

HAEMOLYTIC OF NEWBORN DISEASE-RELATED RED BLOOD CELL ALLOANTIBODIES

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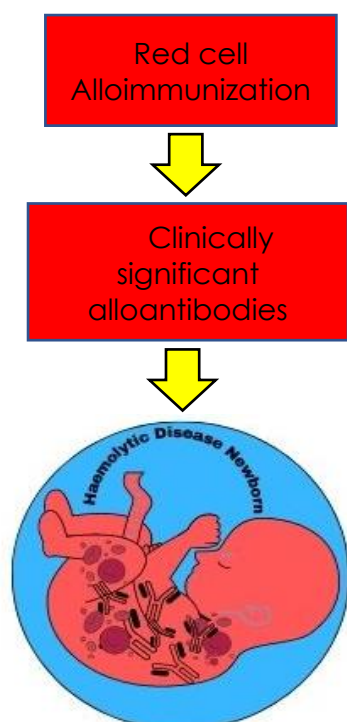
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Graphical abstract



Abstract

Red cell alloimmunisation in pregnancy may result in haemolytic disease of the foetus and newborn (HDN). During pregnancy, there are possibilities of the foetal antigen being exposed to the mother's circulation, thereby leading to the production of antibodies. The immunoglobulin G (IgG) antibodies from the mother pass through the maternal surface placenta, which is then sensitised and obstructs the formation of foetal red blood cells (RBCs). This review discusses the presence of variance alloantibodies during pregnancy, as well as the consequences of HDN and its management. This review revealed that ABO incompatibility is the most common cause of alloimmunisation in pregnancy. The Rh system (anti-D and -E) are the most common alloantibodies found in pregnant women, followed by other alloantibodies such as Kell, Duffy, Kidd and MNSs. Hyperbilirunemia (increased bilirubin levels) is commonly seen in neonatal jaundice due to HDN and it is usually manageable using the phototherapy method. Nevertheless, the clinical significance of alloantibodies can cause complications such as anaemia, and in some extreme cases, kernicterus might require a blood exchange transfusion.

Keywords: Alloimmunisation, HDN, Alloantibodies, ABO incompatibility, hyperbilirunemia, Phototherapy

Abstrak

Alloimmunisasi sel darah merah dalam kehamilan boleh mengakibatkan penyakit hemolitik bayi baru lahir (HDN). Semasa proses kehamilan terdapat kebarangkalian pendedahan terhadap antigen asing dalam peredaran darah ibu yang seterusnya mengakibatkan penghasilan antibodi. Antibodi immunoglobulin G (IgG) dapat merentas plasenta ibu dan menyebabkan pembentukan antigen-antibodi yang menyekat sel darah merah neonatal (RBCs). Penulisan ini membincangkan kepelbagaian alloantibodi yang terhasil semasa proses kehamilan serta kesan dan pengurusan HDN. Penulisan ini menunjukkan yang ketidakserasian ABO adalah penyebab utama alloimmunisasi dalam kehamilan. Sistem Rh (anti-D dan -E) adalah alloantibodi yang paling biasa ditemui dalam kalangan wanita hamil, diikuti dengan alloantibodi lain

seperti Kell, Duffy, Kidd dan MNSs. Hiperbilirunemia (peningkatan paras bilirubin) biasanya ditemui dalam kalangan jaundis neonatal akibat HDN dan boleh dirawat dengan menggunakan kaedah fototerapi. Namun begitu, kepentingan antibodi yang signifikan secara klinikal boleh memberikan komplikasi seperti anemia dan bagi kes yang lebih serius, boleh menyebabkan kernicterus yang mungkin memerlukan pertukaran darah.

