DISTRIBUTION OF MNS BLOOD GROUPS (M AND N ANTIGENS) AMONG PREGNANT WOMEN IN BENIN CITY

BY

OGHOR UZOMA SILVIA

BMS2001186

A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCE, UNIVERSITY OF BENIN, BENIN CITY.

IN PARTIAL FULFILMENT FOR THE REQUIREMENT OF THE AWARD OF BACHELOR OF (B.MLS) DEGREE IN MEDICAL LABORATORY SCIENCE.

SUPERVISED BY: PROF. (MRS.) E.O. OSIME

SEPTEMBER, 2025

DISTRIBUTION OF MNS BLOOD GROUPS (M AND N ANTIGENS) AMONG PREGNANT WOMEN IN BENIN CITY

BY

OGHOR UZOMA SILVIA

BMS2001186

DEPARTMENT OF MEDICAL LABORATORY SCIENCE

SCHOOL OF BASIC MEDICAL SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

SEPTEMBER, 2025

CERTIFICATION

This is to certify that tie project work tiled "DISTRIBUTION OF MNS BLOOD GROUPS (M AND N ANTIGENS) AMONG PREGNANT WOMEN IN BENIN CITY" was carried out by OGHOR UZOMA SILVIA, in the Department of Medical Laboratory Science School of Basic Medical Sciences, University of Benin. Benin City, Edo State.

Prof. (Mrs.) E.O. Osime (Project Supervisor)	**Date**
Prof. (Mrs.) E.O. Osime Head of Department	**Date**
External Examiner	**Date**

DEDICATION

This work is dedicated to God Almighty, whose grace and strength carried me through every stage of this journey. To my loving family, for their constant encouragement, patience, and unwavering support. To all the pregnant women who graciously participated in this study your cooperation made this research possible. And to every student and researcher striving to improve maternal and child health may this work serve as a small stepping stone in your journey.

ACKNOWLEDGEMENTS

My profound gratitude goes to Almighty God, for the wisdom, knowledge and understanding granted me through this work. My sincere appreciation goes to my supervisor, who is also the Head of Department Prof. (Mrs.) E.O. Osime for her effort and commitment to the completion of this work and for her commitments to the department. To all the dedicated staffs of the department for impacting me with knowledge,

My immerse appreciation goes to my parents (MR and MRS IYI-EWEKA), my beloved brothers and sisters for their support, encouragement and prayers.

I will not fail to appreciate my wonderful and supportive friends as well as my Christian brothers and sisters who supported me in different ways. God bless you all, Amen.

TABLE OF CONTENT

CHAPTER ONE: INTRODUCTION

CHAPTER TWO

CHAPTER THREE: MATERIALS AND METHODS

CHAPTER FOUR: RESULTS

CHAPTER FIVE

ABSTRACT

The MNS blood group system is often overshadowed by the ABO and Rh systems; it remains clinically relevant due to its potential role in hemolytic disease of the fetus and newborn (HDFN) and complications related to transfusion therapy. Serological testing was carried out using standard hemagglutination techniques following established protocols. The study aimed to determine the prevalence of M and N antigens, examine their distribution across different trimesters, and assess any associations with parity, gravidity, and ethnic backgrounds. A cross-sectional descriptive design was employed. A total of 110 venous blood samples were collected aseptically into plain tubes during routine antenatal visits. The age range of participants was 20 to 40 years, and the gestational age at the time of sampling ranged from 2 to 8 months, covering the first, second, and third trimesters of pregnant women attending antenatal care at the Central Hospital, Benin City, Edo State. This study provided important regional data that reinforces the clinical significance of incorporating MNS blood group antigen screening into routine antenatal care. Early identification of potential alloimmunization can help prevent serious complications such as fetal anemia and HDFN, ultimately improving both maternal and neonatal outcomes. Out of the 110 samples tested, 62 (56.4%) were positive for M antigens and 73 (66.4%) were positive for N antigens. This study revealed a higher prevalence of the N antigen compared to the M antigen among pregnant women attending antenatal care at Central Hospital, Benin City. These findings underscore the importance of comprehensive blood group antigen screening, including the MNS system, during pregnancy. Early detection of maternal alloantibodies against MNS antigens can help guide appropriate prenatal care, prevent hemolytic disease of the fetus and newborn (HDFN), and improve transfusion safety and perinatal outcomes.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Blood transfusion medicine and immunohematology have remained crucial in modern medical practice, particularly in maternal and child health. One of the most important milestones in medicine was the discovery of blood group antigens, which transformed the safety of transfusions, transplantation, and obstetric care. Blood group antigens are genetically determined polymorphic structures expressed on the surface of red blood cells (RBCs), serving not surface of red blood cells (RBCs), serving not only as markers of immunological identity but also as receptors for pathogens and contributors to membrane integrity (Daniels, 2013).

Since the pioneering work of Karl Landsteiner in 1900 on the ABO system, more than 43 distinct blood group systems have been identified and characterized by the International Society of Blood Transfusion (ISBT) (ISBT, 2022). Each system comprises one or more antigens controlled by specific genes. While the ABO and Rhesus (Rh) systems remain the most clinically significant, other systems such as Kell, Duffy, Kidd, Lewis, and MNS also play crucial roles in transfusion medicine and obstetric care (Westhoff, 2019).

The MNS blood group system stands out for its structural complexity, molecular diversity, and clinical significance. Although often overlooked compared to RhD, MNS alloantibodies can cause hemolytic disease of the fetus and newborn (HDFN) and hemolytic transfusion reactions (HTRs) (Colin & Anstee, 2020). The system's involvement in maternal alloimmunization makes it particularly relevant in antenatal care.

1.1.1 Historical Discovery of the MNS Blood Group System.;

The MNS blood group system was discovered in 1927 by Landsteiner and Levine following the identification of M and N antigens, which were found to be antithetical meaning that individuals could inherit either M, N, or both (Landsteiner & Levine, 1927). Later, in 1947,Walsh and Montgomery identified two additional antigens, S and s, thus expanding the system and establishing its clinical relevance.

Over the years, molecular biology has provided deeper insights into the structural and genetic basis of the MNS antigens. It is now known that M and N antigens are carried on glycophorin A (GPA), while S and s antigens are carried on glycophorin B (GPB), both of which are major sialoglycoproteins located on chromosome 4 (Gassner et al., 1997). The discovery of the molecular basis of these antigens also revealed that they serve as receptors for pathogens such as Plasmodium falciparum, highlighting their evolutionary and epidemiological importance (Pasvol et al., 1982).

1.1.2 Molecular Basis of MNS Antigens;

The MNS blood group system is controlled by two highly homologous genes, GYPA and GYPB, located on chromosome 4q28-q31. These genes encode the major RBC membrane glycoproteins, glycophorin A (GPA) and glycophorin B (GPB). Together, GPA and GPB account for approximately 2% of the total RBC membrane proteins and contribute to the negative surface charge due to their abundant sialic acid residues (Reid & Lomas-Francis, 2004).

M and N Antigens:

The M and N epitopes differ by amino acids at positions 1 and 5 of GPA. The M antigen carries serine and glycine at these positions, while the N antigen carries leucine and glutamic acid.

S and s Antigens:

The S and s epitopes differ by a single amino acid substitution at position 29 of GPB methionine for S and threonine for s. These small structural differences have profound clinical significance because they influence the immunogenicity of the antigens and their susceptibility to alloantibody formation.

1.1.3 Epidemiology and Distribution of MNS Antigens

The distribution of MNS antigens varies significantly among populations and ethnic groups. This variation highlights the importance of studying local populations such as antenatal patients in Nigeria, where limited data is available.

- M antigen: expressed in about 78% of Caucasians, 74% of Africans, and 79% of Asians.
- N antigen: occurs in 72% of Caucasians, 75% of Africans, and 70% of Asians.

- S antigen: more common in Caucasians (55%) but less frequent in Africans (~30%).
- s antigen: very frequent globally, occurring in 89% of Caucasians and 93% of Africans (Daniels, 2013).

These differences imply that alloimmunization risks may vary across regions, and data specific to Nigeria would be crucial for tailoring transfusion and antenatal policies.

1.1.4 Clinical Significance of MNS System

Although antibodies to M and N are often naturally occurring and usually of the IgM class, they can occasionally be IgG, which is capable of crossing the placenta and causing HDFN. On the other hand, anti-S and anti-s antibodies are almost always IgG and are more frequently associated with clinically significant transfusion reactions and severe HDFN (Westhoff, 2019).

Anti-M: Typically cold-reacting, naturally occurring IgM, but IgG anti-M has been implicated in HDFN and transfusion reactions (Ogasawara et al., 1999).

Anti-N: Rarely causes severe clinical effects, but IgG forms have been documented in mild HDFN (Daniels, 2013).

Anti-S and Anti-s: Clinically significant, associated with moderate to severe HDFN and acute hemolytic transfusion reactions (Reid & Lomas-Francis, 2004).

The severity of HDFN due to MNS alloantibodies can vary from mild neonatal jaundice to intrauterine death. Unlike RhD alloimmunization, which is routinely screened for and prevented, MNS alloimmunization is often undetected until adverse outcomes occur.

1.1.5 MNS Antibodies in Antenatal Care

In antenatal patients, the detection of alloantibodies is critical for preventing maternal and neonatal morbidity. Routine antenatal screening programs usually focus on ABO and RhD typing and screening for unexpected antibodies. However, in many developing countries, including Nigeria, the screening panel may not detect antibodies against MNS antigens.

This poses a significant clinical risk. Pregnant women who receive blood transfusions during pregnancy or at delivery are especially prone to alloimmunization. If they develop anti-MNS antibodies, these can cross the placenta in subsequent pregnancies, targeting fetal RBCs and causing HDFN. Case studies have reported severe neonatal outcomes resulting from undetected anti-S and anti-s alloimmunization (Moise, 2008).

The lack of routine MNS screening in antenatal patients thus represents a critical gap in maternal and neonatal care.

1.2 Statement of the Problem

Pregnancy presents unique immunological challenges because it involves the coexistence of two genetically distinct individuals: the mother and the fetus. While the maternal immune system is adapted to tolerate the fetus, complications arise when maternal antibodies target fetal red cell antigens inherited from the father. This immune response, termed alloimmunization, may lead to hemolytic disease of the fetus and newborn (HDFN), which continues to be a significant cause of neonatal morbidity and mortality worldwide (Moise, 2008).

Although RhD alloimmunization is well recognized and routinely screened for in antenatal care, other blood group systems including MNS are often neglected. This neglect poses a hidden but serious threat to maternal and fetal health, particularly in resource-limited settings such as Nigeria, where extended blood group typing and antibody screening are rarely implemented (Akinbami *et al*., 2010).

The problem is multifaceted; these includes the limited awareness of the clinical significance of MNS alloantibodies, the lack of routine antenatal screening beyond RhD, the scarcity of local prevalence data in Nigeria, and the increased risk of HDFN and transfusion reactions among pregnant women who develop MNS antibodies.

1.3. Justification of the Study

Every research project must be anchored in a clear justification that establishes its necessity, feasibility, and expected contribution to knowledge and practice. In the context of maternal and child health, the justification becomes even more critical because the outcomes of such research can directly impact the survival and wellbeing of both mothers and infants.

The present study on the MNS blood group system in antenatal patients is justified by several interrelated factors; this includes the clinical relevance of MNS alloantibodies in causing hemolytic disease of the fetus and newborn (HDFN) and transfusion reactions, the knowledge gap regarding the prevalence of these antibodies in Nigeria and other sub-Saharan African countries, the limitations of current antenatal screening programs, which often exclude MNS antibody detection and the public health burden of neonatal morbidity and mortality, which remains disproportionately high in Nigeria.

The need for evidence-based policy reform to strengthen antenatal care and transfusion practices. The ethical responsibility to safeguard maternal and neonatal health by ensuring early detection of preventable complications. This justification section demonstrates why the study is timely, necessary, and poised to make a significant contribution to both scientific knowledge and healthcare practice.

1.4 Significance of the Study

The MNS blood group system occupies a critical, though sometimes underappreciated, role in maternal and neonatal health. Understanding its relevance—particularly in antenatal patients is essential for safe obstetric care, optimal transfusion practice, and the advancement of immunohematology. The significance of this study can be appreciated from several interrelated perspectives:

1. **Protection Against Hemolytic Disease of the Fetus and Newborn (HDFN);**

Maternal alloantibodies directed against MNS antigens such as M, N, S, s, or U can cross the placenta and attack fetal red blood cells. Although HDFN due to MNS antibodies is less common than Rh(D) disease, it can lead to serious outcomes including fetal anemia, hydrops fetalis, or perinatal death.

- By investigating the frequency and clinical impact of MNS antibodies in pregnancy, this study emphasizes the importance of early antibody detection and surveillance through indirect antiglobulin testing.
- Findings can guide obstetricians to plan timely interventions, such as intrauterine transfusions or early delivery, thereby reducing fetal and neonatal morbidity and mortality.

2. Strengthening Antenatal Screening and Risk Stratification;

Routine antenatal blood group screening in many health systems focuses almost exclusively on the ABO and Rh(D) systems. However, the presence of clinically significant but less common antibodies is increasingly documented across diverse populations.

- Demonstrating the prevalence and clinical relevance of MNS alloimmunization supports the inclusion of extended red cell antibody screening in standard prenatal care protocols.

- For clinicians, this means better risk stratification for mothers and infants, reducing unexpected complications during pregnancy and at birth.

3. Enhancing Transfusion Safety in Pregnancy;

Pregnant women are at heightened risk of requiring transfusion due to complications such as postpartum hemorrhage, placenta previa, or surgical interventions.

- In mothers with MNS antibodies, transfusing antigen-positive red cells can cause acute or delayed hemolytic transfusion reactions and increase the risk of further alloimmunization.
- This study underscores the need for antigen-negative or crossmatch-compatible blood and highlights the importance of maintaining regional and international rare-donor registries to secure compatible units promptly.

4. Advancing Immunohematology and Laboratory Medicine;

The MNS system, first described in 1927, represents a complex interaction of glycophorins on the red cell membrane. Research on its antigens and antibodies contributes to:

- A deeper understanding of red cell membrane biology and the molecular genetics underlying antigen expression.
- Improved diagnostic techniques in transfusion medicine laboratories, such as the refinement of antibody identification panels and molecular genotyping methods.

5. Policy Development and Public Health Impact;

Evidence from this study can inform national and regional blood transfusion policies, particularly in settings where rare blood phenotypes are more prevalent or where transfusion services are developing.

- Data on MNS antigen frequencies within the local population can guide donor recruitment strategies and the establishment of rare donor files.
- At the public health level, integrating MNS screening into maternal care protocols supports broader goals of reducing maternal and neonatal mortality, aligning with global initiatives such as the WHO's targets for safe motherhood and newborn health.

6. Overall Contribution;

By bringing attention to the MNS blood group system in antenatal patients, this study bridges a critical knowledge gap between routine prenatal care and the broader scope of transfusion medicine. It highlights that extended blood group awareness is not merely an academic exercise, but a practical necessity for:

- Safeguarding maternal and fetal health,
- Ensuring transfusion compatibility, and
- Fostering a proactive, evidence-based approach to obstetric care.

Ultimately, the findings of this research can serve as a foundation for policy changes, clinical guidelines, and future studies, benefiting healthcare providers, laboratory

1.5 Aims of the Study

This study is aimed at assessing the distribution of the MNS Blood groups among pregnant women in Benin City

1.6 Specific Objectives

The specific objectives of this study are to:

1. To carry out blood grouping (M and N) on all samples.
2. To determine the distribution of M and N blood groups in pregnant women attending antenatal care in central Hospital, Benin City.
3. To determine the relationship of M and N with ABO and rhesus D blood groups.

1.7 Research Questions

1. Are there differences in the MNS blood groups for pregnant women attending antenatal care in Benin City.
2. Is there a significant relationship between MNS blood group distribution and the parity and gravidity of pregnant women attending antenatal care in Central Hospital, Benin City?

3. What is the pattern of MNS blood group distribution among pregnant women from different ethnic groups attending antenatal care in Central Hospital, Benin City?

CHAPTER TWO

2.1 LITERATURE REVIEW

Blood group systems play a critical role in transfusion medicine, organ transplantation, and obstetric care. Among the more than forty recognized human blood group systems, the MNS system is one of the most complex and clinically significant after the ABO and Rh systems (Daniels, 2021). Although antenatal screening programs traditionally focus on ABO and RhD incompatibility, antibodies directed against MNS antigens can also lead to severe hemolytic disease of the fetus and newborn (HDFN) and other transfusion-related complications (Westhoff and Reid, 2018). For this reason, understanding the MNS system is important for obstetricians, hematologists, and transfusion scientists involved in maternal and fetal health.

