

Translational Trends in Hemolytic Disease of the Fetus and Newborn: Integrating Non-Invasive Prenatal Screening and Novel Biomarkers

Kingsley Chukwuka Amaihunwa¹, Emefiele Favour Chioma², Donald Ibe Ofili³

^{1,2,3}Department of Medical Laboratory Science, Faculty of Allied Health Sciences, Delta State University, Abraka

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ABSTRACT

Background: Hemolytic Disease of the Fetus and Newborn (HDFN) is an alloimmune condition caused by maternal antibodies directed against fetal red blood cell antigens inherited from the father. The disease remains a significant global health challenge regarding neonatal jaundice, mortality, and long-term neurodevelopmental impairment. Recent global burden reports show substantial regional disparities, with the vast majority of severe outcomes and neonatal deaths concentrated in low-resource regions. About three-quarters of neonatal deaths occur in South Asia and sub-Saharan Africa due to healthcare access gaps and shortages in preventative interventions. The disease leads to varying degrees of fetal and neonatal hemolysis, resulting in anemia, hyperbilirubinemia, hydrops fetalis, neurological complications, and in severe cases, perinatal death. Although once associated with high mortality, the introduction of Rh (D) immunoglobulin prophylaxis, improved antenatal screening, and advances in neonatal care have significantly reduced its incidence, especially in developed countries.

Method: A thorough assessment of existing peer reviewed published literatures was conducted using array of database, it deeply appraised recent studies and systematic reviews, and It examined conventional diagnostic methods and recent emerging advances in diagnostic techniques that improve early detection, monitoring, and management of affected pregnancies.

Results: The findings revealed that classical diagnostic methods such as maternal blood grouping, antibody screening, indirect antiglobulin test, direct antiglobulin test, and serum bilirubin estimation are important in detecting maternal sensitization and assessing neonatal hemolysis. However, the method have limited capacity to predict disease severity early in pregnancy while recent emerging diagnostic methods and novel biomarkers including cell-free fetal DNA (cfDNA) analysis, molecular blood grouping, next-generation sequencing (NGS), and middle cerebral artery (MCA) Doppler ultrasonography provide non-invasive and more accurate means of early fetal risk assessment. These methods allow early determination of fetal blood group status and detection of fetal anemia, improving pregnancy management outcomes.

Conclusion: Emerging diagnostic advancement provides far reaching benefits and efficiencies but it is accompanied with challenges of high costs, limited availability and inadequate technical expertise particularly in resource-limited settings. Integrating modern diagnostic approaches with traditional methods can enhance early detection, reduce complications, and improve maternal neonatal outcomes in HDFN with targeted therapeutics.

Keywords; Hemolytic Disease of the Fetus and Newborn, Non-Invasive Prenatal Screening, Novel Biomarker

INTRODUCTION

Hemolytic Disease of the Fetus and Newborn is an alloimmune blood disorder characterized by the accelerated destruction of fetal or neonatal red blood cells due to maternal antibodies. The term hemolytic refers to the

breakdown of red blood cells, erythroblastosis describes the increased production of immature red blood cells and fetalis refers to the fetus. Under normal conditions, red blood cells circulate for about 120 days before being replaced; however, in HDFN, they are destroyed prematurely, resulting in reduced red blood cell survival and anemia (Hall *et al.*, 2025). The recognition of Hemolytic Disease of the Fetus and Newborn dates back to ancient times, with early descriptions of conditions believed to represent fetal hydrops. In 1609, the French midwife Louise Bourgeois reported a case involving twins in which one infant presented with hydrops fetalis, severe jaundice, and kernicterus. Over time, similar cases were described using different terms, including generalized fetal edema, abnormal blood formation, and familial disease conditions. Advances in medicine later resulted in the development of preventive and diagnostic measures such as Rh (D) immunoglobulin prophylaxis, antenatal screening, serological monitoring, and improved prenatal and neonatal care. These developments have significantly reduced the incidence and severity of HDFN. However, despite these improvements, early identification of affected pregnancies and accurate prediction of fetal disease severity remain important challenges (Amaihunwa *et al.*, 2025)

Conventional diagnostic methods including maternal blood grouping, antibody screening, the indirect antiglobulin test, direct antiglobulin test, and bilirubin estimation, have contributed greatly to the detection and management of HDFN. Nevertheless, these methods have limitations, particularly in predicting fetal risk before significant complications occur. Recent advances in diagnostic technology, including cell-free fetal DNA analysis, molecular blood grouping, next-generation sequencing, and middle cerebral artery Doppler assessment, have provided more accurate and non-invasive methods for assessing fetal risk and monitoring pregnancies affected by HDFN (Derek Winter *et al.*, 2023). The understanding and application of these emerging diagnostic approaches are important for improving prenatal surveillance, reducing unnecessary interventions, and enhancing fetal and neonatal outcomes.