Kata kunci: Alloimmunisasi, HDN, Alloantibodi, ketidakserasian ABO, Hiperbilirunemia, Fototerapi

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1.0 INTRODUCTION

Red cell alloimmunisation is defined as the sensitisation of foreign red blood cell (RBC) antigen, which leads to the formation of alloantibodies. This usually occurs after exposure to pregnancy and previous transfusion (1). During pregnancy, the most common events that lead to alloimmunisation is related to ABO and Rh maternal-foetal incompatibility. The incompatibility of blood groups may lead to haemolytic disease of the newborn (HDN) or haemolytic disease of the foetus and newborn (HDFN), which contributes to the haemolysis of RBCs. HDN, also known as erythroblastosis foetus, occurs when foetal RBC inherited antigen that the mother lacks crosses the placenta and stimulates maternal antibody production. HDN can occur both *in vitro* (in utero) and *ex vitro* (right after delivery) resulting in the formation of maternal immunoglobulin, especially immunoglobulin G (IgG) antibodies which are able to cross the placenta into the foetal circulation (2). Following the escape of the maternal antibody into the foetus circulation, the foetal RBCs are attacked and haemolysed (3), which might cause hydrops foetalis, anaemia with reticulocytosis and hyperbilirubinemia(4). HDN is the consequence of sensitisation to RBC antigens such as Kell (K&k), Duffy (Fya& Fyb), Kidd (Jka &Jkb), and other Rh antigens (5). These clinically significant antibodies can exhibit severe outcome due to lack of prophylactic immunoglobulins to prevent these minor irregular alloantibodies(6). Alloimmunisation during pregnancy is still a major problem in the 21st first century(7). Incidence of ABO and maternal red cell antibodies have now develop as a main cause of HDN in various countries(8). Generally, maternal clinical significant alloantibodies against foetal RBC antigen can cause haemolysis or suppress erythropoiesis, leading to foetal anaemia and foetal death as shown in Figure 1 (9). Additionally, 86% of all HDN cases worldwide were due to blood group incompatibility. Foetus blood group antigen is generally inherited from the father; however, the mother lacks such RBC blood group antigen, thus the foetus blood group antigen is considered a foreign antigen (alloantigen) to the mother. Hence, the production of antibodies is stimulated when the alloantigen crosses the placenta into the mother's circulation. The prevalence of irregular red cell alloimmunisation during pregnancy increased from 0.89 % to 5.98% worldwide in 2018 (10).

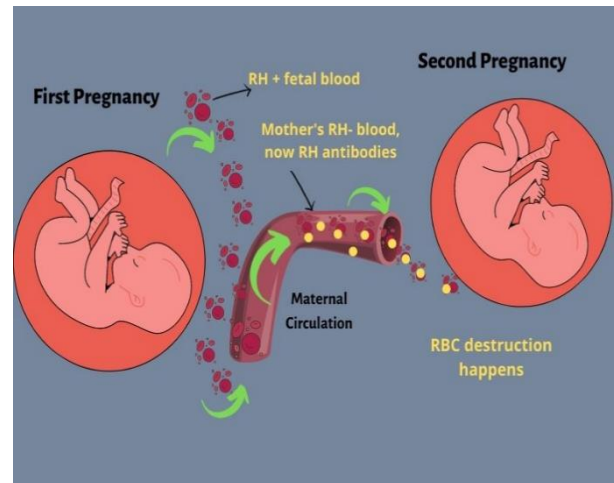


Figure 1 Alloimmunization of maternal-foetal circulation

Alloimmunisation of Maternal-Foetal Circulation

a) First pregnancy

Maternal transportation antibodies actively cross the foetal circulation as early as 13 weeks and the majority of antibodies cross the placenta during the last four weeks of pregnancy (11). Rh-negative blood type mother mixes with the newborn's Rh-positive blood type. Thus, the mother's immune system identifies the baby's blood as a foreign cell. Mother's RBCs produce antibodies to destroy these foreign cells and Rh sensitization occurs.

b) Second Pregnancy

A large number of harmful mother's antibodies cross the placenta and enter the baby's bloodstream. These antibodies destroy the baby's RBCs and cause haemolysis (Figure 1). This process leads to HDN.

