiences a tropical climate with two major seasons: the rainy season (April to October) and the dry season (November to March), moderated by the harmattan winds in the peak of the dry season.

The population of Edo State is estimated to be over 4 million people, with diverse ethnic groups including the Edo (or Bini), Esan, Etsako, Owan, and Akoko Edo. The predominant language is Edo, although English is widely spoken as the official language. Culturally, Edo State is renowned for its rich history, being the seat of the ancient Benin Kingdom, famous for its advanced system of governance, art, and bronze works which remain globally recognized.

Economically, the state is endowed with natural resources such as crude oil, limestone, clay, and other solid minerals. Agriculture also plays a vital role in the economy, with crops such as cassava, yam, maize, and oil palm cultivated across the state.

Health facilities in Edo State include both public and private hospitals, with the University of Benin Teaching Hospital (UBTH) and Central Hospital Benin serving as major referral centers. These institutions provide tertiary healthcare and also serve as research and training centers for medical professionals.

3.2 Study Design

This study employs a cross-sectional descriptive design to determine the distribution of the Rhesus E and e blood group antigens among pregnant women attending antenatal care at Central Hospital, Benin City.

3.3 Study Population

The study population consist of pregnant women attending antenatal care (ANC) clinics at Central Hospital, Benin City. These women are of varying ages, gestational stages, and socio-demographic backgrounds, reflecting the diversity of the pregnant population served by the hospital.

3.4 Inclusion Criteria

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

2.Women aged 18 years and above.

3.Women who provide informed consent to participate in the study.

4.Pregnant women at any gestational age.

3.5 Exclusion Criteria

1.Pregnant women who decline to give informed consent.

2.Women with known hematological disorders or immunological diseases that could affect blood group antigen expression.

3.Women with incomplete antenatal records or those unavailable for follow-up during the study period.

4..Non-pregnant women and pregnant women attending other health facilities.

3.6 Sample Size Determination

The sample size will be calculated using the formula for descriptive cross-sectional studies:

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

Where:

n = required sample size

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

p = estimated prevalence of E and e blood group in the population (5%)

d = margin of error (0.05)

3l

Calculation

$n = (1.96)^2 x\ 0.05\ (1-0.05)/(0.05)^2$

n= 72.96 ~73

A total of one hundred samples would be collected

3.7 Ethical Approval

Ethical approval was obtained from the Ethical Review Committee of Edo state Ministry of Health Benin City.

3.8 Informed consent

Informed consent was obtained from all participants prior to enrollment in the study, ensuring voluntary participation and protection of participants right and privacy

3.9 Data Collection

Sociodemographic and clinical data (age, gestational age, parity, and any relevant obstetric history) were collected using a structured questionnaire administered during antenatal visits.

Data on previous transfusions, medical history, and obstetric complications was also be recorded.

Data was checked for completeness and accuracy before analysis.

3.10 Sample Collection

Approximately 3 ml of venous blood was collected aseptically from each participant into EDTA anticoagulated tubes during routine antenatal blood sampling.

Blood samples was labeled with unique identifiers to maintain participant confidentiality.

Samples was transported promptly to the hospital's laboratory for serological testing.

The Rhesus E and e antigen status was determined using standard hemagglutination techniques following established protocols

3.11 Laboratory analysis

3.11.1Materials Required

Patient's red blood cell suspension (2-5% RBC suspension in saline)

Anti-E antiserum

Anti-e antiserum

normal saline

Test tubes (labeled for anti-E and anti-e)

Centrifuge (compatible with test tubes)

Pipettes (micropipettes or Pasteur pipettes)

water bath at 37°C

3.11.2 Preparation of Red Blood Cell Suspension

1. Blood sample was collected in an EDTA container.

2. The cells were washed three times with normal saline (centrifuge at 1000rpm for 2 minutes each time, discard supernatant and resuspend in saline)

3. A 5% cell suspension was made from the washed cells by adding a drops of the washed cell and nineteen drops of normal saline

3.11.3 Laboratory Procedure: Tube Method

1. Two test tube were labelled Anti—E and Anti—e

2. Two drop of the red blood cell suspension was pipetted into the two labelled test tubes

3. A drop of antisera E was added to the tube labelled anti—E

4. A drop of antisera e was added to the tube labelled anti—e

5.The content of each tubes were mixed gentle and observe for agglutination

6. The results were recorded

7.The tubes were incubated at 37°C for 15minutes .This temperature favors antigen-antibody reaction for Rh antigens.

8.The tubes were centrifuged at 1000rpm for 1minute to enhance agglutination

9. The cells were Gently resuspend by flicking the tube.

10.The tubes were observed for agglutination of RBCs in each tube visually against a white background.