The MNS blood group system is one of the most intricate and clinically significant human red cell antigen systems. Discovered almost a century ago, it is second only to the ABO and Rh systems in historical importance and clinical relevance (Daniels, 2013). More than 40 antigens have now been described within this system, all carried on the sialoglycoproteins known as glycophorin A (GYPA) and glycophorin B (GYPB) on the erythrocyte membrane (Reid & Lomas-Francis, 2004). These antigens are inherited in a codominant fashion and exhibit remarkable genetic and structural diversity arising from point mutations, gene conversions, and hybrid gene formation (Westhoff, 2021).

Pregnancy presents a unique immunological environment where maternal exposure to paternal red cell antigens through fetomaternal hemorrhage can result in maternal alloimmunization. Although routine prenatal screening programs focus primarily on the ABO and Rh(D) systems, antibodies to MNS antigens such as anti-M, anti-S, or the rare anti-U are well-documented causes of hemolytic disease of the fetus and newborn (HDFN) and of hemolytic transfusion reactions (Evers *et al*., 2019; Fung *et al*., 2020). Importantly, the clinical severity of HDFN caused by MNS antibodies can range from mild neonatal jaundice to severe fetal anemia requiring intrauterine transfusion or early delivery (Dean, 2022).

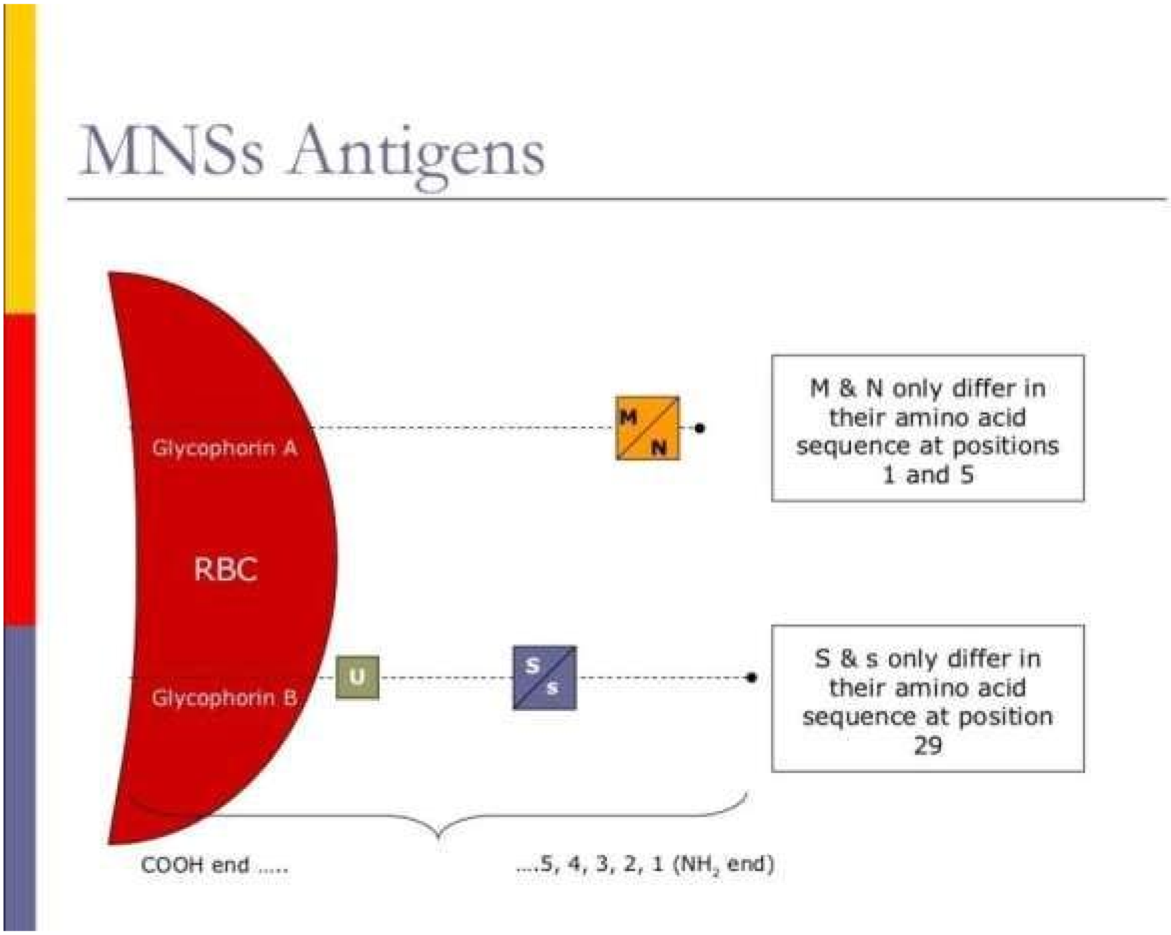
Routine antenatal antibody screening aims to detect unexpected antibodies that could endanger the fetus or newborn. However, in many low-resource settings, testing for antibodies beyond the major ABO and Rh systems remains inconsistent, leading to under-recognition of MNS-related complications (Ameen *et al*., 2021). Documented cases of severe fetal anemia, hydrops fetalis, and even neonatal death due to anti-U or anti-S highlight the need for heightened awareness and improved laboratory capacity (Ogunro *et al*., 2020).In addition, the increasing use of molecular techniques, such as polymerase chain reaction (PCR)-based fetal genotyping from maternal plasma, provides promising non-invasive options for early detection of fetal antigen status and better pregnancy management (Chou *et al*., 2022).

The global distribution of MNS antigens varies widely across populations. Studies in West Africa, for example, show a relatively high frequency of the U-negative phenotype compared with Caucasian populations (Okafor and Owa, 2022; Ukaejiofo *et al*., 2017). This has practical implications for transfusion services in sub-Saharan Africa, where the availability of antigen-negative donor units may be limited and where routine screening for so-called ―minor‖ blood group antibodies is not uniformly practiced. Consequently, undetected maternal alloimmunization to MNS antigens may contribute to preventable perinatal morbidity and mortality.

The purpose of this chapter is to present a comprehensive review of published literature on the MNS blood group system with special reference to antenatal patients. Specific objectives are to:

1. Trace the historical discovery and progressive characterization of the MNS system.
2. Describe the molecular genetics and structural biology underlying its antigenic diversity.
3. Summarize current knowledge of population frequencies and epidemiology of MNS antibodies among pregnant women.
4. Examine laboratory detection methods and antenatal screening practices.
5. Discuss the clinical consequences of maternal MNS alloimmunization and evidence-based management strategies.

Through this synthesis, the chapter highlights the critical importance of incorporating MNS antibody screening into routine antenatal care and identifies research gaps that can inform future policy and clinical practice.



2.2 HISTORICAL BACKGROUND OF THE MNS BLOOD GROUP SYSTEM

Early Discovery of M and N Antigens

The foundation of what is now known as the MNS blood group system was laid in 1927, when Karl Landsteiner and Philip Levine, two pioneers in the emerging field of immunohematology, reported the identification of two previously unrecognized antigens, which they designated ―M‖ and ―N‖ (Landsteiner and Levine, 1927). Working only a few decades after the discovery of the ABO system, their experiments were groundbreaking. By immunizing rabbits with human red blood cells from different donors, they produced antisera capable of detecting subtle antigenic differences among human erythrocytes that could not be explained by the ABO classification alone.

Their observations provided compelling evidence that human red blood cells carry multiple, independent antigenic systems, reshaping contemporary understanding of blood group polymorphism. At the time, transfusion medicine was still in its infancy, and most clinicians believed that the ABO system encompassed the primary determinants of transfusion compatibility. The discovery of the M and N antigens demonstrated that additional antigenic markers could influence transfusion reactions and hemolytic disease, thereby broadening the scope of immunohematology research.

In the 1930s and 1940s, the M and N antigens became a powerful tool in population genetics and paternity testing. Because the gene frequencies for M and N varied between different ethnic and geographic groups, anthropologists used the system to trace human migration patterns and to estimate admixture between populations. For example,early surveys documented a high frequency of M antigen among certain European populations, while some Asian groups showed a relatively balanced distribution of M and N phenotypes. These data were among the first to illustrate how blood group antigens could serve as markers of human diversity long before the era of DNA sequencing (Race and Sanger, 1950).

Landsteiner and Levine’s work not only highlighted the genetic complexity of red cell antigens but also paved the way for the eventual identification of the closely related S, s, and U antigens, which together form the MNS system. Their findings underscored the importance of continued antigenic mapping of human blood, ultimately informing modern practices in blood typing, prenatal care, and transfusion safety (Daniels, 2013; Reid and Lomas-Francis, 2012).

Serologically, the anti-M and anti-N antibodies produced naturally in some individuals were found to be mostly cold-reactive IgM. They caused agglutination at room temperature but rarely at body temperature, a property that generally made them less dangerous for transfusion than antibodies like anti-D of the Rh system. Nevertheless, case reports gradually revealed that anti-M could occasionally occur as immune IgG capable of reacting at 37 °C and crossing the placenta, giving it potential clinical relevance in pregnancy (Westhoff and Reid, 2018).

By the mid-20th century, scientists recognized that the M and N antigens were carried on a specific red-cell membrane glycoprotein later identified as glycophorin A (GYPA) characterized by a high content of sialic acid, which contributes to the negative surface charge of erythrocytes. This biochemical insight explained many serologic findings, including the stability of the antigens and their resistance to common enzyme treatments used in blood banking. These early characterizations of M and N laid the scientific foundation for the subsequent discovery of the S, s, and U antigens and for the eventual classification of the MNS system as one of the major blood group systems recognized by the International Society of Blood Transfusion.

Expansion to Include S and s;

Nearly two decades after the seminal discovery of the M and N antigens, the complexity of the MNS blood group system deepened with the identification of the S antigen. In 1947, Walsh and Montgomery reported a puzzling case of hemolytic transfusion reaction in a patient whose red cells lacked both M and N antigens but who produced an antibody that agglutinated certain donor red cells (Walsh and Montgomery, 1947). Careful serologic investigation revealed a novel antigenic determinant, subsequently named ―S.‖

Shortly thereafter, a second, related antigen designated —s‖ was detected in individuals whose cells lacked S but still reacted with specific antisera. It became clear that S and s represent antithetical antigens, meaning they are encoded by different alleles of the same gene locus and never occur together on the same chromosome. Genetic analyses later demonstrated that the S/s polymorphism is governed by the GYPB gene, which encodes glycophorin B a sialoglycoprotein distinct from glycophorin A that carries the M and N antigens (Daniels, 2013; Reid and Lomas-Francis, 2012).

This discovery completed the classic quartet of MNS antigens M, N, S, and s that defined the system for many years and highlighted its genetic complexity. The recognition of S and s also had immediate clinical relevance: antibodies against these antigens were soon linked to hemolytic transfusion reactions and hemolytic disease of the fetus and newborn (HDFN), underscoring the need for precise serologic typing in transfusion practice (Moore *et al*., 2015).

By establishing that different glycophorins on the erythrocyte membrane could carry distinct but related antigenic determinants, the discovery of S and s broadened scientific understanding of membrane protein diversity and set the stage for subsequent findings such as the U antigen and other rare MNS variants.

Biochemical research in the 1960s and 1970s revealed that the S and s antigens are carried on a different but homologous membrane glycoprotein, later named glycophorin B (GYPB). The discovery that GYPB is closely related to GYPA yet encoded by a separate gene on chromosome 4 elegantly explained the strong genetic linkage between the MN and S/s loci and the occasional occurrence of hybrid phenotypes. Recognition of S and s thus expanded the original —MN‖

system into the —MNS‖ system, reflecting its broadened antigenic landscape and setting the stage for identification of other clinically important antigens such as U.

Identification of the U Antigen and Other Variants

The complexity of the MNS system deepened with the discovery of the U antigen, first reported by Wiener and colleagues in 1951. They described sera from certain individuals that showed no reactivity with either S or s antigens but nevertheless produced an antibody capable of agglutinating the red cells of nearly all humans (Wiener *et al.*, 1951). This antibody defined the U antigen, now classified as a high-incidence (or —public‖) antigen expressed on virtually all erythrocytes. The only known exceptions are rare individuals predominantly of African ancestry who inherit homozygous deletions of the GYPB gene, resulting in the S–s–U– phenotype (Reid and Lomas-Francis, 2004).

The elucidation of the MNS system progressed further with the discovery of the U antigen, a milestone that demonstrated the extraordinary genetic diversity of red cell glycophorins. In 1951, Wiener and colleagues encountered an unusual serum from a patient whose red cells typed negative for both S and s antigens but contained an antibody capable of agglutinating nearly all donor erythrocytes (Wiener *et al.*, 1951). This observation revealed the presence of a previously unrecognized, high-incidence antigen, designated —U,‖ short for —universal,‖ because it is expressed on the red cells of almost all humans.

Genetic and Molecular Basis

Subsequent studies established that the U antigen resides on glycophorin B (GPB), encoded by the GYPB gene on chromosome 4. Individuals of the rare S–s– phenotype seen predominantly in persons of African descent carry homozygous deletions or silencing mutations of GYPB, which result in complete absence of GPB and consequently lack the U antigen (Reid and Lomas-Francis, 2004). Because GPB shares a high degree of sequence homology with glycophorin A (GPA), these loci are prone to unequal crossing-over and gene conversion events, creating an ideal setting for the emergence of hybrid alleles.

Clinical Significance

Anti-U antibodies are of major clinical concern. They are typically IgG and can cause severe hemolytic transfusion reactions as well as hemolytic disease of the fetus and newborn (HDFN). Locating compatible U-negative blood units is particularly challenging, often requiring international rare donor registries or autologous donations for patients with anti-U antibodies (Storry and Olsson, 2009). For pregnant women, early identification of anti-U is crucial so that careful antenatal monitoring and planning for compatible transfusions can be arranged.

Expansion of the Antigenic Spectrum

Following the discovery of U, investigators identified a growing list of low-frequency and high-frequency antigens within the MNS system. These include, but are not limited to:

- Ena: A high-incidence antigen expressed on GPA. Deletions or rearrangements affecting GPA can produce Ena phenotypes, occasionally leading to potent anti-Ena antibodies.

- He (Hil), Vw, Mia, Mur, and GP.Mur: Low-frequency antigens found mainly in certain ethnic groups, such as Southeast Asian populations, where the GP.Mur hybrid is relatively common and clinically significant in transfusion settings.
- Miltenberger (Mi) subclasses: A collection of phenotypes arising from various GYPA–GYPB hybrid genes created through unequal crossover events. These hybrids often co-express epitopes of both parent glycophorins and are responsible for many rare antigenic variants (Issitt & Anstee, 1998; Westhoff, 2021).

Each new variant provided deeper insight into the structural plasticity of glycophorins and the mechanisms of gene recombination. Molecular analysis has shown that the GYPA and GYPB loci form a complex cluster where repetitive sequences facilitate misalignment during meiosis, explaining the high rate of hybrid formation and the extensive antigenic diversity observed in the MNS system.

Advances in molecular diagnostics have transformed detection and classification of these variants. DNA-based blood group genotyping and next-generation sequencing now allow precise identification of hybrid alleles and prediction of antigen expression, even when serologic testing is inconclusive or when patients have been recently transfused (Westhoff, 2021). These technologies are invaluable for managing transfusion therapy in multi-transfused patients, supporting antenatal care, and maintaining rare donor registries.

The detection and characterization of MNS antibodies have progressed dramatically over the past century, reflecting broader innovations in transfusion medicine and molecular genetics.

The Antiglobulin (Coombs) Test

A major breakthrough came in 1945 with the introduction of the antiglobulin (Coombs) test, which employs anti-human globulin to bridge sensitized red cells and produce visible agglutination (Coombs et al., 1945). This innovation enabled the detection of clinically significant IgG alloantibodies including anti-S, anti-s, anti-U, and occasionally anti-M that do not react at lower temperatures. The Coombs test rapidly became a cornerstone of pretransfusion testing, antenatal antibody screening, and hemolytic disease diagnostics.

Refinements in Serology;

- Through the mid to late 20th century, additional refinements improved sensitivity and specificity:
- Enzyme treatment of red cells (e.g., ficin or papain) to enhance or diminish antigen expression and help differentiate MNS antibodies.
- Column agglutination and gel-card methods, which standardized testing and allowed semi-quantitative titration of antibodies.
- Development of monoclonal antisera for routine blood typing of M, N, S, and s antigens.

These advances collectively improved the reliability of detecting antibodies with potential clinical impact.The advent of molecular genetics added a new dimension. Cloning and sequencing of the GYPA and GYPB genes revealed that M and N antigens reside on glycophorin A, whereas S, s, and U antigens are encoded by glycophorin B (Reid and Lomas-Francis, 2004). Identification of the GYP gene cluster on chromosome 4 explained the high rate of unequal crossing-over and gene conversion, mechanisms responsible for the remarkable diversity of hybrid glycophorin molecules and rare MNS phenotypes observed worldwide (Westhoff, 2021).

The integration of serologic and molecular techniques now provides a comprehensive approach to transfusion safety, enabling early detection of clinically significant MNS antibodies, accurate antigen typing, and improved management of both routine and high-risk transfusion scenarios.