2.0 CLINICAL MANIFESTATION

HDN is characterised by a type of anaemia caused by jaundice in newborns or infants, which can be divided into two categories: immune and non-immune causes (3). Etiologically, non-immune HDN is due to the following: (a) acquired defects in RBC caused by cytomegalovirus (CMV) infection, toxoplasmosis, syphilis and some other types of

bacteria (b) congenital defects of RBC related to membrane and enzyme disorders such as haemoglobinopathies, glucose 6 phosphate dehydrogenase (G6PD) deficiency hereditary spherocytosis. Meanwhile, incompatibility of different blood groups such as ABO, Rh and congenital autoimmune disease contribute to immune-related HDN (3). Furthermore, RBCs destruction begins in intrauterine life (12), which causes severe anaemia and neonatal jaundice or even extreme consequences, such as severe brain damage (13). According to Gupta *et al.* (2020), 50% of all fetuses with various aetiologies of HDN died of kernicterus or hydrop foetalis before 1945 (14). The lifespan of the infants' RBCs was shortened as a reaction of maternally cross foreign antibodies being transferred via the placenta and directed against paternally inherited antigens on the RBC.

Feto-maternal haemolysis and previous transfusion are the two possible factors of antigenic exposure resulting in HDN (15). Besides, an indirect haemolytic process has also been shown to lead to anaemia or hyperbilirubinemia which could affect neonatal morbidity and mortality (16). The risk of haemolysis is categorised into three different severity levels: mild, moderate, and severe (Table 1). Milder cases are associated with jaundice and anaemia. The severe reaction could lead to an immature liver of neonates who cannot excrete the accumulated bilirubin in the blood after delivery. Within 24 hours, an increased accumulation of bilirubin may cause kernicterus and potentially fatal, which is associated with permanent neurological damage in the surviving babies (17). On the other hand, HDN is commonly present in a foetus within seven days of life (early-onset anaemia from antibody-dependent haemolysis of RBCs). However, there are also cases reported in two weeks of foetus life (late anaemia of haemolytic disease from antibody-dependent destruction of RBCs precursors) (11). Based on the study, extreme destruction of RBC in the foetus could progress to severe anaemia. Thus, the enlargement of the baby's liver and spleen could cause the production of immature RBC (erythroblast) and spread into the foetus circulation to initiate erythroblastosis foetalis. Therefore, this condition might occur either in maternal circulation or after birth (18)

3.0 ALLOANTIBODIES

Alloantibodies are antibodies that develop upon exposure to a foreign antigen. Pregnant women can potentially become alloimmunised to RBC antigen by blood transfusion and foetal or maternal haemorrhage either during pregnancy or delivery. However, only a small fraction of women can be alloimmunised after antigen exposure (9). Consequently, clinically significant antibodies is found to be slightly higher in European countries than in South Asian and African countries(8).According to Ohio State University study, 8.2% of pregnancies develop more than one alloantibodies during pregnancy (19). These multiple antibodies could cause greater risk for HDN.

The prevalence of alloantibodies varies between population based on their possibilities of blood group within the community (20). In most Western countries, pregnant women are routinely undergoing early screening to assess the formation of antibodies that may cause HDN but not all RBC alloantibodies specificity are involved. no data have been associated with P and Lewis blood groups in HDN (17). Study done in United State of America found that more than 50% of Lewis antibodies expressed on foetal RBC, but they are IgM antibodies, which will not be able to cross the maternal surface(20). The titre of RBC alloantibodies needs to be checked as it correlates with the severity of the disease (21) Routine screening of ABO and Rh is usually conducted at 10 weeks to 16 weeks of gestation, which could help in the early detection of alloantibodies and antenatal management for HDN (22).

3.1 ABO Incompatibility

ABO incompatibility has been recognised as the most common cause of HDN. Risk factors for ABO incompatibility are present in approximately 12% to 15% of pregnancies but only 3% to 4% have been proven to be present with foetal sensitisation using the positive direct Coombs' test. However, less than 1% of cases can cause significant haemolysis, especially after birth (13). Currently, ABO and Rh blood grouping of both paternal and maternal is performed for the early detection of a possible incompatibility that might cause HDN (23). ABO incompatibility is usually a problem of the neonate rather than the foetus because the sensitisation of RBCs occurs when the baby is still in the mother's womb, and a reaction ensues due to the incompatibility of blood between the mother and the neonate. ABO HDN is most commonly found in group A or B babies born to group O mothers with anti-A or anti-B antibodies (24).