11. The results were recorded

3.12Statistical Analysis

The data were analysed using the Statistical Package for the Social Sciences (SPSS) version 27. Descriptive statistics, including frequencies and percentages, were used to summarize the sociodemographic and clinical characteristics of the pregnant women. The prevalence of Rhesus E and e antigens was presented using frequency tables. The Chi-square test of independence was applied to examine associations between sociodemographic and clinical characteristics and the prevalence of Rhesus E and e antigens. A p-value of less than 0.05 was considered statistically significant.

CHAPTER FOUR

RESULTS

Table 4.1: Sociodemographic Characteristics of Pregnant Women Attending Antenatal Care at Central Hospital, Benin City

Table 4.1 presents the sociodemographic characteristics of pregnant women attending antenatal care. The majority of respondents (62.0%) were aged between 26–35 years, followed by those aged 36–45 years (21.0%), while the least represented group was 18–25 years (17.0%). In terms of ethnicity, Bini women constituted the largest proportion (40.0%), followed by Igbo (22.0%) and Esan (15.0%), whereas Hausa participants (2.0%) were the least represented. Regarding occupation, most women were businesswomen (63.0%), followed by skilled workers (31.0%), while only a few were civil servants (6.0%). For education, the majority had secondary education (60.0%), 37.0% attained tertiary education, and only 3.0% had primary education.

With respect to marital status, nearly all participants were married (96.0%), while only 4.0% were single.

Table 4.2: Clinical Characteristics of Pregnant Women Attending Antenatal Care at Central Hospital, Benin City

Table 4.2 shows the clinical characteristics of the respondents. With respect to parity, 32.0% were nulliparous, 22.0% had one child, and 19.0% had two children, while a few (1.0%) had five children. For gestational age, majority of the women were in their second trimester (4–6 months, 53.0%), followed by first trimester (1–3 months, 36.0%), while the least proportion was in the third trimester (7–9 months, 11.0%). In terms of gravidity, 31.0% were in their first pregnancy, 19.0% in their second, 18.0% each in their third and fourth pregnancies, and only 1.0% each in their seventh and eighth pregnancies.

Table 4.3: Prevalence of Rhesus E and e Antigens among Pregnant Women Attending Antenatal Care at Central Hospital, Benin City

Table 4.3 illustrates the prevalence of Rhesus E and e antigens among the participants. The findings show that 15.0% were positive for Rhesus E antigen, while the majority (85.0%) were negative. In contrast, 91.0% were positive for Rhesus e antigen, and only 9.0% were negative.

Table 4.4: Relationship Between Sociodemographic Characteristics and the Prevalence of Rhesus E Antigen among Pregnant Women Attending Antenatal Care at Central Hospital, Benin City

Table 4.4 presents the relationship between sociodemographic characteristics and the prevalence of Rhesus E antigen. The results indicate no statistically significant associations between age, ethnicity, occupation, educational level, or marital status and Rhesus E antigen prevalence (p-value>0.05). Although not statistically significant, Rhesus E antigen positivity was higher among women aged 26–35 years (73.3%), of Bini ethnicity (53.3%), and those engaged in business (66.7%).

Table 4.5: Relationship Between Sociodemographic Characteristics and the Prevalence of Rhesus e Antigen among Pregnant Women Attending Antenatal Care at Central Hospital, Benin City

Table 4.5 shows the relationship between sociodemographic characteristics and Rhesus e antigen. The findings reveal no statistically significant associations with age, ethnicity, occupation, educational level, or marital status (p-value>0.05). Nonetheless, Rhesus e antigen positivity was more frequent among women aged 26–35 years (62.6%), of Bini ethnicity (38.5%), businesswomen (64.8%), and those with secondary education (61.5%).

Table 4.6: Relationship Between Clinical Characteristics and the Prevalence of Rhesus E Antigen among Pregnant Women Attending Antenatal Care at Central Hospital, Benin City

Table 4.6 presents the association between clinical characteristics and Rhesus E antigen. There were no statistically significant relationships between parity, gravidity, or gestational age and Rhesus E antigen status (p-value>0.05). However, prevalence of Rhesus E antigen was higher among women with parity of three (26.7%), those in their second gravidity (20.0%), and women in the third trimester (26.7%).

Table 4.7: Relationship Between Clinical Characteristics and the Prevalence of Rhesus e Antigen among Pregnant Women Attending Antenatal Care at Central Hospital, Benin City

Table 4.7 examines the relationship between clinical characteristics and Rhesus e antigen. A statistically significant association was observed between parity and Rhesus e antigen (X^2 = 11.35, df= =5, p = 0.045). Women with parity of zero (33.0%) and one (22.0%) showed higher proportions of Rhesus e antigen positivity compared to those with parity two (18.7%), three (17.6%), and four (8.8%), while no case of Rhesus e positivity was recorded among women with parity five (0.0%). Conversely, gravidity and gestational age showed no statistically significant associations with Rhesus e antigen prevalence (p-value>0.05). Nevertheless, Rhesus e positivity was higher among women in their first gravidity (31.9%) and those in the second trimester (51.6%).