Automation and High-Throughput Platforms

Modern transfusion services increasingly employ automated platforms (e.g., microplate, gel-card, and solid-phase red-cell adherence systems) for antibody screening and identification. These systems improve reproducibility, reduce hands-on time, and allow simultaneous testing of large sample volumes important for donor centers and busy antenatal clinics.

Flow Cytometry;

Although not routine for MNS typing, flow cytometric red cell phenotyping offers highly sensitive detection of weakly expressed antigens or mixed red-cell populations (e.g., in recent transfusions or fetomaternal hemorrhage). Fluorescently labeled monoclonal antibodies can quantify antigen density and detect subtle mosaic expression.

Digital/Next-Generation Genotyping;

Beyond PCR, high-resolution melting analysis, microarrays, and next-generation sequencing (NGS) enable discovery of rare or novel GYPA/GYPB hybrid alleles and copy-number variations. These tools help explain unusual serologic patterns and support creation of comprehensive rare-donor databases.

Integration with Rare Donor Registries

Molecular typing data feed into national and international rare donor registries, allowing rapid location of U-negative or other rare MNS phenotypes for transfusion support critical for patients with anti-U or complex antibody profiles.

Quality Assurance and Standardization

International standards from bodies such as the AABB and International Society of Blood Transfusion (ISBT) now guide validation of both serologic and molecular tests, ensuring consistency across laboratories and improving transfusion safety worldwide.

Emerging Point-of-Care Options

Research is exploring portable PCR and microfluidic assays for rapid, near-patient MNS genotyping, which could be valuable in remote settings or for urgent transfusion decisions.

The introduction of the antiglobulin (Coombs) test in 1945 further amplified the clinical significance of these antigens by allowing reliable detection of IgG alloantibodies, including anti-S and anti-U, which may cause severe hemolytic transfusion reactions or hemolytic disease of the fetus and newborn (HDFN). In the decades that followed, molecular breakthroughs particularly the cloning and sequencing of GYPA and GYPB genes and recognition of the GYP gene cluster on chromosome 4 revealed the genetic mechanisms behind MNS diversity, including hybrid glycophorin formation and unequal crossing-over (Reid and Lomas-Francis, 2004; Westhoff, 2021).

Collectively, these milestones have direct, ongoing clinical impact:

- Transfusion safety: Routine screening for MNS antibodies and molecular genotyping enable provision of antigen-matched blood, preventing life-threatening hemolytic reactions in multi-transfused patients and those with rare phenotypes.
- Prenatal and perinatal care: Early identification of maternal anti-M, anti-S, anti-s, or anti-U antibodies allows targeted fetal monitoring and timely interventions such as intrauterine transfusion, dramatically improving outcomes in HDFN.
- Population genetics and global donor management: Molecular mapping of GYPA/GYPB variants informs the creation of rare donor registries, ensuring compatible units for individuals with unusual antigen profiles worldwide.

Nearly a century after Landsteiner and Levine's first report, the MNS system remains a model of how careful serologic observation, technological innovation, and molecular genetics converge to improve patient care. Its ongoing study continues to refine transfusion practices, enhance prenatal diagnostics, and illuminate the dynamic nature of human genetic variation.

2.3 MOLECULAR AND GENETIC BASIS OF THE MNS BLOOD GROUP SYSTEM

The molecular foundation of the MNS blood group system lies in the structural and genetic features of two closely linked genes, glycophorin A (GYPA) and glycophorin B (GYPB), which are arranged in tandem on the long arm of chromosome 4 (4q31) (Reid and Lomas-Francis, 2004; Westhoff, 2021). These genes encode highly glycosylated sialoglycoproteins that are among the most abundant integral membrane proteins of the human erythrocyte. Together, glycophorin A (GPA) and glycophorin B (GPB) carry the antigenic determinants for more than 40 serologically recognized MNS antigens,making this one of the most complex blood group systems identified to date.

Gene Organization and Expression

- GYPA spans approximately 7 kilobases and contains seven exons, encoding GPA, which expresses the M and N antigens as codominant traits.
- GYPB, a highly homologous gene adjacent to GYPA, encodes GPB, which bears the S, s, and U antigens.

Both genes are part of a GYP gene cluster that also includes GYPE, a related pseudogene. Their high sequence similarity predisposes the region to unequal crossing-over and gene conversion events, generating hybrid alleles and the remarkable antigenic diversity characteristic of the MNS system (Westhoff, 2021).GPA and GPB are single-pass transmembrane proteins with heavily O-glycosylated extracellular domains rich in sialic acid. This dense negative charge contributes to red cell membrane stability, provides protection against aggregation, and serves as a receptor for certain pathogens, including Plasmodium falciparum, the parasite responsible for malaria (Baum *et al*., 2003). The amino acid differences encoded by the M and N alleles of GYPA occur at positions 1 and 5 of the mature protein, whereas the S/s polymorphism of GYPB reflects a single amino acid substitution at position 29.

The near-identity of GYPA and GYPB promotes frequent recombination and gene conversion, producing a wide range of GYPA–GYPB hybrid genes. These genetic rearrangements underlie numerous Miltenberger (Mi) subclasses and rare phenotypes such as GP.Mur, He, and Sta, each associated with distinct antigenic determinants (Issitt and Anstee, 1998). Such structural variation explains the existence of more than 40 MNS antigens beyond the classic M, N, S, s, and Molecular insights into GYPA and GYPB have transformed laboratory practice. PCR-based genotyping and next-generation sequencing allow precise identification of both common and rare

MNS alleles, even when serologic testing is limited by recent transfusion or weak antigen expression (Storry and Olsson, 2017). This molecular approach enhances transfusion safety, supports noninvasive prenatal testing, and helps maintain rare donor registries critical for patients with antibodies to uncommon MNS antigens.

Gene Organization and Structure

The GYPA gene spans roughly 7 kilobases (kb) and contains seven exons, whereas GYPB is slightly shorter, with five exons (Storry & Olsson, 2017). Despite these small structural differences, the two genes share more than 95 % sequence homology, consistent with the hypothesis that GYPB arose from an ancestral duplication of GYPA millions of years ago (Rahuel *et al*., 2012). Both are situated in close proximity within the GYP gene cluster on chromosome 4q31, along with the related pseudogene GYPE.

This tight physical linkage and striking sequence similarity make the region particularly prone to unequal crossing-over and gene conversion events during meiosis. Such recombination mechanisms generate a broad spectrum of GYPA–GYPB hybrid alleles, leading to an array of low- and high-frequency antigens that account for much of the extraordinary diversity within the MNS blood group system (Westhoff, 2021).

Functionally, both genes encode single-pass transmembrane sialoglycoproteins that are heavily O-glycosylated. These structural features maintain red cell membrane integrity and provide a dense negative charge, which protects erythrocytes against spontaneous aggregation and serves as receptors for certain pathogens, including Plasmodium falciparum (Baum *et al*., 2003).

Importantly, specific point mutations or small insertions/deletions within exons or intron exon junctions can alter the amino-acid sequence of the extracellular domain, creating new antigenic epitopes. For example:

M/N polymorphism: differs by amino acids at positions 1 and 5 of GPA.

S/s polymorphism: results from a single amino acid change at position 29 of GPB.

The dynamic nature of the GYP cluster means that novel variants continue to be discovered through next-generation sequencing and PCR-based genotyping, expanding the recognized MNS antigen repertoire beyond the classical M, N, S, s, and U antigens (Storry and Olsson, 2017).

Molecular Determinants of M and N Antigens

The M and N antigens are classic examples of how minor amino-acid substitutions can create distinct blood group epitopes. They differ by only two residues at positions 1 and 5 of the extracellular N-terminal domain of the glycophorin A (GPA) protein:

M allele: encodes serine at position 1 and glycine at position 5.

N allele: encodes leucine at position 1 and glutamic acid at position 5 (Daniels, 2013).

These seemingly small changes alter the conformation and electrostatic properties of the exposed N-terminal peptide, producing structurally distinct antigenic epitopes readily recognized by specific anti-M or anti-N antibodies in standard serologic assays.

Genetic Inheritance and Phenotypes

Because GYPA is inherited codominantly, each allele is expressed independently on the red-cell surface. This gives rise to four primary phenotypes observed across human populations (Storry and Olsson, 2017):

Genotype	Phenotype	Serologic Profile
MM	M + N –	Only M antigen expressed
NN	M – N +	Only N antigen expressed
MN	M + N +	Both antigens expressed
Null or deletion variants	M – N –	Absence of both antigens (rare)

The M – N – phenotype usually reflects deletions or hybrid GYPA–GYPB genes and is uncommon worldwide but more frequent in certain isolated or admixed populations.

Clinical and Population Relevance

Transfusion medicine: Anti-M antibodies are often naturally occurring IgM and usually clinically insignificant, but immune IgG anti-M can occasionally cause hemolytic disease of the fetus and newborn (HDFN). Anti-N is rarer but has similar implications when reactive at 37 °C.

Population genetics: Global frequencies vary: the MN heterozygous phenotype is most common in many regions, while allele frequencies differ among African, Asian, and European populations, making M and N useful in anthropologic and paternity studies. These molecular details illustrate how two point mutations in GYPA translate into serologically detectable antigens with real clinical and evolutionary significance.

Molecular Determinants of S, s, and U Antigens

The S and s antigens are carried on glycophorin B (GPB) and differ by a single amino-acid substitution at position 29 of the mature protein: methionine specifies the S antigen, while threonine specifies the s antigen (Reid and Lomas-Francis, 2004). Although this change is subtle, it alters the local three-dimensional configuration of the extracellular domain enough to create two clearly distinct serologic epitopes. Like the M and N alleles of GYPA, the S and s alleles are codominant, so red cells can express S, s, both, or rarely neither antigen.

The U Antigen

Located on the same GPB molecule is the U (Universal) antigen, a high-incidence epitope present on the vast majority of human red cells. Expression of U depends on the presence of the membrane-spanning region of GPB; therefore, individuals who inherit homozygous deletions or silencing mutations of GYPB lack GPB entirely and fail to express S, s, and U (Issitt & Anstee, 1998). This S–s–U– phenotype is extremely rare worldwide but occurs with increased frequency among people of African ancestry, reflecting population-specific genetic events such as unequal crossing-over or gene conversion.

Clinical Significance

The S, s, and especially U antigens have major implications for transfusion and obstetrics:

- Transfusion reactions: Alloantibodies to S or s can cause acute or delayed hemolytic transfusion reactions, particularly if the antibody is IgG and reactive at 37 °C.
- Hemolytic disease of the fetus and newborn (HDFN): Anti-S or anti-s occasionally cause HDFN, while anti-U is notorious for severe, sometimes life-threatening HDFN (Evers et al., 2019).
- Blood supply challenges: Because U-negative donors are rare, identifying compatible blood for patients with anti-U often requires coordination with rare donor registries and early antenatal planning.

Allele frequencies vary across populations: S is more prevalent in Europeans, while s is more common globally, and the S–s–U– phenotype is largely confined to certain African and African-descended groups. Molecular analysis has revealed multiple GYPB hybrid alleles that can reduce or alter S/s expression without complete GPB deletion, occasionally complicating serologic typing. These molecular and genetic insights underscore why PCR-based genotyping and next-generation sequencing are now routine for accurately characterizing S, s, and U antigens information that is critical for transfusion safety and management of at-risk pregnancies.

These are hybrids that give rise to a wide spectrum of serologically distinct phenotypes, including:

- Miltenberger (Mi) series: A complex set of variants (e.g., Mi^a, Mur, Hil, Vw)characterized by hybrid GYPA–GYPB alleles. Mi^a is particularly prevalent in East and Southeast Asia, reaching frequencies of 7–9 % in some populations.
- Ena phenotype: Results from partial or complete deletion of GYPA, leading to absence of the M and N antigens and occasionally the En(a) antigen.
- He antigen (GP.He): Another hybrid product combining exons from both genes and recognized as a low-frequency antigen worldwide (Westhoff, 2021).

Clinical Importance

These variant antigens are clinically significant because individuals who lack the corresponding antigen can form alloantibodies after transfusion or pregnancy exposure. Such antibodies anti-Mi^a, anti-Mur, or anti-He, for example have been documented to cause hemolytic disease of the fetus and newborn (HDFN) and hemolytic transfusion reactions. The challenge is amplified in multi-transfused or multiparous patients, where exposure risk is higher and compatible donor units are scarce.

The distribution of hybrid glycophorins is geographically and ethnically variable. For instance, the Mi^a antigen is relatively common in East Asian populations but rare in Europeans and Africans, necessitating region-specific screening protocols and targeted rare-donor registries to ensure safe transfusion and effective antenatal care.

Modern Detection

Traditional serologic methods may fail to detect these variants if the antigenic expression is weak or atypical. PCR-based genotyping and sequencing now allow precise identification of hybrid alleles, facilitating better matching of blood products and more accurate risk assessment during pregnancy

Differences in transcriptional activity translate into quantitative variation in antigen density, which can:

- Affect serologic reactivity, leading to weaker agglutination and potential misclassification of M, N, S, or s status.
- Modify the clinical presentation of alloimmunization, since lower antigen density may reduce but not eliminate the likelihood of maternal antibody binding in pregnancy.
- Contribute to variable severity of hemolytic disease of the fetus and newborn (HDFN) in cases where maternal anti-MNS antibodies are present.

Although these regulatory influences are less well characterized than structural gene mutations, accumulating evidence suggests that promoter polymorphisms and epigenetic variation could help explain inconsistent antigen strength and antibody responses observed in pregnant women and transfusion recipients.

Clinical and Population Relevance

- Transfusion medicine: Alloantibodies to MNS antigens—particularly anti-S, anti-s, and anti-U can cause acute hemolytic transfusion reactions or hemolytic disease of the fetus and newborn (HDFN).
- Ethnic and geographic variation: Frequencies of M, N, S, s, and U differ significantly among populations; for example, U-negative phenotypes are found more often in people

of African ancestry, while Mi^a and related antigens occur at higher rates in parts of Asia.

- Diagnostic complexity: The diversity of hybrid antigens and variable antigen density can complicate serologic typing,often requiring molecular genotyping for accurate blood matching.

The remarkable antigenic diversity of the MNS system reflects the dynamic genetic landscape of the GYPA and GYPB loci, highlighting its continued significance in transfusion practice, prenatal care, and population genetics.

Recognized Phenotypes;

Serologic expression of these antigens gives rise to a wide range of phenotypes beyond the basic M+N– or S+s– patterns. Examples include:

- Common phenotypes: M+N+, M+N–, M–N+, S+s+, S+s–, and S–s+.
- Rare phenotypes: U– (often S–s– as well), Ena–, and various Miltenberger subclasses with unique antigen combinations.
- Null phenotypes: Complete absence of GPA (En(a–)) or GPB (S–s–U–) resulting from large deletions or hybrid arrangements.

Clinical and Population Relevance;

- Transfusion medicine: Alloantibodies to MNS antigens particularly anti-S, anti-s, and anti-U can cause acute hemolytic transfusion reactions or hemolytic disease of the fetus and newborn (HDFN).

- Ethnic and geographic variation: Frequencies of M, N, S, s, and U differ significantly among populations; for example, U-negative phenotypes are found more often in people of African ancestry, while Mi^a and related antigens occur at higher rates in parts of Asia.
- Diagnostic complexity: The diversity of hybrid antigens and variable antigen density can complicate serologic typing, often requiring molecular genotyping for accurate blood matching.

The remarkable antigenic diversity of the MNS system reflects the dynamic genetic landscape of the GYPA and GYPB loci, highlighting its continued significance in transfusion practice, prenatal care, and population genetics.The principal carriers of MNS **Glycophorin** antigens glycophorin A (GPA) and glycophorin B (GPB) are type-I transmembrane sialoglycoproteins with a single membrane-spanning domain and a large extracellular region. Both proteins are characterized by extensive O-linked glycosylation, resulting in a carbohydrate content of nearly 60 % by weight (Reid and Lomas-Francis, 2004).

Structural Features;

Extracellular domain: Rich in sialic acid residues, it projects ~20 nm beyond the erythrocyte membrane. This creates a strong negative surface charge (zeta potential) that prevents red-cell aggregation and contributes to the characteristic smooth flow of erythrocytes through microvasculature (Westhoff, 2021). Transmembrane and cytoplasmic regions: The short cytoplasmic tails interact with the membrane skeleton, particularly band 3 and spectrin, helping maintain red-cell membrane stability.

Antigenic Determinants;

The MNS antigenic epitopes are predominantly located on the N-terminal extracellular segments of GPA and GPB. These regions are highly exposed and conformationally flexible, enabling circulating antibodies to access the epitopes without steric hindrance (Storry & Olsson, 2017). Specific examples include:

M and N epitopes: Amino-acid differences at positions 1 and 5 of GPA.

S and s epitopes: A single amino-acid substitution at position 29 of GPB.

U epitope: Spans the membrane-proximal portion of GPB, requiring intact GPB expression.