Generally, most cases of HDN are caused by naturally formed ABO antibodies and could lead to minimal or mild symptoms (25). A mild degree of haemolysis and anaemia cases are rarely associated with ABO incompatibility because ABO antibodies are prominently IgM pentamer structures, which cannot cross the placenta. However, ABO HDN babies with both anti-A and anti-B are usually susceptible to clinical problems such as jaundice, also known as hyperbilirubinaemia (26). Significant hyperbilirubinemia requires phototherapy while exchange blood transfusion is needed in severe cases (27). Studies have shown that 30.4% ABO incompatibility newborns had significant hyperbilirubinaemia that required a phototherapy session (27).

3.2 Rh Incompatibility

Rh system is the second most important of blood groups after the ABO grouping (28). It has been shown that Rh incompatibility occurs in 276 per 100,000 live births and approximately 50% of untreated foetal HDN cases will either die or develop brain damage (29). One of the common

nomenclature used in the Rh system is the Fisher system which demonstrates three-class epitopes (C, c, D, E, e). Notably, d represents no D or d antigen. Antibodies from the Rh system are primarily from the IgG class, which can pass through the umbilical cord due to their small molecular size (30). Due to the ability of Rh antibodies to cross the placenta, Rh alloimmunisation is considered an emergency condition because it easily causes HDN (3). Studies show that HDN caused by anti-Rh (D) could be prevented or managed by administering Rhogam but unfortunately, this is not applicable for Rh antigens such as C, c, E, and e. Rh (D) is well known as a potent immunogen because as little as 0.1 to 1ml of Rh (D) positive antigen RBC exposure can stimulate the respective antibody production (16).

Approximately 15% of HDN cases are caused by Rh incompatibility (23), which significantly affects the foetus or neonates compared to other blood groups (13). In this condition, an Rh-negative mother with an Rh-positive infant or foetus is at risk of Rh antigen exposure during pregnancy or delivery (23). The Rh (D) positive antigen from the foetus can escape through the placenta and subsequently contribute to the development of Rh (D) IgG antibodies in an Rh (D) negative mother. Thus, the significant alloantibodies in the maternal serum are able to pass through the foetal circulation via the placenta barrier causing major haemolysis (26). Generally, once the mother has been sensitised to the Rh (D) antigen, the mother's serum will contain an anti-Rh (D). Therefore, the direct coomb test (DCT) shows an agglutination with the presence of anti-Rh (D) (17). Clinically, the usage of anti-Rh (D) immunoglobulin (RHIG) prophylaxis has generally decreased the risk of Rh (D) alloimmunisation from 16 to 0.3% in developed countries (31).

Meanwhile, studies show that an Rh (D) positive mother who could produce other antibodies within the Rh system (anti-C, c, E, or e) can be detected postnatally by a positive reaction in the DCT test due to RBCs sensitisation (22). Anti-Rh (D) is the most common aetiology of HDN, whereas 0.5% of pregnancies are associated with IgG antibodies against other Rh antigen epitopes such as (C, c, E, e) (32). On the other hand, a study revealed that mothers who serologically type Rh (D) positive have no risk of causing haemolytic disease, however, rare cases of anti-D isoimmunisation can occur in Rh (D) positive individuals (16). This scenario is seen in an Rh (D) positive mother with a weak D (Du) and partial D. Individuals possessing a weak D phenotype could be bound to D epitopes during transfusion or pregnancy and this could increase the risk of alloantibodies production and RBC sensitisation, leading to fatal HDN in pregnant women (33).

According to a study conducted in Saudi Arabia (34), the prevalence of Rh alloimmunisation in pregnancy is still high resulting in morbidity and mortality in several developing countries. The Rh system alloimmunisation associated with mild HDN includes anti-E, anti-C and anti-e. However, the combination of anti-E and anti-c antibodies are susceptible to severe fetal and neonatal haemolytic disease (35). Anti-c and anti-E are the next most common causes of HDN after anti-D, with anti-c

causing delayed anaemia in neonates (36). In Malaysia, statistics have shown that anti-E is the second most common clinically significant antibody (37) in terms of HDN. The development of anti-E alloantibody can be frequently seen in pregnancies and is associated with HDN with mild to moderate progression (14).