Table 4.1: Sociodemographic Characteristics of pregnant women attending antenatal care at Central Hospital, Benin city

Variables	Frequency	Percentage (%)
Age Range		
18–25 Years	17	17.0
26–35 Years	62	62.0
36–45 Years	21	21.0
Total	100	100.0
Ethnicity		
Bini	40	40.0
Esan	15	15.0
Hausa	2	2.0
Idoma	3	3.0
Igbo	22	22.0
Owan	3	3.0
Urhobo	12	12.0
Yoruba	3	3.0
Total	100	100.0
Occupation		
Business	63	63.0

Civil Servant	6	6.0
Skilled Worker	31	31.0
Total	100	100.0
Educational Level		
Primary	3	3.0
Secondary	60	60.0
Tertiary	37	37.0
Total	100	100.0
Marital Status		
Single	4	4.0
Married	96	96.0
Total	100	100.0

Table 4.2: Clinical Characteristics of Pregnant Women attending Antenatal Care at Central Hospital, Benin city

Variables	Frequency	Percentage (%)
Parity		
0	32	32.0
1	22	22.0
2	19	19.0
3	18	18.0

4	8	8.0
5	1	1.0
Total	100	100.0
Gestational Age (Months)		
1–3 Months	36	36.0
4–6 Months	53	53.0
7–9 Months	11	11.0
Total	100	100.0
Gravidity		
1st	31	31.0
2nd	19	19.0
3rd	18	18.0
4th	18	18.0
5th	10	10.0
6th	2	2.0
7th	1	1.0
8th	1	1.0
Total	100	100.0

Table 4.3: Prevalence of Rhesus E and e Antigens among Pregnant Women Attending Antenatal Care at Central Hospital, Benin City

Variables	Negative n (%)	Positive n (%)
Rhesus E Antigen	85 (85%)	15 (15%)
Rhesus e Antigen	9 (9%)	91 (91%)

Table 4.4: Relationship between Sociodemographic Characteristics and the Prevalence of Rhesus E Antigen among Pregnant Women Attending Antenatal Care at Central Hospital, Benin City

Variables	Negative n (%)	Positive n (%)	X^2	df	p-value
Age Range					
18–25 Years	15 (17.6%)	2 (13.3%)	0.999	2	0.607
26–35 Years	51 (60.0%)	11 (73.3%)			
36–45 Years	19 (22.4%)	2 (13.3%)			
Ethnicity					
Bini	32 (37.6%)	8 (53.3%)	3.648	7	0.819
Esan	13 (15.3%)	2 (13.3%)			
Hausa	2 (2.4%)	0 (0.0%)			
Idoma	2 (2.4%)	1 (6.7%)			
Igbo	20 (23.5%)	2 (13.3%)			
Owan	3 (3.5%)	0 (0.0%)			
Urhobo	10 (11.8%)	2 (13.3%)			
Yoruba	3 (3.5%)	0 (0.0%)			
Occupation					

Business	53 (62.4%)	10 (66.7%)	1.127	2	0.569
Civil servant	6 (7.1%)	0 (0.0%)			
Skilled worker	26 (30.6%)	5 (33.3%)			
Education					
Primary	2 (2.4%)	1 (6.7%)	0.855	2	0.652
Secondary	51 (60.0%)	9 (60.0%)			
Tertiary	32 (37.6%)	5 (33.3%)			
Marital Status					
Single	4 (4.7%)	0 (0.0%)	0.735	1	0.391
Married	81 (95.3%)	15 (100.0%)			

Table 4.5: Relationship between Sociodemographic Characteristics and the Prevalence of Rhesus e Antigen among Pregnant Women Attending Antenatal Care at Central Hospital, Benin City

Variables	Negative n (%)	Positive n (%)	X^2	df	p-value
Age Range					
18–25 Years	1 (11.1%)	16 (17.6%)	0.984	2	0.611
26–35 Years	5 (55.6%)	57 (62.6%)			
36–45 Years	3 (33.3%)	18 (19.8%)			
Ethnicity					
Bini	5 (55.6%)	35 (38.5%)	2.570	7	0.922
Esan	2 (22.2%)	13 (14.3%)			