Functional and Clinical Implications;

Immunogenicity: The protruding, sialylated extracellular domains make MNS antigens highly immunogenic, explaining the frequency of clinically significant antibodies (e.g., anti-S, anti-U) encountered in transfusion and obstetric practice.

Pathogen interactions: The sialic acid–rich architecture serves as a receptor for pathogens, including Plasmodium falciparum merozoites and certain viruses, illustrating a possible evolutionary pressure for antigenic diversity.

Diagnostic relevance: High surface density and accessibility allow reliable detection by routine serologic tests, though hybrid variants can present atypical expression patterns.

These structural and functional attributes highlight why GPA and GPB are not merely passive membrane components but active mediators of cell–cell interactions and immune recognition, underscoring the clinical importance of the MNS system.From a clinical perspective, anti-M antibodies are most often naturally occurring IgM that react optimally at colder temperatures and are therefore usually of limited transfusion importance. Nevertheless, IgG anti-M though uncommon has been documented to cross the placenta and cause hemolytic disease of the fetus and newborn (HDFN), underscoring the need for careful antibody identification during antenatal screening (Evers *et al*., 2019). Routine serologic testing readily distinguishes these antigens, allowing laboratories to provide appropriately matched blood when clinically indicated.

Principal Antigens: S and s;

The S and s antigens are carried on glycophorin B (GPB) and differ by a single amino-acid substitution at position 29: methionine for S and threonine for s (Reid and Lomas-Francis, 2004). Despite this minimal structural change, the alteration modifies the N-terminal extracellular domain sufficiently to create distinct antigenic epitopes detectable by standard serologic testing.

As with the M and N alleles, the S and s genes are codominant, producing the phenotypes S + s – , S – s +, S + s +, and the very rare S – s –. Population surveys reveal striking ethnic differences: the S allele occurs in roughly 50–55 % of most Caucasian populations but averages closer to 30 % in many African groups, whereas the s allele is almost ubiquitous worldwide, often exceeding 85 % in frequency (Daniels, 2013; Okafor and Owa, 2022).

Clinically, anti-S and anti-s antibodies are usually IgG, react best at 37 °C, and are capable of crossing the placenta. Both have been implicated in severe hemolytic disease of the fetus and newborn (HDFN) as well as acute and delayed hemolytic transfusion reactions, making precise antigen typing and antibody screening critical in prenatal care and transfusion medicine (Fung *et al*., 2020). Modern PCR-based genotyping now complements serology, enabling rapid identification of S and s alleles in patients with complex serologic profiles or multiple transfusions.The U antigen is a high-incidence epitope present on nearly all red cells except those with homozygous deletions or silencing of GYPB. Individuals with the S–s–U– phenotype are exceedingly rare but occur more frequently among people of African descent, with reported frequencies up to 1 % in certain West African populations (Ukaejiofo *et al*., 2017). Anti-U antibodies are almost always IgG and can cause severe transfusion reactions and HDFN that may necessitate intrauterine transfusion or early delivery (Evers *et al*., 2019). The scarcity of U-negative donors makes transfusion support for affected patients particularly challenging. Molecular genotyping is therefore recommended when anti-U antibodies are detected in pregnant women to facilitate the timely identification of compatible blood (Westhoff, 2021).

Low-Frequency and Variant Antigens;

Beyond the principal M, N, S, s, and U antigens, the MNS system comprises more than 40 serologically recognized minor antigens, many of which occur at low population frequencies or are restricted to specific geographic regions. Examples include He, Vw, Hil, Hut, and ENEP (Issitt & Anstee, 1998). Others, such as Ena a high-frequency antigen present on nearly all red cells gain clinical importance because antibodies against them are difficult to manage when transfusion is required. A particularly well-studied subset is the Miltenberger series, a group of phenotypes (e.g., Mi^a, Vw, Mur, Hil, Hut) that result from unequal crossing-over and gene conversion events between GYPA and GYPB (Storry and Olsson, 2017). These hybrid genes encode chimeric glycophorins with novel extracellular domains, creating unique antigenic determinants that can provoke alloimmunization in transfused or multiparous individuals.

Geographic distribution is strikingly uneven. For instance, the Mi^a antigen occurs with appreciable frequency in Southeast Asian populations, is less common in Europe, and is rare in most African and North American groups (Daniels, 2013). Clinically significant anti-Mi^a and anti-Mur antibodies have been implicated in hemolytic transfusion reactions and in mild hemolytic disease of the fetus and newborn (HDFN). Because routine antibody screening panels may lack cells expressing these rare antigens, laboratories serving populations where these variants are prevalent must incorporate region-specific reagent red cells or molecular assays to prevent missed alloimmunization and ensure safe transfusion and obstetric management.Epidemiologic surveys demonstrate considerable interethnic variability in MNS phenotypes. In Nigeria and other parts of West Africa, reported M and N allele frequencies are approximately 0.56 and 0.44, respectively, while the S–s–U– phenotype is observed at higher rates than in European populations (Okafor & Owa, 2022; Ukaejiofo *et al*., 2017).

Among European populations, S and s frequencies are roughly 0.55 and 0.89, whereas Asian populations often show higher M allele prevalence and unique variant antigens such as Mi^a (Dean, 2022). Understanding these distributions is essential for planning antenatal screening programs and ensuring the availability of antigen-negative donor blood.

The extraordinary antigenic diversity of the MNS blood group system directly influences obstetric and transfusion practice. Alloantibodies directed against M, S, s, or U antigens can cross the placenta and cause hemolytic disease of the fetus and newborn (HDFN), with severity ranging from mild neonatal jaundice to severe fetal anemia, hydrops fetalis, or intrauterine death (Evers et al., 2019). Although anti-M is often a naturally occurring IgM, clinically significant IgG anti-M antibodies capable of causing moderate to severe HDFN have been well documented.

Antibodies to low-frequency or hybrid antigens such as those in the Miltenberger series (e.g., Mi^a, Mur) also pose challenges. These antibodies may form after fetomaternal hemorrhage or transfusion, and because many standard antibody screening panels lack cells expressing these rare antigens, alloimmunization can be missed, creating a risk of acute or delayed hemolytic transfusion reactions and complicated pregnancies (Fung *et al*., 2020).

Consequently, comprehensive antigen profiling of both pregnant women and prospective blood donors is vital. Modern practice increasingly integrates molecular genotyping with traditional serology to identify MNS alleles, detect silent or weak variants, and secure antigen-matched blood when clinically significant antibodies are present. This combined approach enhances prenatal monitoring, transfusion safety, and perinatal outcomes in settings where MNS antibodies are suspected or confirmed.

2.4 MNS ANTIBODIES AND IMMUNOLOGY

The immunologic response to MNS antigens is heterogeneous and underpins the system's clinical significance in both pregnancy and transfusion medicine. Antibodies may be naturally occurring arising without prior transfusion or pregnancy or immune-stimulated following fetomaternal hemorrhage, transfusion, or transplantation (Daniels, 2013; Fung *et al*., 2020).

Immunoglobulin Class and Thermal Range:

- Anti-M is frequently a naturally occurring IgM antibody that reacts optimally at colder temperatures (≤ 20 °C) and is often clinically insignificant.
- However, IgG anti-M and most anti-S, anti-s, and anti-U antibodies are immune-stimulated and react at 37 °C, making them clinically significant because IgG readily crosses the placenta.

Pathogenic Potential:

- IgG antibodies directed against S, s, or U can cause severe hemolytic transfusion reactions and hemolytic disease of the fetus and newborn (HDFN), leading to fetal anemia or hydrops fetalis.
- Rare antibodies to low-frequency or hybrid antigens (e.g., Mi^a, Mur) have also been implicated in acute and delayed hemolytic reactions.

Detection and Management:

- Indirect antiglobulin testing (IAT) remains the standard for screening clinically significant IgG antibodies, while enzyme-treated panels may enhance detection of certain variants.
- Molecular genotyping of GYPA and GYPB complements serology, helping identify silent or weakly expressed alleles that could otherwise be missed.

The diversity of immunoglobulin class, thermal amplitude, and clinical significance across MNS antibodies necessitates careful interpretation of antibody screens, particularly in antenatal programs, to guide transfusion support and prenatal monitoring.

Classification of MNS Antibodies

MNS antibodies are customarily grouped into naturally occurring and immune-stimulated categories, reflecting their different origins, immunoglobulin classes, and clinical significance.

Naturally Occurring Antibodies

- Most often anti-M or anti-N, these antibodies are usually IgM cold agglutinins that react best at temperatures below 37 °C.
- They can appear without prior transfusion or pregnancy, likely triggered by exposure to environmental antigens such as certain bacteria or plant proteins that share structural homology with M or N epitopes (Dean, 2022).
- Although typically clinically insignificant, naturally occurring anti-M can occasionally present as an IgG component or demonstrate broad thermal amplitude, warranting careful evaluation before transfusion or during pregnancy.

Immune Antibodies

- IgG anti-M, anti-S, anti-s, and anti-U antibodies arise after sensitization through blood transfusion, fetomaternal hemorrhage, or transplantation.
- These antibodies react optimally at 37 °C and can cross the placenta, posing a risk of hemolytic disease of the fetus and newborn (HDFN) and hemolytic transfusion reactions.
- Among these, anti-U is particularly concerning because compatible U-negative donor blood is rare, making management of affected pregnancies and transfusions challenging.

Laboratory and Clinical Considerations

- Routine antibody screening with the indirect antiglobulin (Coombs) test helps differentiate clinically significant IgG antibodies from benign cold agglutinins.
- Molecular genotyping of the GYPA and GYPB genes can identify silent or hybrid alleles when serologic results are ambiguous, ensuring safe transfusion planning and prenatal care.

This classification underscores the need to assess both antibody type and thermal amplitude during antenatal screening to distinguish benign cold agglutinins from antibodies capable of causing serious fetal or transfusion-related complications.

The clinical importance of an antibody is closely linked to its immunoglobulin class and thermal amplitude:

IgM antibodies commonly anti-M or anti-N usually react at cold temperatures and are often clinically insignificant. However, a subset demonstrates broad thermal amplitude, remaining reactive at 37 °C and capable of causing hemolysis or HDFN (Storry and Olsson, 2017).

IgG antibodies typical of anti-S, anti-s, anti-U, and some anti-M react at body temperature and are clinically significant. IgG antibodies efficiently cross the placenta and can lead to fetal anemia, hydrops fetalis, or stillbirth if unrecognized (Fung *et al.*, 2020).

Mechanisms of Alloimmunization in Pregnancy

Alloimmunization in pregnancy arises when fetal red blood cells (RBCs) carrying paternal MNS antigens enter the maternal circulation and stimulate a maternal immune response.

Fetomaternal Hemorrhage (FMH):

Small transplacental bleeds may occur spontaneously during gestation, particularly in the late second or third trimester, and more substantial FMH commonly accompanies labor and delivery.

Additional risk factors include trauma, placental abruption, external cephalic version, and invasive prenatal procedures such as amniocentesis or chorionic villus sampling (Dean, 2022).

Immunologic Pathway:

Maternal antigen-presenting cells process the foreign MNS antigens and present them to $CD4^+$ T helper cells, which in turn activate antigen-specific B cells. B cells undergo class switching from IgM to IgG, aided by cytokines such as IL-4 and IL-21, and differentiate into plasma cells and memory B cells.

Because IgG readily crosses the placenta, these antibodies are capable of causing fetal hemolysis.

Anamnestic Response in Subsequent Pregnancies:

In later pregnancies with an antigen-positive fetus, even minimal FMH can trigger a rapid, high-affinity IgG response from maternal memory B cells, often earlier and more robust than the primary reaction (Moise, 2018). The resulting maternal IgG binds to fetal RBCs, leading to extravascular hemolysis in the fetal spleen and liver, which can manifest as fetal anemia, hydrops fetalis, or intrauterine death if not managed promptly.

Understanding these mechanisms underscores the importance of routine antibody screening and timely interventions such as serial maternal antibody titers, Doppler assessment of fetal middle cerebral artery flow, and intrauterine transfusion when indicated to prevent or treat hemolytic disease of the fetus and newborn (HDFN) associated with MNS alloantibodies.

Anti-M Antibody

While anti-M is most often a naturally occurring IgM cold agglutinin of limited clinical significance, a growing number of reports document pathogenic IgG anti-M capable of causing moderate to severe hemolytic disease of the fetus and newborn (HDFN) (Evers *et al*., 2019; Moise, 2018).

Clinical Detection;

IgG anti-M often displays variable thermal amplitude and reactivity, sometimes reacting only weakly or intermittently at 37 °C. Indirect antiglobulin testing (IAT) is essential for detection, and enzyme-treated red cell panels or molecular genotyping of GYPA may be required to confirm specificity when serology is inconclusive (Storry and Olsson, 2017).

Management Considerations;

Pregnancies complicated by IgG anti-M warrant serial maternal antibody titers and middle cerebral artery Doppler ultrasonography to monitor for fetal anemia. Severe cases may require intrauterine transfusion with M-negative blood, and at delivery, neonates should be observed for late-onset anemia, which can persist for several weeks. The potential for IgG anti-M to suppress erythropoiesis makes early recognition critical, even when routine screening shows only low antibody titers.

Anti-N Antibody

Anti-N is most often a naturally occurring IgM cold agglutinin, reacting optimally at temperatures below 37 °C and rarely associated with hemolytic disease of the fetus and newborn (HDFN) (Daniels, 2013).

However, important exceptions warrant clinical attention:

Immune IgG Anti-N;

Documented but uncommon, immune-stimulated IgG anti-N can arise after blood transfusion or fetomaternal hemorrhage.

Such antibodies have been linked to isolated hemolytic transfusion reactions and to mild neonatal jaundice in a few reported pregnancies.Because anti-N typically behaves as a cold-reactive IgM, serologic testing at 37 °C with an indirect antiglobulin test (IAT) is essential to uncover a clinically significant IgG component.

Enzyme-treated panels may enhance detection when IgG anti-N is suspected.

Clinical Implications;

While most anti-N antibodies are benign, the rare IgG form can cross the placenta, so clinicians should not dismiss anti-N solely for its usual cold-reactive nature during antenatal screening. When an IgG component is present, monitoring maternal titers and ensuring N-negative, crossmatch-compatible blood for transfusion are prudent. These precautions help prevent the occasional but clinically relevant complications associated with immune anti-N.

2.5 LABORATORY DETECTION AND CHARACTERIZATION OF MNS ANTIBODIES

Accurate detection and characterization of MNS antibodies in antenatal patients require a combination of serologic testing and, when appropriate, molecular techniques. Proper laboratory evaluation is essential for identifying clinically significant antibodies, guiding transfusion decisions, and assessing the risk of hemolytic disease of the fetus and newborn (HDFN).

Indirect Antiglobulin Test (IAT);

The IAT, performed using tube or gel-based methods, remains the gold standard for detecting IgG antibodies against MNS antigens in maternal serum (Fung et al., 2020). This test allows the identification of antibodies that react at 37 °C, which are more likely to be clinically significant in transfusion or pregnancy settings.

Enzyme-Treated Red Cells;

Treating reagent red cells with enzymes such as papain or ficin can enhance or reduce reactivity of certain MNS antibodies. For example, enzyme treatment often enhances detection of anti-S and anti-s while diminishing anti-M activity, thereby helping distinguish among antibody specificities (Daniels, 2013).

Molecular Assays;

Polymerase chain reaction (PCR)–based genotyping and other molecular techniques (e.g., sequencing, PCR-RFLP) allow precise identification of GYPA and GYPB alleles, including rare or hybrid variants. Molecular testing is particularly valuable in cases where recent transfusion may obscure serologic typing, or when weak or variant antigen expression complicates antibody identification. Genotyping also assists in providing antigen-matched blood for transfusion and can complement serology in high-risk pregnancies where fetal antigen status may influence management decisions.

Other Advanced Serologic Techniques;

Solid-phase red cell adherence assays and flow cytometry can provide enhanced sensitivity for low-titer antibodies. These methods are useful for detecting clinically significant antibodies early in pregnancy or in complex serologic cases.

Clinical Relevance;

Combining serologic and molecular approaches ensures accurate characterization of maternal antibodies, enabling proactive monitoring of at-risk pregnancies and safe transfusion support. This integrated approach is especially critical for antibodies against S, s, and U, which are associated with severe HDFN and are challenging to manage if unidentified.

Antenatal Management of Pregnancies Complicated by MNS Antibodies

Effective management of pregnancies affected by clinically significant MNS antibodies requires a risk-based, multidisciplinary approach that combines laboratory monitoring, fetal surveillance, and transfusion planning.

Maternal Antibody Screening and Monitoring;

All pregnant women should undergo routine antibody screening during early antenatal visits. If a clinically significant MNS antibody (e.g., IgG anti-S, anti-s, anti-U, or IgG anti-M) is detected, serial titration of maternal antibody levels is recommended to assess the risk of fetal hemolysis. Rising titers or levels exceeding critical thresholds indicate an increased risk of hemolytic disease of the fetus and newborn (HDFN).