Besides, anti-E can be categorised as an insensitive and poor predictor of HDN severity as all foetus or newborns of a mother who has been diagnosed with anti-E alloimmunisation should be considered potentially high risk of HDN (3). Moreover, anti-E alloimmunization could lead to severe haemolytic anaemia, hyperbilirubinaemia, cholelithiasis and thrombocytopenia(38). The most common antibodies was reported anti-E in non-RHD group 10% of these antibodies cause severe HDN and exchange transfusion has been given to 21% of the neonates (6). Anti-e is usually considered a rare cause of HDN, the disease is generally milder while anti-C was found to be associated with anti-D. Under these conditions. Intrauterine death is likely to occur and all cases require intrauterine transfusion. Moreover, the combination between anti-C and anti-D could progress to HDN (39).

3.3 Kell

The third most immunogenic antigen besides ABO and Rh system is Kell (K). K antigen is probably 8 to 10 times less immunogenic compared to Rh (D) system with an incidence rate ranging from 14% to 28% (40). The most common antigens found from the Kell system are K and k (25). It has been predicted that alloimmunisation of the Kell antigen mostly occurs in one every 1000 pregnancies, equivalent to 29% of women producing alloimmunisation and capable of causing neonatal haemolytic disease (41). Furthermore, it has been estimated that about 10% of individuals are at risk of developing an anti-K after transfusion with at least one unit of K antigen-positive packed cell (40). The production of Kell alloimmunisation is mainly due to previously packed cell transfusion and foetal-maternal haemorrhage induced during pregnancy (36).

K antigen is produced in foetal RBCs at 10 to 11 weeks, however, the small allele k antigen is expressed at 6 to 7 weeks of gestation (42). Nevertheless, there is approximately a 50% chance of an individual having a Kell-positive foetus in second pregnancy because most Kell-positive individuals are heterozygous (41). Moreover, milder cases of naturally occurring anti-K are mostly due to transfused packed cells and pregnancy (43). Nonetheless, predominantly, anti-K consists of IgG class antibody. Thus it could cause severe haemolytic transfusion reaction due to the ability to cross the placenta (40). One of the major effects of anti-K HDN is the suppression of red cell production in the foetus rather than causing haemolysis (44).

Alloimmunisation occurring due to anti-K will lead to the suppression of erythropoiesis (41). This incidence could also induce severe anaemia and foetal death (36). HDN with anti-K alloimmunisation can cause severe anaemia due to the destruction of erythrocyte precursors in the bone marrow and

mature erythrocytes in foetal RBCs (45). Alloimmunisation of Kell antibodies can be predicted according to the titre of amniotic bilirubin level, as the production of Kell antigen is well expressed in the early stage of erythropoiesis in the bone marrow (46). This could result in hyperbilirubinemia in neonates, which increases progressively on the 3rd day of life, thus haemoglobin and haematocrit levels will drop and result in moderate anaemia (47).

3.4 Duffy

The Duffy blood group system (Fy) is the fourth immunogenic antigen in the human blood group system that causes HDN (48). Duffy blood group system consists of two antigens: anti-Fy^a and anti-Fy^b. The anti-Fy^a and anti-Fy^b antibodies are both immunogenic, however, anti-Fy^a alloantibody is more frequently found compared to anti-Fy^b. These alloimmunisations can cause HDN and sensitisation to the antigen (49). Furthermore, the Duffy antigen is one of the clinically significant blood group systems due to its IgG class and its ability to cross the placenta barrier into the foetal circulation and cause HDN, as well as haemolytic transfusion reaction (HTR) due to complement activation (48).