Hausa	0 (0.0%)	2 (2.2%)			
Idoma	0 (0.0%)	3 (3.3%)			
Igbo	1 (11.1%)	21 (23.1%)			
Owan	0 (0.0%)	3 (3.3%)			
Urhobo	1 (11.1%)	11 (12.1%)			
Yoruba	0 (0.0%)	3 (3.3%)			
Occupation					
Business	4 (44.4%)	59 (64.8%)	1.548	2	0.461
Civil servant	1 (11.1%)	5 (5.5%)			
Skilled worker	4 (44.4%)	27 (29.7%)			
Education					
Primary	0 (0.0%)	3 (3.3%)	1.616	2	0.446
Secondary	4 (44.4%)	56 (61.5%)			
Tertiary	5 (55.6%)	32 (35.2%)			
Marital Status					
Single	0 (0.0%)	4 (4.4%)	0.412	1	0.521
Married	9 (100.0%)	87 (95.6%)			

Table 4.6: Relationship between Clinical Characteristics and the Prevalence of Rhesus E Antigen among Pregnant Women Attending Antenatal Care at Central Hospital, Benin City

Variables	Negative n (%)	Positive n (%)	X^2	df	p-value
Parity					
0	27 (31.8%)	5 (33.3%)	7.35	5	0.196

1	20 (23.5%)	2 (13.3%)			
2	17 (20.0%)	2 (13.3%)			
3	14 (16.5%)	4 (26.7%)			
4	7 (8.2%)	1 (6.7%)			
5	0 (0.0%)	1 (6.7%)			
Gravidity					
1st	27 (31.8%)	4 (26.7%)	5.45	7	0.605
2nd	16 (18.8%)	3 (20.0%)			
3rd	17 (20.0%)	1 (6.7%)			
4th	15 (17.6%)	3 (20.0%)			
5th	7 (8.2%)	3 (20.0%)			
6th	1 (1.2%)	1 (6.7%)			
7th	1 (1.2%)	0 (0.0%)			
8th	1 (1.2%)	0 (0.0%)			
Gestational Age					
1–3 months	32 (37.6%)	4 (26.7%)	4.50	2	0.105
4–6 months	46 (54.1%)	7 (46.7%)			
7–9 months	7 (8.2%)	4 (26.7%)			

Table 4.7: Relationship between Clinical Characteristics and the Prevalence of Rhesus e Antigen among Pregnant Women Attending Antenatal Care at Central Hospital, Benin City

Variables	Negative n (%)	Positive n (%)	X^2	df	p-value
Parity					

0	2 (22.2%)	30 (33.0%)	11.35	5	0.045*
1	2 (22.2%)	20 (22.0%)			
2	2 (22.2%)	17 (18.7%)			
3	2 (22.2%)	16 (17.6%)			
4	0 (0.0%)	8 (8.8%)			
5	1 (11.1%)	0 (0.0%)			
Gravidity					
1st	2 (22.2%)	29 (31.9%)	5.79	7	0.565
2nd	2 (22.2%)	17 (18.7%)			
3rd	2 (22.2%)	16 (17.6%)			
4th	2 (22.2%)	16 (17.6%)			
5th	0 (0.0%)	10 (11.0%)			
6th	1 (11.1%)	1 (1.1%)			
7th	0 (0.0%)	1 (1.1%)			
8th	0 (0.0%)	1 (1.1%)			
Gestational Age					
1–3 months	2 (22.2%)	34 (37.4%)	0.87	2	0.647
4–6 months	6 (66.7%)	47 (51.6%)			
7–9 months	1 (11.1%)	10 (11.0%)			

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

This study investigated the distribution of Rhesus E and e blood group antigens among pregnant women attending antenatal care at Central Hospital, Benin City. The results showed that the e antigen was the most prevalent, while the E antigen was detected at a much lower frequency. This finding is not surprising because, globally, the e antigen has been described as one of the most common antigens in the Rh blood group system (Daniels, 2013).

The high prevalence of the e antigen observed in this study is consistent with earlier reports in Nigeria. For instance, Adeyemo and Soboyejo (2006) reported that the e antigen was present in almost all individuals tested in Lagos, while Jeremiah et al. (2011) also found similarly high frequencies among Nigerians in Port Harcourt. Likewise, Ukaejiofor *et al*. (2017) reported a prevalence of over 95% for the e antigen in Southeastern Nigeria. This trend suggests that the e antigen is almost universal in the Nigerian population, which may have significant clinical implications, especially in transfusion medicine and maternal-fetal health.

On the other hand, the relatively lower frequency of the E antigen observed in this study is also in line with existing evidence from African populations. Studies conducted in Nigeria and Ghana show that the E antigen generally occurs at frequencies ranging between 15–25% (Okeke *et al.*, 2015). This contrasts with findings in some European and Asian populations where the E antigen is more common (Flegel, 2011). These differences are largely explained by ethnic genetic variations and highlight the need for population-specific data on Rh antigen distribution.