Fetal Monitoring;

Non-invasive surveillance includes ultrasound assessment for signs of fetal anemia, such as hydrops fetalis, ascites, or cardiomegaly. Middle cerebral artery peak systolic velocity (MCA-PSV) Doppler studies are the current standard for detecting moderate to severe fetal anemia, allowing timely intervention without immediate invasive testing.

Invasive Interventions;

When severe fetal anemia is suspected, cordocentesis (percutaneous umbilical blood sampling) can quantify fetal hemoglobin and confirm antibody mediated hemolysis. Intrauterine transfusion (IUT) with antigen-negative, crossmatch-compatible red cells may be required to correct fetal anemia and prevent hydrops or demise.

Delivery Planning;

Timing and mode of delivery are tailored based on gestational age, fetal status, and severity of anemia. Neonates should be delivered at centers equipped for immediate phototherapy, exchange transfusion,or intensive care, as HDFN may persist after birth.

Transfusion Considerations;

Maternal and fetal/neonatal transfusions must use antigen-negative blood matching the clinically significant MNS antibody profile. Molecular genotyping of donor and recipient blood is increasingly utilized to ensure safe matches, particularly for rare or low-frequency antigens (e.g., U-negative blood for anti-U antibodies).

Postnatal Management;

Neonates with HDFN require close monitoring of hemoglobin and bilirubin levels, with interventions such as phototherapy, immunoglobulin therapy, or transfusions as indicated. Continued surveillance is necessary, as late-onset anemia can occur in the first several weeks of life, especially in cases with IgG anti-M antibodies that suppress erythropoiesis. This framework integrates laboratory findings, immunologic risk, and fetal/neonatal monitoring to optimize outcomes in pregnancies complicated by MNS antibodies.

2.6 RISK FACTORS FOR ALLOIMMUNIZATION IN PREGNANCY

The development of clinically significant MNS antibodies in pregnant women is influenced by a combination of immunologic exposure, genetic factors, and maternal health conditions. Key risk factors include:

Previous Pregnancies;

Maternal exposure to paternally inherited fetal red cell antigens during prior pregnancies, particularly in heterozygous fetuses, increases the risk of alloimmunization in subsequent pregnancies (Dean, 2022). Even small amounts of fetomaternal hemorrhage (FMH) can prime maternal B cells to produce IgG antibodies, leading to a more rapid and robust anamnestic response in later pregnancies.

Blood Transfusions;

Prior allogeneic transfusions with red cells not fully matched for MNS antigens can sensitize women to foreign epitopes, particularly S, s, and U. Multi-transfused women, including those with chronic anemia or hemoglobinopathies, are at higher risk for developing clinically significant alloantibodies.

Ethnic and Genetic Background;

Certain populations have a higher prevalence of rare or null phenotypes, such as S–s–U– in women of African ancestry. These women are particularly susceptible to developing anti-U antibodies, which are clinically significant and challenging to manage due to the scarcity of compatible donor blood.

Autoimmune Conditions and Trauma;

Maternal disorders that disrupt the placental barrier or promote inflammation such as autoimmune vasculitis or events causing placental or fetal trauma can increase the risk of fetal red cell leakage into the maternal circulation (Moise, 2018). Invasive prenatal procedures (e.g., amniocentesis, chorionic villus sampling) similarly elevate the likelihood of sensitization if protective measures (e.g., anti-D prophylaxis for Rh-negative women) are not taken.

Other Contributing Factors;

Late or inadequate antenatal antibody screening may allow early alloimmunization to go undetected. Maternal immunologic status, including previous sensitizations or hyperactive immune responses, can influence the magnitude of the antibody response. Understanding these risk factors is critical for targeted antenatal monitoring, timely serologic or molecular testing, and planning antigen-matched transfusions to prevent HDFN and transfusion-related complications in sensitized pregnant women.

2.7 HEMOLYTIC DISEASE OF THE FETUS AND NEWBORN (HDFN) AND MNS SYSTEM

Hemolytic disease of the fetus and newborn (HDFN) occurs when maternal IgG antibodies cross the placenta and destroy fetal red cells carrying the target antigen. While RhD remains the most common cause of severe HDFN, antibodies to MNS antigens have also been implicated in both mild and severe disease (Bowman, 1997).

Anti-M mediated HDFN: Usually mild but can cause intrauterine growth restriction and neonatal anemia (Kozlowski et al., 2015).

Anti-S and Anti-s mediated HDFN: Often more severe, sometimes requiring intrauterine transfusions or exchange transfusion after delivery (Moise, 2008).

The risk of HDFN due to MNS antibodies underscores the need for extended antibody screening in antenatal patients.

Current Practices in Antenatal Alloantibody Screening

In developed countries, extended antibody screening is often performed during pregnancy, allowing early detection and monitoring of clinically significant alloantibodies. Pregnant women with antibodies such as anti-S are monitored with serial antibody titers and fetal Doppler ultrasound, and interventions such as intrauterine transfusions are available (Moise, 2008).

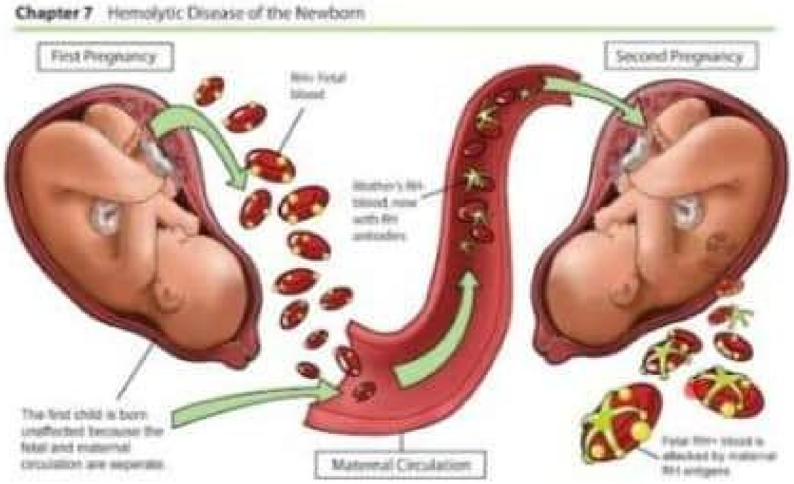
However, in Nigeria and many African countries, antenatal antibody screening is largely limited to RhD typing. The absence of routine extended screening means that alloantibodies such as anti-M and anti-S are rarely detected. This contributes to underreported cases of HDFN and preventable neonatal deaths (Akinbami et al.,2010).

Despite the clinical relevance of the MNS system, there is limited research on its prevalence and significance in Nigerian antenatal populations. Most studies on red cell alloimmunization in Nigeria focus on RhD and ABO systems, neglecting other clinically significant antigens. As a result, healthcare providers may underestimate the burden of alloimmunization caused by MNS antibodies.Without local epidemiological data, it is difficult to design appropriate antenatal care strategies or transfusion protocols. This knowledge gap justifies the need for research on the MNS system in antenatal patients in Nigeria.

The MNS blood group system, discovered nearly a century ago, remains clinically relevant in transfusion medicine and obstetric care. Its antigens—M, N, S, and s—are carried on glycophorins A and B, and their antibodies, particularly anti-S and anti-s, are capable of causing clinically significant hemolysis. Despite their importance, MNS antibodies are often overlooked in antenatal care programs, especially in developing countries.

For antenatal patients, undetected MNS alloantibodies pose a real threat, contributing to cases of hemolytic disease of the fetus and newborn. There is thus a pressing need to investigate the prevalence of MNS antigens and antibodies in Nigerian antenatal populations to improve maternal and neonatal health outcomes.

Hemolytic disease of the newborn...



Case Presentation

A preterm male infant (35 weeks) appropriate for gestational age with birth weight of 2.20 kg, was born to a 28-year G2 P0 mother. The mother's blood group was A positive and the father's blood group was B positive. This pregnancy was complicated by gestational diabetes mellitus which was controlled on diet. Her first pregnancy was an intrauterine fetal death due to immune hydrops. The mother's blood was positive for indirect Coomb's test (ICT) with 1:32 dilution and anti-M antibodies. In this pregnancy too, the mother's ICT was positive. Antenatal scans showed polyhydramnios (amniotic fluid index-18) but no hydrops, Doppler ultrasound of middle cerebral artery MCA) revealed peak systolic velocity in zone B of Marie's curve. Pregnancy was induced at 35 weeks of gestation. The baby cried immediately at birth and had Apgar's of 8/9/9 at 1, 5 and 10 min, respectively. Investigations from the cord blood revealed A positive blood group, positive direct Coomb's test (DCT), haematocrit of 41.4%, reticulocyte count of 5.3% and total serum bilirubin (TSB) of 2.7 mg/dL. On examination the infant was healthy with no pallor, no splenomegaly and was started on breastfeeding.

2.8 CLINICAL SIGNIFICANCE AND MANAGEMENT IN ANTENATAL CARE

The clinical importance of the MNS blood group system in pregnancy lies primarily in its potential to cause hemolytic disease of the fetus and newborn (HDFN) and to complicate transfusion therapy. MNS antibodies particularly IgG anti-S, anti-s, anti-U, and occasionally IgG anti-M can cross the placenta, bind to fetal red cells, and trigger extravascular hemolysis or, in the case of anti-M, suppression of fetal erythropoiesis. Untreated, these mechanisms may result in severe fetal anemia, hydrops fetalis, or intrauterine death (Moise, 2018; Fung *et al.*, 2020). This immune reaction may lead to varying degrees of hemolysis, which in severe cases, can result in:

- Fetal anemia
- Hydrops fetalis (severe fluid accumulation in fetal compartments)
- Intrauterine growth restriction (IUGR)
- Preterm labor
- Perinatal morbidity or mortality

While anti-M is often naturally occurring and sometimes considered clinically insignificant, cases have been reported where anti-M IgG antibodies caused severe, even fatal HDFN, especially when detected early in pregnancy. Antibodies like anti-S and anti-s, if IgG in nature, are more likely to result in moderate to severe HDFN and warrant closer monitoring

Management Strategies in Antenatal Care:

Proper identification and management of MNS antibodies during pregnancy are vital to reduce the risk of hemolytic disease of the fetus and newborn (HDFN). Although not as common as Rh alloimmunization, antibodies in the MNS system especially anti-M, anti-S, and anti-s can be significant. The management approach includes screening, monitoring, diagnostics, and intervention, as explained below:

Antenatal Screening and Risk Assessment;

Routine antibody screening during the first prenatal visit and again at 28 weeks is recommended to identify clinically significant MNS antibodies early. Once an antibody is detected, serial titration (every 2–4 weeks) helps determine whether the pregnancy is at risk of HDFN. A critical titer commonly 1:16 or 1:32, depending on local protocols signals the need for intensified surveillance**.**

Paternal antigen typing or molecular genotyping can clarify whether the fetus is at risk (e.g., heterozygous vs.homozygous for the corresponding antigen).

- If the father is negative for the implicated antigen, the fetus is unlikely to be at risk.
- If the father is heterozygous or positive, fetal antigen status can be determined via cell-free fetal DNA (cffDNA) testing or amniocentesis.

Fetal Surveillance and Intervention;

Pregnancies at or beyond the critical titer are monitored with middle cerebral artery peak systolic velocity (MCA-PSV) Doppler to detect fetal anemia non-invasively. When severe anemia is suspected, cordocentesis may confirm fetal hemoglobin levels, and intrauterine transfusion (IUT) with antigen-negative red cells can prevent hydrops or fetal demise.

Transfusion Planning;

Pregnant patients with clinically significant MNS antibodies who require transfusion must receive antigen-negative, crossmatch-compatible blood. For rare antibodies such as anti-U, sourcing compatible U-negative units often requires coordination with rare donor registries or molecularly typed inventories.

Neonatal Management;

After delivery, affected infants may need phototherapy, intravenous immunoglobulin, or exchange transfusion to manage hyperbilirubinemia and anemia. Ongoing follow-up is critical because late anemia may develop even when initial postnatal hemoglobin is stable. Early identification of maternal MNS antibodies and a multidisciplinary care plan including obstetricians, transfusion medicine specialists, and neonatologists are key to reducing morbidity and mortality associated with MNS alloimmunization in pregnancy.

2.9 NEGLECT OF MNS ALLOIMMUNIZATION IN ANTENATAL CARE

Globally, antenatal antibody screening programs are designed to detect unexpected red cell antibodies that may endanger the fetus. In high-resource countries, extended antibody panels capable of detecting antibodies against Rh, Kell, Kidd, Duffy, and MNS systems are routinely employed (Colin & Anstee, 2020). However, in many developing countries including Nigeria the practice is limited to ABO and RhD typing, with little or no provision for identifying other clinically significant alloantibodies (Akinbami et al., 2010).

This narrow focus means that MNS antibodies, which are capable of causing moderate to severe HDFN, often remain undetected until after neonatal complications have occurred. Consequently, cases of unexplained neonatal jaundice, anemia, or stillbirths may actually be due to undiagnosed MNS alloimmunization.

The MNS system includes four major antigens M, N, S, and s carried on glycophorins A and B. Although anti-M and anti-N antibodies are often naturally occurring and of the IgM class, they can occasionally occur as IgG and cross the placenta, causing mild to moderate HDFN (Daniels, 2013). More importantly, anti-S and anti-s antibodies are typically IgG and are well-documented causes of clinically significant hemolytic transfusion reactions and HDFN (Reid & Lomas-Francis, 2004).

Yet, despite their documented significance, these antibodies are not routinely screened for in antenatal patients in Nigeria. This gap in clinical practice poses a danger, particularly for women requiring blood transfusion during pregnancy, who may be sensitized to MNS antigens and later produce antibodies harmful to the fetus.

Pregnancy and childbirth are often associated with conditions such as postpartum hemorrhage, anemia, sickle cell disease crises, and surgical interventions, which increase the demand for blood transfusion in women of reproductive age (WHO, 2019). In Nigeria, where maternal morbidity is already high, transfusion support is frequently required.

Unfortunately, most blood transfusion services match only for ABO and RhD antigens, ignoring MNS compatibility. This oversight increases the risk of alloimmunization in pregnant women, who may later develop antibodies such as anti-S or anti-s. In subsequent pregnancies, these antibodies may cross the placenta, resulting in HDFN and contributing to Nigeria’s already high neonatal mortality rate (UNICEF, 2020).

Hemolytic disease of the fetus and newborn (HDFN) is often attributed to RhD incompatibility. However, studies show that non-Rh antibodies, including MNS alloantibodies, contribute significantly to HDFN in both developed and developing countries (Moise, 2008; Bowman, 1997). In Nigeria, cases of severe neonatal jaundice, kernicterus, and unexplained stillbirths are often misattributed to infections or poorly managed neonatal sepsis, when in fact undetected maternal alloantibodies could be responsible.

Because MNS antibody screening is not routine, many cases go unrecognized, leading to repeated adverse pregnancy outcomes in affected women. This represents a significant diagnostic and clinical problem. While global studies have the frequency of MNS antigens and antibodies, there is limited data on the prevalence of MNS alloantibodies among Nigerian antenatal patients. Most available studies in Nigeria focus on ABO and RhD incompatibility (Akinbami et al., 2010). This lack of local data creates a knowledge gap, making it difficult for clinicians and policymakers to appreciate the true burden of MNS alloimmunization.Without baseline prevalence data, it is impossible to develop evidence-based guidelines for antibody screening in antenatal care. This gap further perpetuates preventable maternal and neonatal complications.

Public Health Burden

Nigeria accounts for a significant proportion of the global burden of maternal and neonatal mortality. According to the World Health Organization (WHO, 2019), Nigeria contributes nearly 20% of all global maternal deaths, while neonatal deaths remain unacceptably high. Although multiple factors contribute to this burden, preventable causes such as alloimmunization-related HDFN should not be overlooked. The lack of routine MNS screening contributes to preventable neonatal morbidity and mortality. In addition, the economic burden of treating severe HDFN through interventions such as exchange transfusions, phototherapy, and prolonged hospital stays places significant strain on families and the healthcare system.

A critical part of the problem lies in the policy and infrastructure gap. In Nigeria, blood transfusion services are underfunded, and extended red cell typing is rarely available. Antenatal clinics lack the resources and trained personnel to perform routine antibody screening beyond RhD. Furthermore, national guidelines do not mandate screening for MNS or other clinically significant antibodies. This policy gap results in a healthcare system that is unprepared to prevent or manage cases of MNS-related alloimmunization and HDFN, leaving mothers and newborns vulnerable.

Ethical and Social Implications

There are also ethical implications associated with the neglect of MNS alloimmunization. Pregnant women have the right to comprehensive antenatal care that safeguards both their health and that of their unborn child. Failure to detect and prevent MNS alloimmunization represents a breach of standard medical ethics, particularly in comparison to countries where such screening is routine. Additionally, the recurrence of adverse pregnancy outcomes due to undiagnosed alloimmunization has profound social consequences for women and families, contributing to psychological distress, stigma, and loss of trust in the healthcare system.