Besides, anti-Fy^a is also known as an uncommon factor and is usually seen in mild cases. It rarely causes significant HDN but minor cases are reported occasionally in the literature, where the presence of anti-Fy^a could cause severe HDN or even intrauterine death (50). Antigen Fy^b is about 20 times less immunogenic compared to antigen Fy^a. Approximately 5% of pregnant women are able to develop Duffy alloimmunisation, which subsequently leads to the production of anti-Fy^a antibodies. In addition, anti-Fy^a antibodies are associated with mild to severe HDN while anti-Fy^b is rarely seen and could only cause a mild HDN (51). According to (52), although HDN related to anti-Fy^a is usually mild, it has the potential to induce significant haemolytic disease requiring transfusion therapy. Approximately 11% of cases cause significant HDN reaction, necessitating intrauterine or neonatal exchange transfusion. Research showed that there is no history of hydrops foetalis or

mortality as a result of these alloimmunisation antibodies.

3.5 Kidd

The Kidd blood group system is known as the fifth important immunogenic blood group system on human erythrocytes. This antigen system has been divided into common specificities such as Jk^a and Jk^b, which can produce neither anti-Jk^a nor Jk^b that are considered as clinically significant antibodies, that could cause acute or delayed transfusion reaction (DHTR) as well as HDN (5). Kidd antibodies are usually IgG type that able to cross the maternal placenta, bind and activate the complement which then could cause either rapid intravascular in the infant. Furthermore, antigen Jk^a can be developed on erythrocytes as early as 11 weeks of pregnancy but is rarely seen as severe HDN due to poor immunogenicity (53).

Importantly, Kidd antibodies are also not commonly detected in sera that contain other antibodies. These antibody titres are often found to be low and will only react with homozygous cells with the double dosage of k antigen. Occasionally, due to the low titre of the antibody, it could remain undetected with only one-third being responsible for delayed haemolytic transfusion reaction (DHTR) (54). According to a previous report (55), exposure to Jk^a antigen in pregnant women could risk their infant with a mild haemolytic disease even without a history of transfusion or transplantation. Moreover, HDN can be associated with any type of antibodies with mild alloimmunization but antigen Jk^a shows an increased level of clinically significant cases which contributes to 1.5% of an alloimmunization incidents in pregnant women (51). Nevertheless, anti-Jk^b can cause haemolytic disease that is usually mild and diagnosed with a benign condition (5). Generally, the Kidd blood group system is rarely seen in HDN and it is usually a mild reaction (54).

3.6 MNSS

The anti-M is known as a common naturally occurring antibody, and it is rarely associated with HDN because anti-M is IgM are unable to cross the placenta due to their larger pentamer size.

Table 1 Summary of Alloantibodies related to HDN

Antibodies	Types of Immunoglobulin	Percentage of IgG	Severity	Diseases	Study
ABO	IgG	20-40%	Mild	Jaundice Hyperbilirunaemia	(13)
Rh	IgG	Anti-E: 36.3% Anti-D: 16% Anti-c: 6.4%	Severe	HDN Kernicterus Hydrop foetalis	(56)
Kell	IgG	29%	Moderate	HDN	(41)
Duffy	IgG	5.4%	Moderate	HDN	(35)
Kidd	IgG	1.5%	Mostly mild	Mild hemolytic disease	(35)
MN	IgM (occasionally) IgG)	0.01-0.7%	Mostly mild	HDN (rare)	(12)
Ss	IgG	3.9%	Mostly mild	Mild jaundice	(57)

HDN is usually caused by the production of maternal IgG passing through the placenta barrier, hence HDN caused by anti-M is rarely documented (58). Anti-M is known as a cold reacting antibody and predominantly IgM class, however, there are cases of IgG anti-M reacting at 37°C. Hence, a study reported that the HDN and HTR could be associated with IgG of anti-M that can be observed by agglutination of DAT and saline phase, demonstrating the presence of a strong immunogenic antigen on the RBC (59). Nevertheless, anti-M can cause alloimmunisation through three conditions: transfusion, pregnancy, and transplantation, which might stimulate the production of anti-M (IgG) despite being considered a rare event (60). Anti-M reported in 15% of the patients and possibilities to cause fetal anemia and intrauterine death (61). In addition, there are a few sporadic cases where the fetus or newborn could be asymptomatic to hydropic with severe HDN depending on the anti-M titre (62). Although anti-M is a low clinical significance antibody, there are occasionally cases of HDN regarding this antibody (59). Nevertheless, there are reports of immunogenic IgG anti-M that could be active at 37°C with the probability of causing HDN in the newborn.