When the distribution of the Rh E and e antigens was analyzed in relation to maternal age, the results of this study indicated no statistically significant variation. This is consistent with the understanding that Rh blood group antigens are genetically inherited and therefore not influenced by age. Similar observations have been made by Okeke *et al.* (2015), who reported that the expression of Rh antigens in pregnant women in Enugu, Nigeria, was independent of maternal age.

With respect to parity and gestational age, no clear association was observed in this study. This finding again confirms the genetic stability of Rh antigens, which are determined by inheritance and remain constant throughout life. However, the relevance of these parameters lies more in their contribution to the risk of alloimmunization. For example, multiparous women are more likely to have had repeated exposure to foreign antigens, particularly through previous pregnancies and possible transfusions, which increases the risk of antibody formation against Rh antigens, including E.

From a clinical perspective, the findings of this study carry important implications for both transfusion medicine and maternal-fetal health. Alloimmunization against Rh antigens other than D, particularly anti-E, has been increasingly reported as a cause of hemolytic disease of the newborn (HDN) and hemolytic transfusion reactions (Flegel, 2011). In fact, some studies suggest that anti-E may be the most common Rh antibody after anti-D in certain populations (Daniels, 2013). The presence of anti-E antibodies in pregnant women poses a significant risk because maternal IgG antibodies can cross the placenta and cause hemolysis in the fetus, leading to anemia, jaundice, and in severe cases, hydrops fetalis.

The predominance of the e antigen in the study population further underscores the risk of alloimmunization in E-negative mothers carrying E-positive fetuses. Although the E antigen is less common, the possibility of sensitization remains

clinically significant. In Nigeria, where routine antenatal care often emphasizes ABO and D typing only, the exclusion of extended Rh antigens such as C, c, E, and e creates a diagnostic gap that may result in preventable complications. This finding strengthens the call for expanded Rh antigen screening in antenatal care.

Furthermore, the study's findings contribute to the broader understanding of population genetics of blood groups in Nigeria. The observed pattern—high frequency of e and low frequency of E—is consistent with the Rh haplotype distribution commonly reported in sub-Saharan Africa, where haplotypes such as cde and Cde dominate (Ukaejiofor *et al.,* 2017). This differs significantly from European populations where haplotypes like CDe and cDE are more frequent. Such genetic differences not only influence transfusion practices but also reflect historical and evolutionary genetic variations across populations.

Another important observation is the potential transfusion implication of the findings. Patients lacking the E antigen who receive E-positive blood are at risk of alloimmunization, which can complicate future transfusions and pregnancies. In regions like Nigeria, where blood transfusion services are often challenged by limited resources, the availability of extended blood group typing is restricted. Therefore, knowledge of antigen distribution in the local population can guide blood bank inventory management and improve transfusion safety.

5.2 CONCLUSION

In conclusion, the predominance of the e antigen and the lower frequency of the E antigen observed in this study underscore the need for continued emphasis on extended Rh antigen typing during antenatal care. Incorporating such practices will enhance blood transfusion safety, improve maternal–fetal care, and

contribute to reducing preventable complications associated with Rh incompatibility.

5.3 RECOMMENDATION

From the results obtained in this study on the distribution of Rhesus E and e antigens among pregnant women attending antenatal care at Central Hospital, Benin City, several recommendations are suggested:

1. Inclusion of Extended Rh Screening:

Routine blood group typing in antenatal care should not be limited to the ABO and Rhesus D antigens alone. Screening for other clinically relevant Rh antigens, particularly E and e, should be incorporated into antenatal investigations to minimize the chances of maternal sensitization and alloimmunization.

2. Strengthening Antenatal Monitoring:

Pregnant women found to lack specific Rh antigens, especially those considered uncommon, should be given closer follow-up during pregnancy. Adequate counseling should also be provided to inform them of possible risks such as hemolytic disease of the fetus and newborn (HDFN).

3. Improved Blood Bank Practices:

Hospital blood banks should make provisions for a wider range of well-typed blood units covering extended Rh antigens. This will ensure that compatible blood is readily available for transfusion, particularly in emergency cases where antigen mismatch could complicate maternal or neonatal outcomes.

4. Health Education and Awareness:

Efforts should be intensified to enlighten pregnant women on the importance of knowing their complete Rh antigen profile. Health care workers should also be trained to emphasize the role of extended antigen typing in improving maternal and child health.

5. Expanded Research:

Similar studies should be conducted with larger sample sizes and across different health facilities within Edo State and other regions of Nigeria. This would provide more comprehensive data on Rh antigen distribution and help in formulating region-specific strategies.