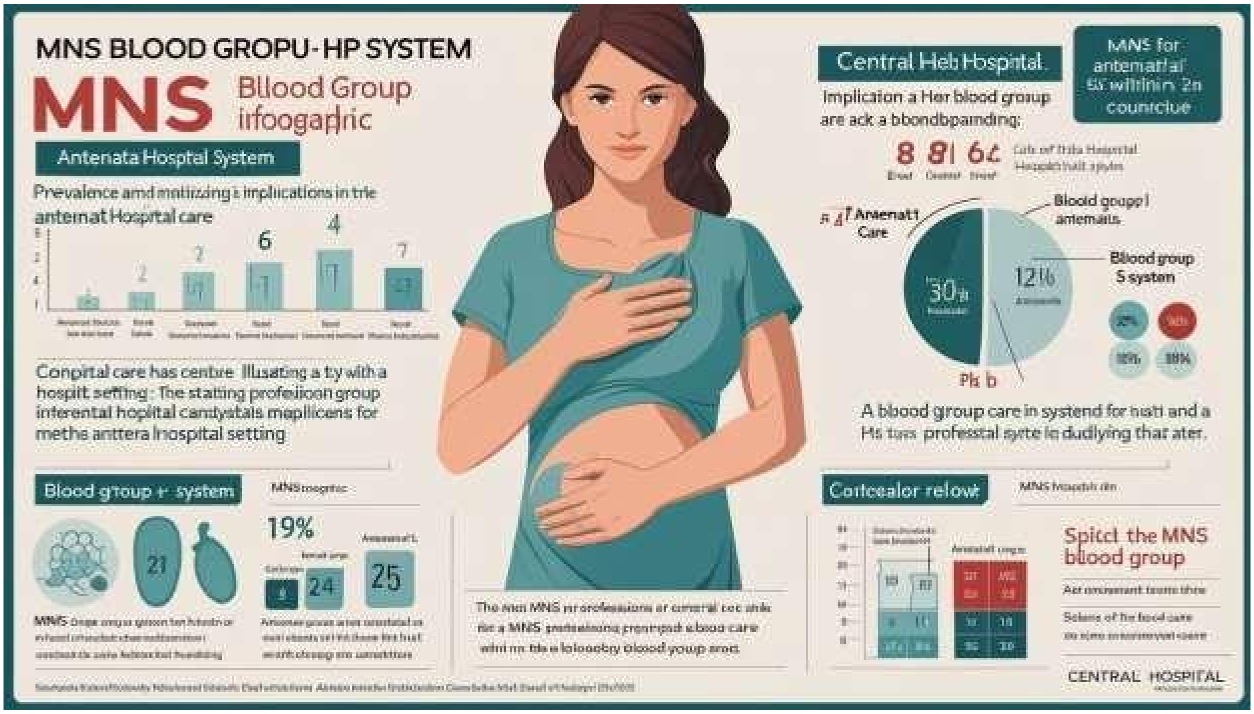
The Core of the Problem

The statement of the problem can therefore be summarized as follows:

1. MNS alloantibodies are clinically significant and can cause HDFN and transfusion reactions.
2. Routine antenatal screening in Nigantibodies among antenatal women in Nigeria.
3. As a result, many cases of neonatal morbidity and mortality may be linked to undetected MNS alloimmunization.

4. There is a scarcity of local data on the prevalence of MNS antigens and antibodies among antenatal women in Nigeria.
5. This knowledge gap hinders evidence-based policy-making and perpetuates preventable adverse outcomes in mothers and newborns.

Addressing this problem requires research to generate local epidemiological data, policy reform to include extended antibody screening in antenatal care, and investment in laboratory infrastructure to improve maternal and neonatal outcomes.



Clinical Importance of MNS Alloantibodies

The MNS blood group system is the second most complex system after ABO and Rh, with over 40 recognized antigens. Of particular clinical relevance are the M, N, S, and s antigens, which are located on glycophorin A and B. The immunological responses directed against these antigens may lead to alloantibody formation, with clinical consequences for both transfusion medicine and obstetric practice (Daniels, 2013).While anti-M and anti-N are frequently naturally occurring and usually IgM in nature, they can occasionally be of the IgG class and capable of crossing the placenta, causing mild to moderate HDFN. More importantly, anti-S and anti-s antibodies are typically IgG and are known to cause both hemolytic transfusion reactions and moderate to severe HDFN (Reid & Lomas-Francis, 2004).

Given this background, the clinical justification for the study is straightforward: pregnant women carrying these antibodies are at risk of adverse pregnancy outcomes, yet most antenatal care systems in Nigeria do not screen for them. By documenting their prevalence, this research addresses an immediate and clinically relevant problem.

Knowledge Gap in Nigeria and Sub-Saharan Africa

Much of the available data on MNS antigen distribution and alloimmunization prevalence comes from Europe, North America, and Asia (Colin & Anstee, 2020; Moise, 2008). In contrast, very few studies have focused on sub-Saharan Africa, particularly Nigeria, despite its high birth rate and burden of neonatal mortality. Existing Nigerian studies on blood group incompatibility tend to emphasize ABO and RhD systems (Akinbami et al., 2010), with little or no attention given to other clinically significant blood group systems such as MNS, Kell, Kidd, or Duffy. This leaves a critical gap in epidemiological knowledge, which hampers the development of context-specific guidelines for antibody screening in antenatal patients.

This study is justified because it will generate baseline data on the prevalence of MNS antigens and alloantibodies among pregnant women in Nigeria. Such data is indispensable for:

- informing clinical decision-making,
- guiding blood transfusion practices, and

- shaping national antenatal screening policies.

Limitations of Current Antenatal Screening Practices

In developed countries, routine antenatal care includes indirect antiglobulin testing (IAT) to detect unexpected antibodies, followed by detailed antibody identification panels when results are positive. This ensures that clinically significant antibodies, including those against MNS antigens, are identified early (Bowman, 1997). However, in Nigeria, antenatal screening is largely limited to ABO and RhD typing, with antibody screening either unavailable or inconsistently performed (Akinbami et al., 2010). Consequently, cases of alloimmunization due to MNS antibodies may remain undetected until the newborn presents with jaundice, anemia, or hydrops fetalis. The justification here is that by highlighting the prevalence of MNS alloantibodies, this study can provide the evidence needed to advocate for more comprehensive antibody screening in antenatal clinics, thereby improving maternal-fetal outcomes.

Public Health Relevance

Nigeria contributes disproportionately to the global burden of maternal and neonatal mortality. According to the World Health Organization (WHO, 2019), Nigeria accounts for about 20% of global maternal deaths and has one of the highest neonatal mortality rates worldwide. While many of these deaths are linked to hemorrhage, sepsis, and birth asphyxia, immune-mediated conditions such as HDFN also play a role and are often underreported or misdiagnosed.

By failing to screen for MNS alloantibodies, the healthcare system perpetuates preventable causes of neonatal jaundice, anemia, kernicterus, and even stillbirth. The public health justification of this study is that it can provide the missing data to design interventions that reduce avoidable maternal and neonatal deaths, thereby contributing to Nigeria's progress toward achieving the Sustainable Development Goals (SDGs), particularly SDG 3.1 (reduce maternal mortality) and SDG 3.2 (end preventable deaths of newborns and children under 5).

Contribution to Blood Transfusion Safety

Pregnant women are at higher risk of requiring blood transfusions due to conditions such as postpartum hemorrhage, anemia, sickle cell crises, and obstetric surgeries. Unfortunately, most blood banks in Nigeria match only ABO and RhD groups, neglecting other clinically significant antigens (WHO, 2019). This increases the risk of alloimmunization in women who receive mismatched blood. If MNS alloantibodies are generated, they can complicate future transfusions and pregnancies. Documenting the prevalence of MNS antibodies in antenatal women will help blood transfusion services to adopt extended typing practices, thereby improving transfusion safety.

Thus, the study is justified not only for antenatal care but also for strengthening transfusion medicine in Nigeria.

Ethical Justification

From an ethical perspective, pregnant women deserve access to comprehensive antenatal care that safeguards both maternal and fetal wellbeing. The failure to detect MNS alloantibodies despite their known clinical significance represents a gap in ethical responsibility and standard of care. By conducting this study, healthcare providers can be better informed and equipped to prevent alloimmunization-related complications. This aligns with the ethical principles of beneficence (acting in the patient’s best interest), non-maleficence (avoiding harm), and justice (equitable access to care) (Beauchamp & Childress, 2013).

Therefore, the justification also rests on the moral obligation to prevent avoidable harm to mothers and infants in Nigeria.

Policy Relevance

Policy makers in Nigeria require local data to justify reforms in healthcare practice. International guidelines cannot be adopted wholesale without evidence of local relevance. For example, if this study shows a high prevalence of MNS antibodies in antenatal women, it will provide the evidence base to push for policy reforms such as: mandatory antibody screening beyond RhD, investment in laboratory infrastructure for extended phenotyping, and training of healthcare workers in managing alloimmunization.

Thus, the study is justified as a policy-enabling research effort that can drive systemic improvements in maternal and child health services.

Academic and Scientific Contribution

Beyond clinical and policy implications, the study will also make significant contributions to academic and scientific knowledge. The MNS blood group system remains understudied in African populations, and the genetic diversity of Africans makes this an especially important area of research (Tishkoff et al., 2009).

This study will add to global literature by providing epidemiological data on MNS antigen distribution in Nigerian antenatal women, documenting the frequency of alloantibodies, and identifying potential risk factors for alloimmunization. Such findings will not only benefit Nigeria but also enrich global understanding of blood group immunohematology.

The justification for this study rests on multiple pillars:

1. Clinical significance of MNS alloantibodies in causing HDFN and transfusion reactions.
2. Knowledge gap in Nigeria regarding their prevalence among antenatal patients.
3. Limitations of current antenatal screening programs that exclude MNS antibodies
4. Public health urgency given Nigeria’s high maternal and neonatal mortality rates.

5. Need for safer blood transfusion practices for pregnant women.
6. Ethical responsibility to provide comprehensive antenatal care.
7. Policy relevance for designing evidence-based guidelines.
8. Scientific contribution to global immunohematology research.

By addressing these interconnected justifications, the study promises to generate data with far-reaching implications for maternal health, neonatal survival, transfusion safety, and healthcare policy in Nigeria and beyond.

2.10 PATHOPHYSIOLOGY OF HDFN IN MNS ALLOIMMUNIZATION

Hemolytic disease of the fetus and newborn (HDFN) associated with the MNS blood group system occurs when maternal IgG antibodies directed against antigens such as M, S, s, U, or rare hybrid variants cross the placenta and target fetal erythrocytes bearing the corresponding antigen. After transplacental passage, these antibodies bind to fetal red-cell membrane glycoproteins (glycophorin A or B), forming immune complexes that are recognized by Fc receptors on fetal macrophages in the spleen and liver. The result is extravascular hemolysis, progressive fetal anemia, and indirect hyperbilirubinemia. A distinctive feature of MNS-mediated disease is seen with anti-M antibodies. Research by Evers, Middelburg, de Haas, and colleagues (2019) demonstrated that some IgG anti-M antibodies not only promote red-cell destruction but also suppress fetal erythroid progenitor cells in the bone marrow. This mechanism can cause profound anemia with relatively little evidence of hemolysis, complicating diagnosis and management.

As fetal anemia worsens, compensatory mechanisms such as increased cardiac output and extramedullary hematopoiesis can be overwhelmed. If unrecognized, the fetus may develop hydrops fetalis,characterized by generalized edema, ascites, and pleural or pericardial effusions.

Severe cases may progress to congestive heart failure or stillbirth, as emphasized by Dean (2022) in his review of red cell alloimmunization.

The pathophysiology therefore involves a spectrum:

- Classic extravascular hemolysis mediated by Fc receptor–bearing macrophages.
- Suppressed erythropoiesis, particularly with anti-M, leading to hyporegenerative anemia.
- Secondary complications such as hydrops fetalis and fetal demise if anemia is untreated.

Early detection of maternal antibodies, careful titer monitoring, and timely fetal surveillance are essential to interrupt this cascade and prevent life-threatening outcomes.

Placental Transfer Dynamics;

Maternal IgG is actively transported across the placenta by the neonatal Fc receptor (FcRn) expressed on syncytiotrophoblasts. Transport efficiency rises sharply after 28 weeks' gestation, explaining why fetal anemia from MNS alloimmunization usually becomes apparent in the third trimester.

Mechanisms of Red Cell Destruction;

After entering the fetal circulation, IgG antibodies bind glycophorin A or B antigens on the fetal red cell membrane. Fc receptor–bearing macrophages in the spleen and liver recognize these immune complexes, resulting in extravascular hemolysis, progressive fetal anemia, and indirect hyperbilirubinemia.

Unique Role of Anti-M Antibodies;

Research by Evers, Middelburg, de Haas, and colleagues (2019) demonstrated that IgG anti-M can also suppress fetal erythroid progenitor cells, producing severe anemia with relatively little hemolysis a presentation that can delay recognition. Bone marrow suppression adds to the severity of anemia and may delay recovery even with transfusions.

Maternal Immune Response;

Initial fetomaternal hemorrhage elicits a primary immune response, but subsequent pregnancies with an antigen-positive fetus trigger a rapid anamnestic response, rapidly increasing high-affinity IgG1 or IgG3 antibodies, which are the subclasses most strongly associated with severe disease.

Complement Activation;

Although most MNS antibodies are non-complement-fixing, rare IgG1 or IgG3 antibodies may activate complement, contributing to occasional intravascular hemolysis.

Fetal Compensatory Changes;

Severe anemia drives high-output cardiac failure, hepatosplenomegaly, and extramedullary hematopoiesis, often accompanied by polyhydramnios. If uncorrected, these changes lead to hydrops fetalis, characterized by generalized edema and effusions, and may cause congestive heart failure or stillbirth. Maternal ―mirror syndrome,‖ with edema paralleling the fetal hydrops, can also develop.

Postnatal and Pathologic Findings;

Newborns may present with persistent anemia, hyperbilirubinemia, and require exchange transfusion. Placental or autopsy findings can include erythrophagocytosis and hemosiderin deposition in the fetal spleen and liver.

Clinical Monitoring;

Disease severity correlates with both maternal antibody titers and IgG subclass. Rising titers or a middle cerebral artery (MCA) Doppler peak systolic velocity greater than 1.5 multiples of the median is a reliable, noninvasive marker of fetal anemia (Dean, 2022).

2.11 TRANSFUSION SUPPORT FOR THE MOTHER

Pregnant women who harbor clinically significant MNS antibodies require meticulous planning for any transfusion whether during pregnancy, at delivery, or in the event of postpartum hemorrhage.

Antigen-Negative Blood Selection

Extended Phenotyping/Genotyping: Before transfusion, maternal red-cell antigen status and antibody specificity should be fully characterized. Crossmatch Compatibility: Only antigen-negative, crossmatch-compatible red cell units for example, S-negative or U-negative as appropriate must be selected to prevent anamnestic antibody responses or acute hemolysis. Advance Reservation: Blood should be crossmatched and reserved in advance of scheduled procedures such as cesarean delivery or external cephalic version, where bleeding risk is higher.

Rare Antibodies and Donor Sourcing

For antibodies such as anti-U or antibodies to uncommon hybrid antigens, compatible donors are extremely rare. Locating suitable units may require regional or international rare-donor registries such as the American Rare Donor Program (ARDP) or the International Blood Group Reference Laboratory (IBGRL) networks (Westhoff, 2021).

When feasible, autologous donation or directed family donation may be considered early in pregnancy if transfusion is anticipated.

Coordination and Planning

Early Communication: Immediate notification of the hospital transfusion service or blood bank once a significant MNS antibody is identified is essential. Multidisciplinary Approach: Collaboration among the obstetric team, transfusion medicine specialists, and maternal–fetal medicine consultants ensures prompt availability of compatible blood in emergencies. Contingency Planning: A written transfusion plan should be included in the patient's chart and birth plan, detailing antibody specificity, required antigen negativity, and contact information for rare-donor resources. Supportive Measures to Minimize Transfusion Needs

Iron and folate supplementation to optimize maternal hematologic status.

Active management of the third stage of labor and rapid treatment of postpartum hemorrhage to reduce transfusion volume.

Proactive coordination, early donor identification, and vigilant crossmatching are critical to prevent delays and ensure safe transfusion support for mothers with MNS alloimmunization.

The MNS blood group system, with its intricate genetic architecture and diverse antigenic expression, exemplifies how subtle molecular variations can have profound clinical consequences. Beyond its historical and serologic significance, the system underscores the delicate interplay between maternal immunity and fetal vulnerability, revealing that even minor antigenic differences like a single amino acid can determine neonatal outcomes. The literature reveals a paradox: while MNS antibodies are less frequent and often overshadowed by RhD and Kell alloimmunization, their potential for silent yet severe hemolytic disease demands vigilance. Moreover, the prevalence of rare phenotypes and hybrid glycophorins in specific populations highlights an essential truth: blood group science cannot be universalized; it must be contextualized to the population it serves.