Furthermore, cases related to anti-N are less common than anti-M. Anti-N is not clinically significant, and only a few studies have been reported to cause any haemolytic transfusion reaction or HDN (63). Anti-M alloimmunisation can be seen during delivery with severe late-onset anaemia due to haemolysis, whereas a combination with anti-MN related to HDN is rarely reported. Next, alloimmunisation of anti-M will target the red cell precursors and could lead to neonatal hypogenerative anaemia (62). Severe cases such as hydrops foetalis require frequent transfusion or immunoglobulin (59). Based on the literature, it has been concluded that anti-M that is caused by IgG alloimmunisation can induce severe HDN with anaemia, following the suppression of erythropoiesis (64). Additionally, the suppression of RBCs could be the main reason for foetal anaemia and the alloimmunisation might be responsible for foetal death.

Lastly, anti-S antibodies, an IgG class, are usually developed after sensitisation of RBCs in antigen-negative mothers carrying S antigen-positive foetus. Research has demonstrated that anti-Ss is a rare cause of HDN (65), however, this alloimmunisation could lead to mild jaundice. Thus, antigen-negative blood should be used for transfusion to prevent the production of alloantibodies in future pregnancies (63)

4.0 CLINICAL MANAGEMENT

The most common treatment of HDN during pregnancy is Intrauterine Blood Transfusion(IUT) n of RBCs into the foetal circulation. Foetal condition can be determined by using a Doppler ultrasound scan to estimate middle cerebral artery peak systolic velocity and to examine foetal growth.

Consequently, the outcome of foetal blood sampling shows varies haemolysis of RBC, hemoglobinopathies, alloimmunization in severe anaemia cases. Thus, procedure of IUT recommended to be done to prevent hydrops fetalis in order to allow the pregnancy continue and delivery to be at least 36 weeks of gestation (66). This is achieved by using a needle to transfer the RBCs through the mother's uterus, which is directly passed into the foetus's abdominal cavity. Next, when the lungs are matured, early delivery of the foetus is induced to prevent the foetus from developing any complications (67). This technique known as most successful for foetal anaemia due to irregular antibodies. The survival rate of hydropic fetuses with IUT reached almost 80.5-93.5% (68,69). Even IUT considered as a safe method to manage severe foetal anaemia however it also could lead to complication such as foetal distress, emergency delivery with the risk of preterm labour and may result foetal death. It is also shown that 0.9 to 4.9% foetal loss per procedure (68). Furthermore, neonates requiring phototherapy sessions were considered to have severe hyperbilirubinemia and need exchange transfusion procedures (27). Based on an earlier report (70), 60% of all newborns will go through phototherapy within 24 hours after birth to maintain their total serum bilirubin (TSB) levels (3). Next, intensive phototherapy is performed by exchange transfusion that could prevent kernicterus. This procedure decreases the circulating bilirubin level by replacing antibody-coated RBCs with antigen-negative RBCs (71). Exchange transfusion should also be undertaken immediately when the neonate shows signs of acute bilirubin and encephalopathy, including hypertonia, arching, retrocollis, opisthotonos and high pitch crying (3). Besides, the diagnosis of hydrops foetalis demonstrates a sign of anaemia with reticulocytosis or hyperbilirubinaemia that requires emergency phototherapy or even exchange transfusion (4). The purpose of exchange transfusion is to remove unwanted toxic bilirubin and provide additional albumin to be bonded with the bilirubin (72). Technology has been improved, hence, the plasmapheresis method is considered an alternative therapy. The Plasmapheresis method eliminates IgG and the development of red cell alloimmunisation in the maternal circulation, thereby resulting in the reduction of the number of alloantibodies passing through the foetus. Therefore, the survival rate of severe HDN with the plasmapheresis method is about 75% (38). The types of diagnosis in HDN based on laboratory management shown in Table 2. Based on a previous report (7), despite several preventions and treatments procedures for sensitisation of alloimmunisation in HDN, precaution is still required, as well as the assessment by gynaecologists and obstetricians.