Etiology of Hemolytic Disease of the Newborn

Hemolytic Disease of the Fetus and Newborn occurs when a mother produces antibodies against red blood cell antigens inherited by the fetus from the father. These antigens may belong to several blood group systems, including Rhesus (Rh), ABO, Kidd, Kell, MNS, Duffy, and Lewis systems. Maternal sensitization occurs when fetal red blood cells enter the maternal circulation, stimulating the production of antibodies. These maternal antibodies can cross the placenta and bind to fetal red blood cells, resulting in their destruction through immune-mediated hemolysis, following birth, affected newborns may develop elevated bilirubin levels because the immature neonatal liver is unable to efficiently process the excess bilirubin produced from red blood cell destruction. If untreated, severe hyperbilirubinemia may result in kernicterus, neurological damage, or cerebral palsy (Dean, 2005)

Pathophysiology of Hemolytic Disease of the Newborn

HDFN is an alloimmune condition in which maternal antibodies attack fetal red blood cells. The condition usually occurs when the fetus inherits a red blood cell antigen from the father that is absent in the mother. The maternal immune system recognizes the antigen as foreign and produces immunoglobulin G (IgG) antibodies against it. These IgG antibodies cross the placenta and enter the fetal circulation, where they attach to fetal red blood cells and promote their destruction by macrophages, mainly in the spleen. This process leads to fetal anemia, increased production of immature red blood cells, hydrops fetalis, and neonatal hyperbilirubinemia. After birth, continued hemolysis results in increased bilirubin production, increasing the risk of jaundice and bilirubin-induced neurological complications if not managed promptly (Hall *et al.*, 2025).

Prevalence of Hemolytic Disease of the Newborn

Globally, RhD-related HDFN remains an important cause of fetal and neonatal morbidity. However, the introduction of Rh (D) immunoglobulin prophylaxis and improved antenatal care has greatly reduced its occurrence, especially in developed countries. In Nigeria, studies have shown that RhD alloimmunization contributes less frequently to neonatal jaundice compared with other causes such as infection and prematurity.

However, challenges remain regarding routine maternal alloantibody screening and access to Rh immunoglobulin prophylaxis, which may increase the risk of preventable complications.

Morbidity and Mortality Statistics

HDFN may result in significant fetal and neonatal complications, including anemia, hydrops fetalis, severe jaundice, neurological damage, and death if untreated. In Nigeria, neonatal jaundice remains one of the major causes of neonatal admission. Mortality is higher among premature infants and those with severe neonatal complications. Early diagnosis, appropriate monitoring, and timely intervention are therefore essential in reducing morbidity and mortality associated with HDFN. (Amaihunwa *et al*, 2025)

Maternal Blood Grouping and Antibody Screening

Maternal immunization occurs when a pregnant woman develops antibodies against fetal red blood cell antigens that differ from her own. These antibodies can cross the placenta and destroy fetal red blood cells, resulting in HDFN. Routine antenatal screening is therefore performed during early pregnancy to identify maternal blood type and detect potentially harmful antibodies. Women who are RhD-negative undergo additional evaluation to determine whether the fetus is RhD-positive and therefore at risk of developing HDFN. Early identification of maternal antibodies enables timely monitoring and intervention to reduce fetal complications. (Moise *et al*, 2008)

Limitation

Non-ABO red blood cell antibodies are frequently identified during the first trimester of pregnancy with prevalence rates ranging from about 1% to 2%. Among the clinically significant antibodies associated with hemolytic disease of the fetus and newborn, the most frequently encountered are anti-D, anti-Kell, anti-E, and anti-c. These antibodies arise through a process known as RBC alloimmunization, which may occur following blood transfusion, pregnancy, or episodes of fetomaternal hemorrhage. Although antibody titres are useful for screening and monitoring purposes, they do not reliably predict the severity of disease in the fetus or newborn. Instead, they indicate the potential risk for HDFN. When antibody titres reach critical levels, typically between 1:16 and 1:64, or when a rapid increase in titre is observed, referral to a high-risk obstetric team is recommended for specialized fetal surveillance and management.