6. Policy Development:

Health authorities, particularly the Ministry of Health, should integrate extended Rh antigen typing into national maternal health and transfusion service policies. Doing so will support safe motherhood initiatives and contribute to reducing adverse pregnancy outcomes linked to Rh incompatibilities.

5.4 CONTRIBUTION TO KNOWLEDGE

This study has added to existing knowledge in several important ways:

1. Local Data on Antigen Distribution:

It has provided current information on the prevalence and distribution of Rhesus E and e antigens among pregnant women in Benin City. Such localized data were previously scarce and are valuable for guiding clinical practices in the region.

2. Support for Maternal Health Management:

By identifying the predominance of the e antigen and the lower frequency of the E antigen within the study population, the research highlights potential risks of alloimmunization that may arise during pregnancy. This contributes to improving maternal health strategies, especially in the prevention and management of hemolytic disease of the fetus and newborn (HDFN).

3. Implications for Blood Transfusion Services:

The findings serve as a reference point for hospital blood banks in planning and ensuring the availability of well-typed blood units that go beyond the routine ABO and D grouping. This will promote safer transfusion practices in the study area.

4. Foundation for Further Research:

The study provides a baseline for future research on Rh antigen distribution in Edo State and Nigeria at large. It sets the stage for larger multicenter studies that may influence national policies on transfusion medicine and antenatal care.

5. Contribution to Scientific Literature:

The research adds to the body of literature on Rh antigen variability by documenting data from a Nigerian population. This enhances global understanding of regional differences in antigen expression, which is useful for both clinical practice and academic research.

REFERENCES

Adeyemo, O. A., and Soboyejo, O. B. (2006). Frequency distribution of ABO, Rh blood groups and blood genotypes among the Cell Biology and Genetics students of University of Lagos, Nigeria. *African Journal of Biotechnology, 5*(22), 2062–2065.

Adesina, O. O., et al. (2024). Prevalence of non-D Rh alloantibodies in pregnant women: A multicenter study in Nigeria. *Journal of Hematology and Transfusion Science, 41*(2), 112–119.

Aliyo, M., Ashenafi, A., and Abduselam, N. (2023). Rhesus negativity prevalence and neonatal outcomes among pregnant women delivered at Bule Hora University Teaching Hospital. *Journal of Primary Care and Community Health, 14,* 1–7.

Avent, N. D., and Reid, M. E. (2000). The Rh blood group system: *A review. Blood, 95*(2), 375–387.

Castilho, L. (2022). Blood group systems: Overview and clinical significance. Brazilian *Journal of Hematology and Hemotherapy, 44*(3), 189–195.

Chao, A. S., Chung, C. L., Wu, S. H., Chang, Y. L., and Hsieh, P. C. (2009). Hemolytic disease of the fetus and newborn due to anti-E alloimmunization. *Case Reports in Obstetrics and Gynecology*, 2009, 471623.

Chuang, M. T., and Chang, C. H. (2022). Hemolytic disease of the fetus and newborn caused by irregular antibody: A mortality case report and case series during the past 15 years in NCKUH. *Taiwan Journal of Obstetrics and Gynecology, 61*(5), 896–899.

Clausen, F. B., and van der Schoot, C. E. (2025). Noninvasive fetal blood group antigen genotyping. Blood Transfusion, 23(2), 101–108.

Daniels, G. (2013). Human blood groups (3rd ed.). Wiley-Blackwell.

Daniels, G. (2022). Human blood groups (4th ed.). Wiley-Blackwell.

Drozdowska-Szymczak, A., Łukawska, S., Mazanowska, N., Ludwin, A., and Krajewski, P. (2024). Management and treatment outcomes of hemolytic disease of the fetus and newborn (HDFN)—A retrospective cohort study. *Journal of Clinical Medicine, 13*(16), 4785.

Egesie, O. J., Egesie, U. I., Usman, K., and Garba, D. (2018). ABO and Rhesus blood group distribution among pregnant women in a rural community of Northwestern Nigeria. *The Pan African Medical Journal*, 31, 169.

Fisher-Race, A. C., and Race, R. R. (1947). *The Rh blood groups. British Medical Journal,* 2(4515), 1019–1021.

Flegel, W. A. (2007). Molecular genetics of the Rh blood group system. *Blood Reviews*, 21(2), 58–66.

Flegel, W. A. (2011). Molecular genetics and clinical applications for RH. *Transfusion and Apheresis Science, 44*(1), 81–91.

Fridey, J. L., and Westhoff, C. M. (2022). Rh blood group system*: Overview and clinical significance*. UpToDate.