4.1 Screening Program

Regular check-ups must be carried out to detect alloimmunisation during pregnancy. Moreover, pregnant women should follow routine antenatal anti-D prophylaxis(RAADP) at 28 weeks of gestation.

In addition, Rhogam (Rhlg), which is commonly obtained from the human plasma, should be given within 72 hours after delivery of an Rh (D) positive foetus. The function of RhoGAM is to prevent alloimmunisation and is effective when an adequate dosage is administered (36). Previously, it was shown that the incidence of Rh (D) alloimmunisation reduced after the administration of Rhlg (15). Based on earlier reports (4), the usage of immunoprophylaxis with anti-D immunoglobulin during the antenatal and postnatal phase reduced the incidence of Rh(D) alloimmunisation from 14% to 2% worldwide. Pregnant women with Rh system antibodies or anti-K must monitor antibody concentration titre during their second trimesters (44). In Netherlands, the Rh(D) negative pregnant women offered with initial antibody screening cell-free foetal DNA testing at 9 weeks gestation, to test RBC(Rh phenotype), currently this screening have been used in United States and has shown to be positive feedbacks in all three trimester of pregnancy(20). Therefore cell-free DNA screening for these Rh phenotypes of foetal are not a compulsory examination in Malaysia.

5.0 DIAGNOSIS

Table 2 Diagnosis HDN based on laboratory management (73, 74)

During pregnancy	After birth
<ul style="list-style-type: none"> ○ Ultrasound-to identify organ enlargement ○ Amniocentesis- to estimate the amount of bilirubin in the amniotic fluid and to determine the chromosomal and genetic disorders in neonates 	<ul style="list-style-type: none"> ○ Neonates sample shows severe hyperbilirunaemia ○ Formation of alloantibodies in maternal surface
<ul style="list-style-type: none"> ○ Sample of the foetal umbilical cord to examine antibodies, bilirubin level and anaemia 	<ul style="list-style-type: none"> ○ Positive Direct Coombs Test (DAT) in neonatal samples
	<ul style="list-style-type: none"> ○ The presence of haemolysis on blood film findings ○ Foetal hydrops (fluid presents completely in body's tissues including lung, heart, and abdominal cavity)

6.0 LABORATORY MANAGEMENT

According to the British Committee for Standard in Haematology guideline, it is recommended that pregnant women at 12-16 weeks of gestation should get their blood grouping typing (ABO and Rh) and

repeat the antibody screening test, to avoid late development of alloantibodies. Similarly in Malaysia, based on Ministry of Health Malaysia guidelines, pregnant women need to be ABO and Rh grouping checked before 12 weeks gestation, and in case negative the father need to be checked for both ABO and Rh grouping as well. Indirect Coombs test is needed to detect any prior sensitization in negative Rh mother (75). In addition, determining the father's phenotype helps to assume the likelihood of a foetus carrying compatible red cell antigen. Besides, the foetus genotype can be confirmed by performing the polymerase chain reaction method of foetal DNA in the maternal circulation. This method is highly recommended for detecting the Rh system and Kell antibodies in pregnancies. Alloimmunisation in pregnancy is predicted by an increased antibody titre that has the potential to be diagnosed as HDN or hydrops foetalis (76). Therefore, a cord blood sample of Rh(D) negative mother of a child should be sent to the laboratory in order to determine the baby's ABO and Rh group. If the baby's grouping cannot be determined or unclear, it should be assumed to be RhD positive for the Anti-D Ig administered. Therefore, a direct antiglobulin test (DAT) should be performed if the diagnosis of HDN is uncertain (44).

7.0 CONCLUSION

Conclusively, the presence of various alloantibodies in haemolytic disease of the newborn (HDN) could significantly affect the foetal outcome. Therefore, alloantibodies should be screened to prevent alloimmunisation in pregnancy. Hence, the awareness of significant HDFN with the presence of multiple alloantibodies should be helpful to the clinician and management of the patient.

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