This chapter also illuminates gaps in knowledge—particularly in low-resource settings where antenatal screening is inconsistent and molecular genotyping remains aspirational. Addressing these gaps is not merely academic; it is a matter of preventing preventable morbidity and mortality in mothers and newborns.

By integrating molecular insights, population epidemiology, and clinical management strategies, this review positions the MNS system not as a historical curiosity but as a living challenge in modern obstetric care. The pathway forward is clear: precision in blood group typing, proactive monitoring, and population-informed transfusion planning can transform outcomes. Ultimately, the MNS system teaches a broader lesson: in medicine, the smallest molecular details can have the largest impact and understanding them is both a scientific pursuit and a moral imperative.

CHAPTER THREE

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 research adopt a cross-sectional/descriptive study design aimed at determining the distribution of M and N antigens among pregnant human attending antenatal care at Central Hospital, Benin City. By collecting data at a single point in time, this design allows for the assessment of the prevalence and pattern of MNS blood group antigens within the study population. It is particularly suitable for identifying associations between blood group distribution and demographic or clinical variables providing understanding.

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

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

$n= 72.96 \sim 73$

A total of 110 (one hundred and ten) samples was 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 were labeled with unique identifiers to maintain participant confidentiality. Samples were transported promptly to the hospital's laboratory for serological testing. The MNS blood group (M and N antigens) was adheringto established protocol for accurate serological identification 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-M antisera

Anti-N antisera

Normal saline

Test tubes (labeled for anti-M and anti-N)

Centrifuge (compatible with test tubes)

Pipettes (micropipettes or Pasteur pipettes)

water bath at 37°C

Tuberack

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.

Laboratory Procedure: Tube Method

1. Two test tube were labelled one for Anti M and one for Anti N
2. Two drop of the red blood cell suspension was pipetted into the two labelled test tubes
3. A drop of antisera M was added to the tube labelled anti M (Tube 1)
4. A drop of antisera N was added to the tube labelled anti N (Tube 2)
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

3.12 Statistical 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 M and N antigens from the MNS blood group system was presented using frequency tables. The Chi-square test of independence was employed to assess associations between sociodemographic and clinical characteristics and the distribution of M and N antigens. A p-value less than 0.05 was considered statistically significant.

CHAPTER FOUR

RESULTS

A total of 110 respondents were studied. The sociodemographic characteristics revealed that the mean age of respondents was 30.45 ± 5.02 years, with the majority (67.3%) within the 27–35 years age bracket, followed by 18.2% aged 18–26 years, and 14.5% aged 36-45 years. Most of the respondents were married (94.5%) while a minority were single (5.5%), with no divorced or widowed respondents recorded. Regarding religion, the majority were christians (94.5%) and a smaller proportion practiced islam (5.5%), with no respondents reporting adherence to Traditional or other religions. The ethnic distribution showed that 36.4% were Bini, 14.5% Esan, 1.8% Yoruba, 3.6% Hausa, while 43.6% belonged to other ethnic groups including Delta, Auchi, Igbo, and Kwale. In terms of educational status, most respondents had secondary education (64.5%), followed by tertiary education (33.6%) and a minority had primary education (1.8%), with none being uneducated. Regarding occupation, the majority were self-employed (67.2%), 25.5% were employed, and 7.3% were unemployed (Table 4.1).

Table 4.1. Sociodemographic Characteristics of Participants

Variable	Number (n=110)	Percentage (%)
Age (Years)	Mean =30.45±5.02	
18-26	20	18.2
27-35	74	67.3
36-44	16	14.5
Marital Status		
Single	6	5.5
Married	104	94.5
Divorced	0	0
Widowed	0	0
Religion		
Christianity	104	5.5
Islam	6	94.5
Traditional	0	0
Others	0	0
Ethnicity		
Bini	40	36.4
Esan	16	14.5
Yoruba	2	1.8
Hausa	4	3.6
Others*	48	43.6
Education		
None	0	0
Primary	2	1.8
Secondary	71	64.5
Tertiary	37	33.6
Occupation		
Unemployed	8	7.3
Employed	28	25.5
Self Employed	74	67.2

*Others: Delta, Auchi, Igbo, Kwale

The blood transfusion and obstetric history revealed that a minority of respondents (10.9%) had ever received a blood transfusion, while the majority (89.1%) had never received one. Regarding gravidity, 29.1% of respondents were nulligravida, 43.6% were gravida 1–2, and 27.3% were gravida 3–4. The parity distribution showed that 29.1% were nulliparous, 65.5% had 1–2 previous deliveries, and 5.5% had 3–4 previous deliveries. A smaller proportion of respondents (18.2%) reported experiencing pregnancy complications, whereas the majority (81.8%) had no history of complications. Concerning gestational age, most respondents were in their second trimester (54.5%), followed by those in the first trimester (38.2%), and a minority in the third trimester (7.3%) (Table 4.2).

Table 4.2. Blood Transfusion and Obstetric History of Participants

Variable	Number (n=110)	Percentage (%)
Ever Had Blood Transfusion		
Yes	12	10.9
No	98	89.1
Gravida		
None	32	29.1
1-2	48	43.6
3-4	30	27.3
Parity		
None	32	29.1
1-2	72	65.5
3-4	6	5.5
Ever Had Pregnancy Complications		
Yes	20	18.2
No	90	81.8
Gestational Age		
1st Trimester	42	38.2
2nd Trimester	60	54.5
3rd Trimester	8	7.3

Phenotypic Distribution of ABO, Rhesus (Rh) D Blood Groups

A total of 110 respondents were studied. The phenotypic distribution of ABO and Rhesus (Rh) D blood groups revealed that the majority of respondents had blood group O (60.9%), followed by blood group B (20.9%), A (16.4%), and a minority with AB (1.8%). Regarding Rhesus D status, most respondents were Rh positive (91.8%), while a smaller proportion were Rh negative (8.2%) (Table 4.3).

Table 4.3. Phenotypic Distribution of ABO and Rhesus (Rh) D Blood Groups

Blood Group	Number (n=110)	Percentage (%)
ABO		
A	18	16.4
B	23	20.9
AB	2	1.8
O	67	60.9
Rhesus		
Positive	101	91.8
Negative	9	8.2

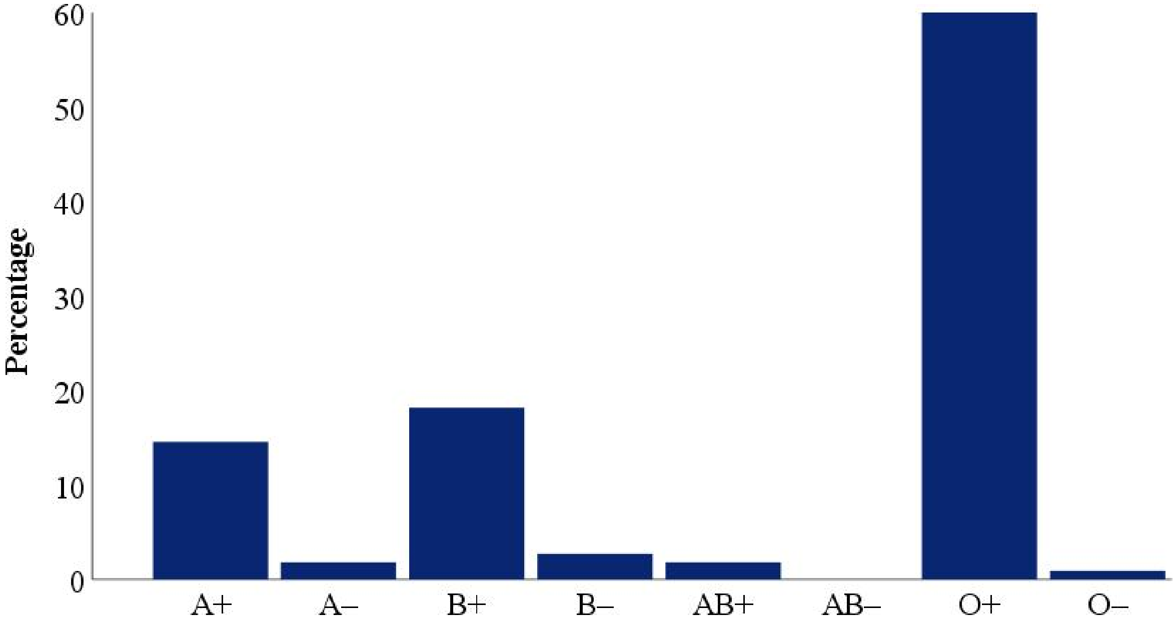


Figure 4.1. Distribution of ABO and Rhesus Blood Group among Participants.

The phenotypic distribution of M and N blood group antigens among the 110 participants showed that the M–N+ phenotype was the most prevalent, observed in 40 (36.4%) participants. This was followed by the M+N+ phenotype, present in 33 (30.0%), and the M+N– phenotype, seen in 29 (26.4%) participants. The rare M–N– phenotype was observed in 8 (7.3%) participants. Overall, the majority of participants expressed at least one of the M or N antigens, with the absence of both antigens (M–N–) being uncommon.

Table 4.4. Phenotypic Distribution of M and N Blood Group Antigens among Participants

MN Phenotypes	Number (n=110)	Percentage (%)
M+N+	33	30.0
M+N–	29	26.4
M–N+	40	36.4
M–N–	8	7.3
Total	**110**	**100.0**

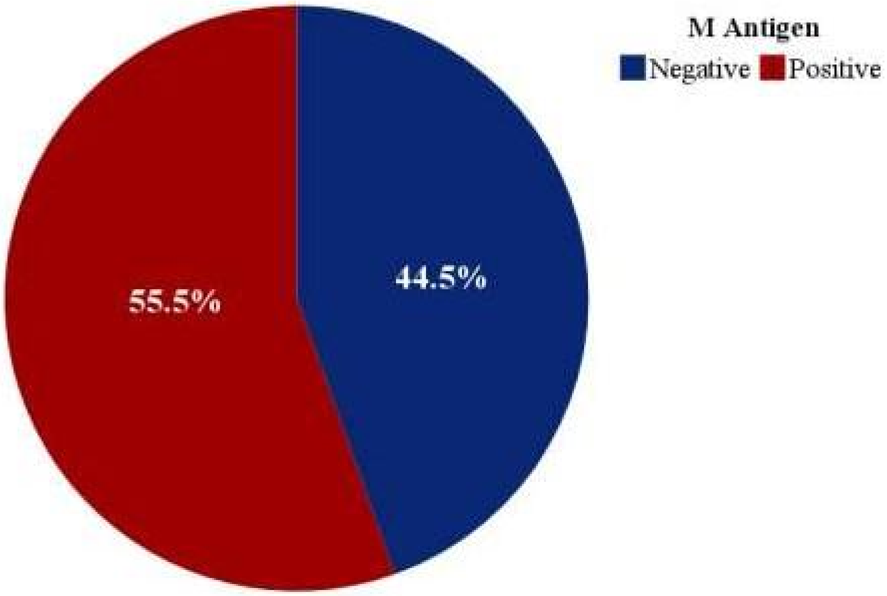


Figure 4.2. Prevalence of M antigen among participants. Out of the 110 participants 62 (55.5%) tested positive for the M antigen while 49 (44.5%) tested negative.

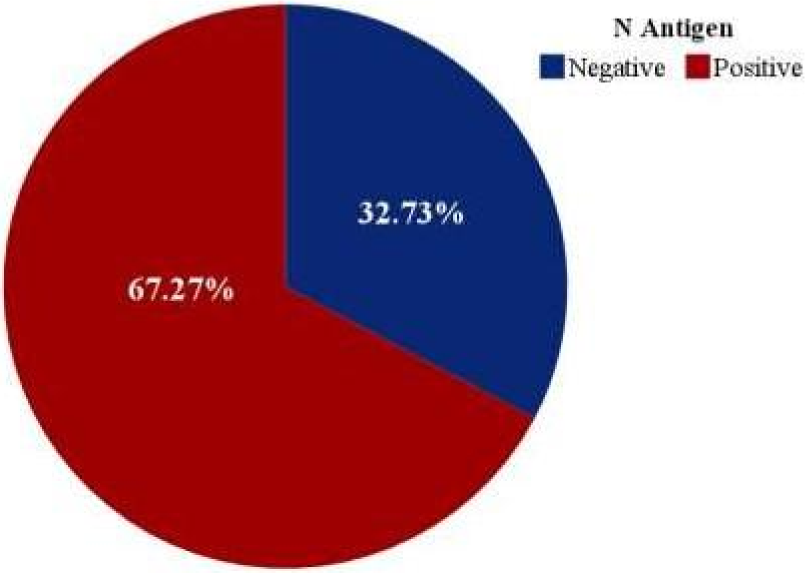


Figure 4.3. Prevalence of N antigen among participants. Out of the 110 participants 73 (67.27%) tested positive for the N antigen while 36 (32.73%) tested negative.

Relationship Between M Antigen with ABO and Rhesus D Blood Grouping

The distribution of the M antigen across ABO blood groups showed that among respondents with blood group A, 9 (14.3%) were M positive and 9 (18.4%) were M negative. Among respondents with blood group B, 11 (18.0%) were M positive, while 12 (24.5%) were M negative. For blood group AB, 1 (1.6%) was M positive and 1 (2.0%) was M negative. Among respondents with blood group O, 40 (65.6%) were M positive, and 27 (55.1%) were M negative. The association between ABO blood group and M antigen was not statistically significant ($\chi^2 = 1.272$, $p = 0.736$). Regarding Rhesus (Rh) D status, 5 (8.2%) of RhD positive respondents were M positive and 45 (91.8%) were M negative. Among RhD negative respondents, 4 (8.2%) were M negative and 5 (8.2%) were M positive. There was no statistically significant association between RhD status and M antigen ($\chi^2 = 0.001$, $p = 0.995$) (Table 4.5).

Table 4.5. Relationship Between M Antigen with ABO and Rhesus D Blood Grouping

Groups	M Negative (%)	M Positive (%)	χ^2	p value
ABO				
A	9 (18.4)	9 (14.3)	1.272	0.736
B	12 (24.5)	11 (18.0)		
AB	1 (2.0)	1 (1.6)		
O	27 (55.1)	40 (65.6)		
Rhesus				
RhD–	4 (8.2)	5 (8.2)	0.001	0.995
RhD+	45 (91.8)	56 (91.8)		

Relationship Between N Antigen with ABO and Rhesus D Blood Grouping

The distribution of the N antigen across ABO blood groups showed that among respondents with blood group A, 14 (18.9%) were N positive and 4 (11.1%) were N negative. Among respondents with blood group B, 15 (20.3%) were N positive and 8 (22.2%) were N negative. For blood group AB, 2 (2.7%) were N positive, while none (0 [0%]) were N negative. Among respondents with blood group O, 43 (58.1%) were N positive, and 24 (66.7%) were N negative. The association between ABO blood group and N antigen was not statistically significant ($\chi^2 = 2.211$, $p = 0.530$). Regarding Rhesus (Rh) D status, among RhD negative respondents, 6 (8.1%) were N positive, and 3 (8.3%) were N negative. Among RhD positive respondents, 68 (91.9%) were N positive, and 33 (91.7%) were N negative. There was no statistically significant association between RhD status and N antigen ($\chi^2 = 0.002$, $p = 0.968$) (Table 4.6).