Indirect Coombs Test

The Indirect Coombs Test is used to detect free antibodies circulating in maternal serum. During the test, maternal serum is mixed with reagent red blood cells carrying known antigens. Agglutination indicates the presence of antibodies capable of reacting with red blood cell antigens. This test is routinely included in prenatal screening programs because it identifies clinically significant antibodies that may cause fetal hemolysis. Positive results help determine the need for closer monitoring and further fetal assessment. (Murray and Roberts, 2007).

Limitation

A positive Direct Antihuman Globulin Test in a newborn who does not exhibit pathological jaundice has limited predictive value for HDFN. Furthermore, some infants may develop delayed hemolysis despite having reassuring initial laboratory findings, provided that the direct antihuman globulin test is positive. Furthermore, newborn red blood cells possess fewer antigenic sites, which are more widely spaced, making agglutination with the Coombs reagent less effective. The Coombs reagent, also known as antihuman globulin, contains polyclonal antibodies directed against human immunoglobulins and components of the complement system. In addition, infants with HDFN who have undergone intrauterine transfusions for the management of fetal anemia or hydrops fetalis may produce a negative direct antihuman globulin test result. This occurs because the transfused donor red blood cells can interfere with the agglutination process, thereby reducing the test's ability to detect sensitized erythrocytes. (Murray and Roberts *et al.*, 2022).

Serum Bilirubin Estimations

Measurement of total serum bilirubin is an important investigation in newborns affected by HDFN. Excessive red blood cell destruction leads to increased bilirubin production, resulting in hyperbilirubinemia. Elevated bilirubin levels increase the risk of bilirubin-induced neurological damage, including acute bilirubin encephalopathy and kernicterus. (Murray and Roberts, 2007). Regular bilirubin monitoring allows early initiation of treatment. Common therapeutic interventions include phototherapy and exchange transfusion in severe cases to prevent complications associated with excessive bilirubin accumulation. (Hall *et al.*, 2025)

Limitations

During phototherapy, serum bilirubin levels should be monitored frequently to assess the effectiveness of treatment and guide further management. Infants with hemolytic disease of the fetus and newborn are particularly prone to rebound hyperbilirubinemia, meaning that bilirubin concentrations can rise rapidly after phototherapy is discontinued. Consequently, many affected newborns require repeated courses of phototherapy to maintain safe bilirubin levels. Although visible jaundice may indicate increasing bilirubin levels, it is not a reliable measure of the actual bilirubin concentration in the bloodstream or the central nervous system. Therefore, laboratory assessment of neonatal bilirubin levels is essential, and visual examination alone should not be used to monitor or manage infants with HDFN (Murray *et al.*, 2022).

Cell Free Fetal Dna

Prenatal cell-free DNA (cfDNA) screening is a non-invasive blood test performed during pregnancy to analyze small fragments of fetal DNA that circulate within the mother's bloodstream. This screening method is primarily used to assess the likelihood that the fetus has certain chromosomal abnormalities resulting from an abnormal number of chromosomes, including Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), and Patau syndrome (trisomy 13). In addition to screening for chromosomal disorders, cfDNA testing can be used to determine the sex of the fetus, particularly when ultrasound findings are inconclusive regarding external genitalia. The test may also be employed to identify the fetal Rhesus (Rh) blood type. Rh factor is a protein located on the surface of red blood cells; individuals who possess the protein are classified as Rh-positive, while those who lack it are Rh-negative. Determining fetal Rh status is especially important when the mother is Rh-negative because an Rh incompatibility can trigger maternal immune responses against fetal red blood cells. Early identification allows timely administration of preventive treatment to reduce the risk of serious complications associated with hemolytic disease of the fetus and newborn (Gil *et al.*, 2017)