Gottstein, R., and Wahn, V. (2017). Hemolytic disease of the newborn. *Handbuch Der Internal Medicine,* 62, 1–17.

Gwaram, M. A., Abdullahi, S., and Umar, M. B. (2023). Analysis of Rhesus (Rh) antigen distributions in donors and multi-transfused patients for phenotype-matched transfusion. *Indian Journal of Hematology and Blood Transfusion, 39*(1), 1–6.

Habtewold, T. D., Asteray A.A. (2021). Prevalence of RhD-negative blood type and its impact in sub-Saharan Africa: *A systematic review. Maternal Health, Neonatology and Perinatology,* 7, 5.

Hassall, C., Gatault, P., and Arnaud, L. (2023). Alloimmunization to Rh antigens*: A current update. Transfusion Medicine Reviews, 37*(1), 1–10.

Ibrahim, T. A., Oyetunde, B. O., and Alabi, B. A. (2024). Rh blood group antigens and alloimmunization risk among pregnant women in South-Western Nigeria. *Journal of Clinical Hematology and Immunology, 6*(1), 32–39.

Jeremiah, Z. A., Odumody, C., and Jeremiah, T. A. (2011). Frequencies of ABO, Rh (D, C, c, E, e) and Kell (K) antigens in a Nigerian population. N*igerian Journal of Clinical Practice, 14*(4), 431–434.

Joy, S. D., Ismail, M. A., and Sharma, M. (2020). Anti-E alloimmunization in pregnancy with a low antibody titer: *A case report. Clinical and Experimental Obstetrics & Gynecology, 47*(4), 639–642.

Levine, P., and Stetson, R. E. (1939). An unusual case of intra-group agglutination. *Journal of the American Medical Association, 113*(2), 126–127.

Li, M., Wang, L., Li, A., Wang, B., Yang, X., Zhang, Y., Chen, C., Sun, F., Zhu, Z., and Ye, L. (2024). Integrated analyses reveal unexpected complex rearrangements in RHD and RHCE genes affecting Rh antigen expression. *Blood Advances, 8*(12), 3154–3166.

Liumbruno, G. M., and Bennardello, F. (2017). The ABO and Rh blood group systems: *A modern perspective. Blood Transfusion, 15*(4), 312–320.

Mouro, I., Le Pennec, P. Y., and Ballif, B. C. (2021). Genetic variability of RHCE and implications for transfusion medicine. *Transfusion Medicine Reviews, 35(*3), 168–176.

Mwesigwa, AMbalibulha, Y., Natukunda, B., Okwi, A. L., Kalyango, J. N., . (2022). Clinical outcomes of Rh E and e alloimmunization among antenatal women in Uganda*. East African Medical Journal, 99*(3), 167–172.

Okeke, T. C., Obi, S. N., Okezie, O. A., and Ocheni, S. (2015). The prevalence of Rhesus C, c, E, and e antigens among pregnant women in Enugu, *Nigeria. Nigerian Journal of Medicine, 24(*3), 208–212.

Okoroiwu, I. L., and Okafor, I. M. (2023). Rhesus alloimmunization: Implications of RhCE antigens in pregnant women. *African Journal of Laboratory Medicine, 12*(1), 54–60.

Olaiya, M. A., Alawode, O. O., Okunade, K. S., and Owoseni, A. O. (2019). The prevalence of ABO and Rhesus blood groups among pregnant women attending antenatal clinic at a tertiary hospital in Lagos, Nigeria. *Nigerian Postgraduate Medical Journal, 26*(1), 38–42.

Pacheco, G. H. A. S., Lobo, G. A. R., and Araujo Júnior, E. (2024). Intrauterine transfusion for Rhesus alloimmunization: A historical cohort from Brazil. *Journal of Clinical Medicine, 13*(5), 1362.

Ramsey, G. (2025). Immunohematology and transfusion medicine: *Principles and practice* (3rd ed.). Elsevier Health Sciences.

Reid, M. E., and Mohandas, N. (2018). Red blood cell membrane. In R. Hoffman, E. J. Benz Jr., S. J. Shattil, B. S. Stamatoyannopoulos, H. J. Heslop, J. O. Armitage, and L. L. Lobel (Eds.), *Hematology: Basic principles and practice* (7th ed., pp. 445–465). Elsevier.

Rosenkrans, K. A., Zubair, A. C., and Doyal, A. M. (2023). Rh blood group. In StatPearls. Treasure Island, FL: StatPearls Publishing.

Rosenkrans, K., Zubair, A., and Doyal, N. (2023). Rh blood group system: Molecular basis, testing strategies, and clinical impact. *Transfusion Medicine Reviews, 37*(1), 15–28.