Table 4.6. Relationship Between N Antigen with ABO and Rhesus D Blood Grouping

Groups	N Negative (%)	N Positive (%)	χ^2	p value
ABO				
A	4 (11.1)	14 (18.9)	2.211	0.530
B	8 (22.2)	15 (20.3)		
AB	0 (0)	2 (2.7)		
O	24 (66.7)	43 (58.1)		
Rhesus				
RhD–	3 (8.3)	6 (8.1)	0.002	0.968
RhD+	33 (91.7)	68 (91.9)		

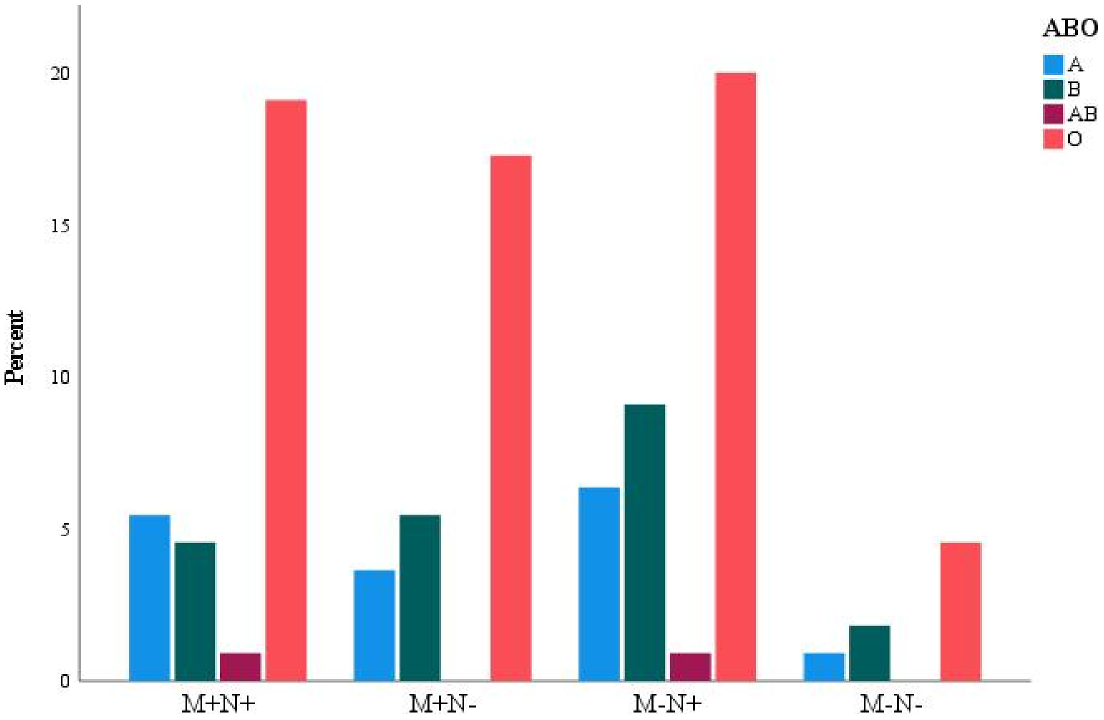


Figure 4.4. Chart showing MN phenotype in relationship to ABO blood group.

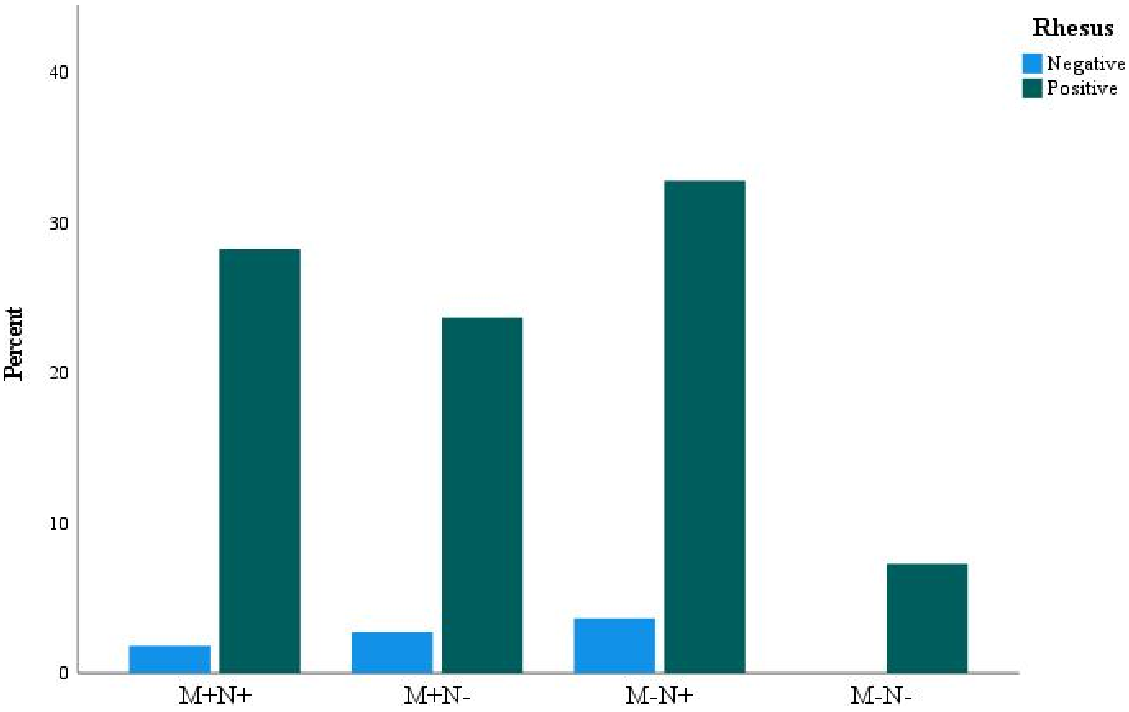


Figure 4.5. Chart showing MN phenotype in relationship to Rhesus.

CHAPTER FIVE

5.1. Discussion

The MN blood group system is clinically significant due to the ability of antibodies within the system to cause hemolytic disease of the fetus and newborn (HDFN) as well as transfusion reactions (Delaney and Matthews, 2015). In this present study, we investigated the prevalence of MN antigens among antenatal subjects attending Central Hospital, Benin City, Edo State, Nigeria. Understanding the distribution of MN blood group antigens in this population is important for safe blood transfusion practices and for anticipating potential immunological complications during pregnancy.

The majority of participants were within the age range of 27–35 years (67.3%) indicating that most antenatal women were within the active reproductive age of 20–40 years. This finding is comparable to the study by Buhari *et al*. (2022) in northern Nigeria and Anyiam *et al*. (2022) in Ilorin, where the majority were aged 21–40 years. The distribution of study subjects based on ethnicity reveals that Bini and Esan were predominant, followed by other ethnic groups. These findings differ from Buhari *et al*. (2022), where Hausa/Fulani (90%) were predominant, followed by the Yoruba (6%) and Igbo (4%) ethnic groups, indicating the a regional variation in participants demographics and possibly reflecting differences in population distribution or hospital attendance patterns across study location. The high prevalence of Bini and Esan in this study reflects the cultural and geographical context of Benin City, Edo State, similar to how Hausa/Fulani dominance reflected northern Nigeria in the study of Buhari *et al*. (2022).

The distribution of participants based on gestational age shows that most women booked during the second trimester (54.5%). This pattern may be due to a combination of factors, including previous pregnancy experience leading to delayed booking among multigravida women, limited awareness of the importance of early antenatal care, and potential cultural or logistical barriers such as work and transportation (Tesale and Tesema, 2020; Warri and George, 2020; Gaikwad *et al*., 2024). Gravidity and parity distribution further indicate that the majority had 1–2 pregnancies and deliveries, consistent with the reproductive trends in the age distribution of the participants and also in other similar populations (Anyiam *et al*., 2022). Only a small proportion reported previous pregnancy complications or a history of blood transfusion, suggesting generally uncomplicated obstetric histories and limited exposure to conditions necessitating transfusion. However, this finding was not in agreement with the study of Law *et al*. (2015) who reported in his study that 46.9% of women had at least one pre-specified pregnancy complication.

The phenotypic distribution of ABO blood groups among the antenatal participants showed that blood group O was the most prevalent, followed by B, A, and AB being the least common. This finding aligns with the study of Buhari *et al*. (2023) who reported similar patterns. This finding is due to genetic inheritance patterns and the high frequency of the O allele in these populations. Blood group AB is typically rare globally, which explains its low occurrence in this study (Ristovska *et al*., 2022). Regarding the Rhesus (Rh) D factor, the majority of participants were Rh-positive, with only a few being Rh-negative. This aligns with the study of Duangchan *et al*. (2024) in Thailand and Medugu *et al*. (2016) who reported the prevalence of Rh positive to be 99.01% and 97.1% respectively. The low prevalence of Rh-negative blood may be attributed to the predominantly African ancestry of the study population, as the Rh-negative phenotype is more common among Caucasian populations (Sandler *et al*., 2017).

The prevalence of the M antigen among participants in this study was 55.5%, while 44.5% tested negative. For the N antigen, 67.27% of participants were positive, with 32.73% negative. These results indicate that both M and N antigens are relatively common among antenatal patients in Central Hospital, Benin City, with N antigen showing a slightly higher prevalence than M antigen.

When compared with the study by Halawani *et al*. (2021), the prevalence of M antigen in this study (55.5%) is lower than the 89.26% reported in their population, whereas the N antigen prevalence (67.27%) is higher than their 51.67%. These differences may be attributed to variations in ethnicity, genetic background, and sample size between the populations studied.

The phenotypic distribution of MN antigens among participants showed that M–N+ was the most common phenotype (36.4%), followed by M+N+ (30.0%), M+N– (26.4%), and M–N– being the least common (7.3%). This pattern reflects a higher prevalence of N antigen as observed previously, resulting in more individuals expressing the M–N+ phenotype. The variation in phenotype frequencies is primarily due to the codominant inheritance of M and N alleles on chromosome 4, where each individual expresses antigens according to the combination of alleles inherited from their parents (Chatzikyriakidou, 2024). Additionally, population genetics, including historical migration patterns and natural selection, may influence the distribution of these antigens, as certain phenotypes can become more common in response to regional genetic diversity and environmental pressures (Zamudio *et al*., 2016). The finding from this study is in disagreement with the studies of Halawani *et al*. (2021) in India and Duangchan *et al*. (2024) who both reported the highest phenotypic prevalence to be M+N+.

Clinically, the distribution of MN phenotypes has significant implications for maternal and child health. Women who lack either the M or N antigen (M–N+ or M+N–) are at risk of alloimmunization if exposed to the corresponding antigen through blood transfusion or during pregnancy from foetal red blood cells carrying the antigen they lack (Rai *et al*., 2016). This immune response can lead to the formation of anti-M or anti-N antibodies, which may cross the placenta and cause haemolytic disease of the foetus and newborn (HDFN) in subsequent pregnancies (Karim *et al*., 2015). Although anti-M and anti-N antibodies are generally less aggressive than anti-D antibodies, they can still contribute to neonatal anaemia, hyperbilirubinemia, and, in rare cases, severe haemolysis if maternal antibody titres are high (Yasuda *et al*., 2014; Nahendran *et al*., 2023). For maternal health, alloimmunization can complicate future transfusions, as women with antibodies against M or N antigens may require antigen-matched blood to prevent transfusion reactions (Fasano *et al*., 2019).

In this study, the lack of significant association between MN antigens and ABO or RhD blood groups indicates that the expression of M and N antigens occurs independently of other major blood group systems. This independence arises because the genes encoding the MN antigens are located on chromosome 4 (Kim, 2024), whereas ABO and RhD antigens are controlled by genes on different chromosomes (chromosome 9 for ABO and chromosome 1 for RhD) (Abbas and Ali, 2022). As a result, the inheritance of MN antigens follows a separate Mendelian pattern, unaffected by the presence or absence of ABO or RhD antigens (Kim, 2024). This genetic independence explains why individuals with the same ABO or RhD blood group may exhibit different MN phenotypes, and conversely, individuals with the same MN phenotype may belong to different ABO or RhD groups. Overall, these findings highlight the independent distribution of MN antigens in this population and show the importance of MN typing in antenatal care and transfusion practices to prevent alloimmunization and ensure maternal and foetal safety.

5.2. Recommendations

1. Routine MN antigen typing should be incorporated into antenatal care to identify women at risk of alloimmunization.
2. Blood banks should consider MN phenotypes when matching blood for transfusions to minimize the risk of hemolytic reactions.
3. Health education programs should emphasize the importance of early antenatal booking to monitor and manage potential immunological complications.
4. Further research should be conducted on MN antigen distribution in different regions to improve transfusion safety and maternal-fetal outcomes.
5. Clinicians should maintain detailed records of maternal antibodies, including anti-M and anti-N, to guide safe transfusion and pregnancy management.

REFERENCES

Abbas, A. A. A. and Ali, A. S. F. A. (2022). Occurrence of ABO and RhD Blood Groups in Sudanese Population Attending Blood Bank, Gezira State, Wad Madani, Sudan, 2021. *World Journal of Advanced Research and Reviews*. 16(2): 236-251.

Anyiam, A. F., Obeagu, E. I., Obi, E., Omosigho, P. O., Irondi, E. A., Arinze-Anyiam, O. C. and Asiyah, M. K. (2022). ABO blood groups and gestational diabetes among pregnant women attending University of Ilorin Teaching Hospital, Kwara State, Nigeria. *International Journal of Research and Reports in Hematology*. 5(2): 113-121.

Buhari, H. A., Sagir, A., Akuyam, S. A., Erhabor, O. and Panti, A. A. (2022). Blood Group Antigens Phenotype Frequencies among Pregnant Women in Sokoto State, Nigeria. *African Journal of Laboratory Haematology and Transfusion Science*. 2(3): 232-238.

Buhari, H. A., Sagir, A., Akuyam, S. A., Erhabor, O., Panti, A. A. and Garba, N. (2023). ABO, Rh, and Kell Blood Group Antigen Frequencies among Pregnant Women in Sokoto, Nigeria. *African Journal of Laboratory Haematology and Transfusion Science*. 2(3): 232-238.

Chatzikyriakidou, A. (2024). Beyond the ―Dominant‖ and ―Recessive‖ Patterns of Inheritance. *International Journal of Molecular Sciences*. 25(24): 13377.

Delaney, M. and Matthews, D. C. (2015). Haemolytic disease of the fetus and newborn: managing the mother, fetus, and newborn. *Hematology 2014, the American Society of Hematology Education Program Book*. 2015(1): 146-151.

Duangchan, T., Suklim, N., Pornpisanvijit, V., Ratmunee, S., Plyduang, T., Kooltheat, N. and Charong, N. (2024). Distribution of ABO, Rh, and MNS blood groups from students in Walailak University, Thailand: A descriptive cross-sectional study. *Clinical Epidemiology and Global Health*. 28(1): 101625.

Fasano, R. M., Meyer, E. K., Branscomb, J., White, M. S., Gibson, R. W. and Eckman, J. R. (2019). Impact of red blood cell antigen matching on alloimmunization and transfusion complications in patients with sickle cell disease: a systematic review. *Transfusion Medicine Reviews*. 33(1): 12-23.

Gaikwad, V., Bhadoriya, A., Gaikwad, S. and Gaikwad, S. V. (2024). A comparative study of foetal and maternal outcomes in registered and unregistered antenatal cases in a tertiary care centre. *Cureus*. 16(8): 1-5.

Karim, F., Moiz, B. and Kamran, N. (2015). Risk of maternal alloimmunization in Southern Pakistan–A study in a cohort of 1000 pregnant women. *Transfusion and Apheresis Science*. 52(1): 99-102.

Kim, C. H. (2024). Non-ABO Blood Group Systems. In *Glycoimmunology in Xenotransplantation*. Singapore: Springer Nature Singapore. Pp. 47-60.

Law, A., McCoy, M., Lynen, R., Curkendall, S. M., Gatwood, J., Juneau, P. L. and Landsman-Blumberg, P. (2015). The prevalence of complications and healthcare costs during pregnancy. *Journal of Medical Economics*. 18(7): 533-541.

Medugu, J. T., Abjah, U., Nasir, I. A., Adegoke, S. and Asuquo, E. E. (2016). Distribution of ABO, Rh D blood groups and haemoglobin phenotypes among pregnant women attending a Tertiary Hospital in Yola, Nigeria. *Journal of Medicine in the Tropics*. 18(1): 38-42.

Nahendran, P., Budin, S. B., Saat, N. Z. M., Yusop, M. F., Yazid, T. N. T. and Anuar, N. N. M. (2023). Haemolytic of newborn disease-related red blood cell alloantibodies. *Jurnal Teknologi (Sciences and Engineering)*. 85(1): 1-9.

Rai, R., Saha, S. C., Jain, A., Bagga, R., Kumar, P. and Marwaha, N. (2016). Anti-M alloimmunization in pregnancy: an unusual cause of bad obstetric history. *The Journal of Obstetrics and Gynecology of India*. 66(2): 607-609.

Ristovska, E., Bojadjieva, T. M., Velkova, E., Dimceva, A. H., Todorovski, B., Tashkovska, M. and Bosevski, M. (2022). Rare blood groups in ABO, Rh, Kell systems–Biological and clinical significance. *Prilozi*. 2(1): 21-26.

Teshale, A. B. and Tesema, G. A. (2020). Prevalence and associated factors of delayed first antenatal care booking among reproductive age women in Ethiopia; a multilevel analysis of EDHS 2016 data. *PLoS One*. 15(7): e0235538.

Warri, D. and George, A. (2020). Perceptions of pregnant women of reasons for late initiation of antenatal care: a qualitative interview study. *BMC Pregnancy and Childbirth*. 20(1): 70.

Yasuda, H., Ohto, H., Nollet, K. E., Kawabata, K., Saito, S., Yagi, Y. and Ishida, A. (2014). Hemolytic disease of the fetus and newborn with late-onset anaemia due to anti-M: a case report and review of the Japanese literature. *Transfusion Medicine Reviews*. 28(1): 1-6.

Zamudio, K. R., Bell, R. C. and Mason, N. A. (2016). Phenotypes in phylogeography: Species' traits, environmental variation, and vertebrate diversification. *Proceedings of the National Academy of Sciences*. 113(29): 8041-8048.