Molecular Blood Grouping

The Molecular Diagnostics provides fetal genotyping services to support maternity and transfusion medicine. Using cell-free fetal DNA (cffDNA) obtained from maternal blood samples, the method offers a rapid, non-invasive, reliable, and convenient method for determining the fetal D, C, c, E, and K antigen status in pregnancies complicated by maternal alloantibodies. This testing enables the early identification of fetuses that carry the relevant red cell antigens, allowing healthcare providers to counsel mothers appropriately and implement close monitoring throughout pregnancy when necessary. Conversely, the service can also identify fetuses that lack the corresponding antigens, thereby confirming that they are not at risk of developing hemolytic disease of the fetus and newborn. The use of fetal genotyping improves antenatal care by allowing clinicians to concentrate surveillance efforts on pregnancies with antigen-positive fetuses while minimizing unnecessary interventions for women carrying antigen-negative fetuses. As a result, mothers whose fetuses are not at risk can experience a less stressful pregnancy with reduced monitoring requirements. In addition, this approach lowers healthcare costs and reduces the need for repeated clinic visits, Doppler ultrasound examinations, and referrals for serial antibody quantification and titre assessments (NHS Blood and Transplant, 2024).

Next Generation Sequencing

Next-generation sequencing (NGS) is widely used in non-invasive prenatal screening for fetal chromosomal abnormalities, although only a limited number of studies have explored its application in fetal blood group genotyping. NGS technology operates through two principal processes: the clonal amplification of DNA fragments in vitro and the simultaneous sequencing of millions to billions of DNA templates. Amplification of individual DNA molecules is achieved either through emulsion-based polymerase chain reaction (PCR) on beads, as utilized by the IonTorrent platform, or through bridge amplification on a solid surface, as employed by the Illumina platform. The sequencing process involves the incorporation of nucleotides by DNA polymerase, with different platforms using distinct detection methods. The IonTorrent system identifies hydrogen ions released during nucleotide incorporation, whereas the Illumina platform detects fluorescently labeled nucleotides through optical imaging. Other sequencing approaches, such as nanopore sequencing, have also been developed but are beyond the scope of this discussion. The application of NGS in fetal blood group genotyping was first demonstrated by Rieneck et al., who used cell-free fetal DNA (cffDNA) for antenatal prediction of the fetal Kell (K) blood group phenotype. However, their method did not include measurement of the fetal DNA fraction.(Wienzek-Lischka *et al.*, 2023).

Middle Cerebral Artery Doppler Assessment

Middle cerebral artery Doppler ultrasonography is a non-invasive technique used to measure the peak systolic velocity of blood flow within the fetal middle cerebral artery. This assessment is valuable in monitoring fetuses at risk of anemia, particularly in cases of hemolytic disease of the fetus and newborn. Routine prenatal screening enables the identification of pregnancies at risk for HDFN during the first antenatal visit, usually before 10 weeks of gestation. In women with a previous history of HDFN, the risk is recognized from the outset of subsequent pregnancies. For pregnancies considered at high risk of HDFN, MCA Doppler evaluation is generally recommended after 20 weeks of gestation, especially when there is a prior history of HDFN, confirmed maternal alloimmunization, elevated maternal antibody levels, or ultrasound evidence of hydrops fetalis. To date, no harmful effects on the fetus have been associated with the use of MCA Doppler ultrasound during pregnancy, making it a safe and effective method for fetal surveillance (Allo Hope Foundation, 2024).

Comprehensive Comparison of Conventional and Emerging Methods of Hemolytic Disease of the Foetus and Newborn Screening

	Conventional Methods	Emerging Methods
Purpose	Routine blood typing, antibody screening, direct detection of neonatal erythrocyte coating, and invasive staging of fetal anemia or bilirubin buildup.	Early non-invasive risk stratification, high-precision fetal genotyping, dynamic monitoring of fetal anemia, and targeted invasive testing.
Specimen or Procedure	Maternal venous blood, neonatal cord blood, amniotic fluid (via amniocentesis), and fetal cord blood (via cordocentesis).	Maternal peripheral blood (for cell-free fetal DNA), paternal/maternal genomic DNA, and high-resolution color Doppler ultrasound.
Timing	First prenatal visit, repeated at 28 weeks (or postnatally via cord blood). Amniocentesis and cordocentesis are typically reserved for mid-to-late gestation	cffDNA testing is viable early in pregnancy (from ~10 weeks); MCA-PSV Doppler tracking is initiated around 18–20 weeks and repeated serially.
Diagnostic Capability	Confirms maternal alloimmunization and postnatal infant hemolysis; historical amniotic fluid spectrophotometry provides indirect assessments.	High diagnostic accuracy for fetal RHD and minor blood group antigens (e.g., Kell, C, c, E); MCA-PSV Doppler accurately detects fetal anemia non-invasively.