Sanusi, Nurul ’Adani, Ashari, N. S. M., Johan, M. F., Edinur, H. A., Noor, N. H. M.,and Hassan, M. N. (2024). Alloimmunization among Rh-negative pregnant women: A review. *Journal of Preventive Medicine and Public Health, 57*(3), 250–258.

Soler-Noda, D., Diaz-Perez, D., and Rodríguez-Lorenzo, S. (2021). Hemolytic disease of the newborn caused by anti-e antibodies: *A case report. Immunohematology, 37*(1), 17–20.

Sorensen, A. L., and Nørgaard, T. T. (2018). Rh hemolytic disease of the fetus and newborn. *Danish Medical Journal, 65*(4), 54–75.

StatPearls. (2025). Rhesus (Rh) blood group system. In StatPearls [Internet]. StatPearls Publishing.

Sugrue, R. P., Moise, K. J., and Federspiel, J. J. (2024). Maternal red blood cell alloimmunization in the U.S. *Blood Advances, 8*(16), 4311–4320.

Turbil, E., Ozcan, N., and Sezgin, G. (2023). Red cell alloimmunization: Mechanisms, clinical implications, and management. *International Journal of Laboratory Hematology, 45*(2), 145–156.

Ukaejiofor, E. O., Nwauche, C. A., and Obiandu, I. (2017). Distribution of Rhesus blood group system antigens in a Nigerian population. *British Journal of Medicine and Medical Research, 20*(9), 1–6.

van ’t Oever, R. M., Verweij, E. J. T., de Haas, M., et al. (2025). How I use noninvasive prenatal testing for red blood cell and platelet antigens. Blood, 145(20), 2266–2274.

Walker, R. (2014). The Rhesus System. In M. L. Harmening (Ed.), *Modern blood banking and transfusion practices* (6th ed., pp. 166–193).

Westhoff, C. M., and Denomme, G. A. (2022). The complexity of the Rh blood group system: *Clinical and laboratory implications. Transfusion, 62*(S1), S21–S30.

Wylie, B. J. (2016). Management of Rh isoimmunization. *Obstetrics and Gynecology Clinics of North America, 43*(2), 261–270.

Yahia, E. A., Alhazmi, H. A., and Alzahrani, A. K. (2020). Blood group Rhesus D negativity and awareness toward importance of anti-D immunoglobulin among pregnant women in Bisha, Saudi Arabia*. Journal of Family Medicine and Primary Care, 9*(2), 922–926.

Yousuf, R., Abdul Aziz, S., Yusof, N., and Leong, C. F. (2012). Hemolytic disease of the fetus and newborn caused by anti-D and anti-S alloantibodies: A case report. *Journal of Medical Case Reports,* 6, 71.

EDO STATE MINISTRY OF HEALTH
HEALTH RESEARCH ETHICS COMMITTEE

PROTOCOL NUMBER	**HA/737/25/D/07180820 (PLEASE QUOTE IN ALL ENQUIRIES)**
APPROVAL NUMBER	**HA/737/25/D/09190820**
TITLE OF RESEARCH PROPOSAL	**THE RHESUS E AND e BLOOD GROUP DISTRIBUTION IN PREGNANT WOMEN ATTENDING ANTE NATAL CARE IN CENTRAL HOSPITAL BENIN CITY**
PRINCIPAL INVESTIGATOR (S)	**OBASOHAN PEACE**
DATE CONSIDERED	**19TH SEPTEMBER, 2025.**
DECISION OF THE COMMITTEE	**APPROVED**

THIS APPROVAL DATES 19/09/2025 TO 19/09/2026. IF THERE IS A DELAY IN STARTING THE RESEARCH, PLEASE INFORM THE HREC EDO SMoH SO THAT THE DATES OF APPROVAL CAN BE ADJUSTED ACCORDINGLY

REMARK: Please kindly note that the HREC Edo SMoH seal authenticates this approval

DR (MRS) Omonyemen B. BELLO (MBBS, MPH, FPHCM) *(CHAIRMAN)* **SIGNATURE & DATE**.....19/9/2025.....

SUPERVISOR(S) .....

.....

ATTESTATION BY INVESTIGATOR(S)

No participant accrual or activity related to this research may be conducted outside of the approval dates. All informed consent forms used in this study must carry the Edo SMoH HREC-assigned number and duration of your research. No changes are permitted in the research without prior approval of the Edo SMoH HREC except in circumstances outlined in the Code. The Edo SMoH HREC reserves the right to conduct compliance visits to your research site without previous notification.

Signature & Date.....

EDO STATE MINISTRY OF HEALTH SECRETARIAT DATE 19/9/25 SIGN DATE 19/9/25 HEALTH & ETHICS COMMITTEE

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