Limitations	Invasive procedures carry risks of fetal loss, bleeding, and worsening maternal sensitization. Indirect marker interpretation can be easily confounded. Cannot predict fetal antigen status without invasive testing	Allows extremely early detection and risk stratification but Higher initial setup cost and specialized equipment requirements. Molecular tests require advanced laboratory infrastructure. False negatives are possible with very low cffDNA fractions.
Cost	Generally lower per routine serologic test, but cumulative costs rise if complications from invasive tests occur.	Moderate-to-high initial test cost, though overall healthcare expenditure is offset by avoiding unnecessary interventions and NICU stays.
Potential Clinical Applications	Highly standardized and globally accessible. Essential for postnatal blood banking and cross-matching. Provides direct evaluation of neonatal red blood cells. Routine prenatal blood grouping and antibody screening. Postnatal Direct Antiglobulin Test (DAT). Emergency neonatal cross-matching. Diagnostic confirmation.	Drastically reduces the need for invasive, Enables targeted antenatal prophylaxis. Allows extremely early detection and risk stratification. Routine non-invasive prenatal screening (NIPS) for fetal antigens in alloimmunized pregnancies. Serial non-invasive surveillance of fetal peak systolic velocity for intrauterine transfusion planning.

Feasibility of the New Trends of Diagnosis of Hemolytic Disease of the Newborn in Nigeria

Study carried in Nigeria emphasized the importance of implementation of routine maternal alloantibody screening during pregnancy to reduce the burden of hemolytic disease of the fetus and newborn. Previous reports indicated that the prevalence of neonatal jaundice in Abuja was approximately 21.4%; however, information regarding the frequency of maternal red cell alloantibodies and uncommon unexpected antibodies remains limited because alloantibody screening is not routinely performed in antenatal care or before blood transfusions. A prospective cross-sectional study conducted at a specialist hospital between September 2023 and March 2024, where 250 pregnant women aged 16–45 years were enrolled, all of whom provided blood samples for blood group typing and antibody screening. Most participants (60.4%) were in their second trimester of pregnancy, while 64% had no previous history of miscarriage or abortion, nearly one-third of the women (29.8%) had experienced more than two pregnancies, and a small proportion (1.2%) had been pregnant five or more times. The findings revealed that 95.2% of the women tested negative for irregular alloantibodies, whereas 4.8% were positive. Among those with detectable antibodies, anti-E was the most common (1.2%), followed by anti-K (0.8%), while anti-C and anti-Jsb were each detected in 0.4% of participants. Notably, all women who tested positive for these antibodies were RhD-positive (Ijeoma *et al.*, 2025). The prevalence of maternal irregular antibodies observed in this studied population showed (4.8%) was considerably higher than rates reported in countries such as the Netherlands (2.7%) (Koelewijn, 2008) and Sweden (0.5%) (Filbey, 1995). The researchers suggested that some women with a history of delivering jaundiced newborns may have had undiagnosed HDFN caused by non-RhD antibodies, highlighting the need for routine alloantibody screening during pregnancy. Emerging diagnostic advancement provides far reaching benefits and efficiencies but it is accompanied with challenges of high costs, limited availability and inadequate technical expertise particularly in resource-limited settings such as in South Asia and sub-Saharan Africa due to healthcare access gaps and shortages in preventative.

CONCLUSION

Hemolytic Disease of the Fetus and Newborn remains an important cause of fetal and neonatal morbidity despite screening methods in preventive care. While conventional diagnostic methods are useful for screening and monitoring of affected pregnancies, they have limitations in predicting disease severity and fetal outcomes. New diagnostic technologies such as cell-free fetal DNA testing, molecular blood grouping, next-generation sequencing, and MCA Doppler assessment provide earlier, more accurate, and non-invasive detection of fetal risk. The integration of these modern approaches into clinical practice can improve prenatal management, reduce

complications, and enhance fetal and neonatal survival. Continued efforts to increase access to these diagnostic tools, particularly in Nigeria and other resource-limited settings, are essential for improving outcomes in HDFN.

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