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ANTI-D ANTIBODY ISOLATION AND IDENTIFICATION FROM FETUSES AND NEONATES WITH HEMOLYTIC DISEASE IN THE ERBIL CITY

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Yüksek Lisans Tezi

ERBİL ŞEHRİNDE HEMOLİTİK HASTALIKLI FETÜS VE YENİDOĞANLARDAN ANTİ-D ANTİKORU İZOLASYONU VE TANIMLANMASI

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Rhesus D (Rh-D) antijen alloimmünizasyonu hematologlar ve doğum uzmanları için bir endişe odağı olmaktadır. Bu durum perinatal morbidite ve mortaliteye katkıda yol açmaktadır. Çalışmada Erbil ilinde Anti-D antikoru üreten Rh(D) negatif annelere yönelik coğrafi veri profilinin tasarlanması amaçlanmıştır. Çalışmamızda Rh(D) negatif Anne ve Rh(D) pozitif Babalardan 1028 (kan örneği) toplandı. Tüm numunelere (Baba ve Anne) ABO ve RhD tipleme testleri uygulandı, ardından anne numunelerinde Du testi gerçekleştirildi ve anne numunelerine antikor taraması yapıldı. Tüm annelerden alınan kan örnekleri yaş gruplarına göre 20-25: 35 (%7), 26-30: 165 (%32), 31-35: 191 (%37), 36-40: 112 (%22 ve 40 yaş üstü: 11 (%2) katagorilerine ayrıldı. Rh negatif annelerde en sık görülen kan grubu O grubu (%42,4) olurken, bunu sırasıyla %27,6, %26,7 ve %3,7 ile A, B ve AB grupları izledi. Anti-D pozitif antikor yüzdesi en yüksek % 44 ile 36-40 yaş kategorisinde görülürken, bunu %22 ile hem (26-30) hem de (31-35) yaş kategorisi ve %11 ile 40 yaş üstü kategorisinde izledi. 20-25 yaş kategorisinde ise pozitif vakaya rastlanmadı. ABO uyumsuzluğunun ve diğer alloantikorların neden olduğu hemolitik hastalık, Fetüs ve yenidoğanın hemolitik hastalığının ana nedeni haline gelmektedir. Yeni doğanlar üzerindeki olumsuz etkileri önlemek ve antijen negatif kan teminin acilen gerçekleştirilmesi için bu verileri sağlanması önem taşımaktadır.

Anahtar Kelimeler: Hemolitik hastalık, Antikor tanımlama, Anti-D antikoru, Du testi, Anti-D titresi.

ABSTRACT

MSc Thesis

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Rhesus D (Rh**-**D) antigen alloimmunization has been a focus of concern for hematologists and obstetricians. It contributes to perinatal morbidity and mortality. In this study, it was aimed to design a geographic data profile for mothers who are Rh(D) negative and produced Anti-D antibody at Erbil-city. In our study, we collected 1028 (blood samples) from Mothers who is Rh(D) negative and Fathers who are Rh(D) positive. We performed ABO and RhD typing tests for all samples (Fathers and Mothers), then tested all mother samples for Du test and performed antibody screening for all mothers sample. All the mothers' blood samples have been divided into five different Age category 20-25: 35 (7%), 26-30: 165 (32%), 31-35: 191 (37%), 36-40: 112 (22%), and over 40: 11 (2%), Age groups. The most common blood type among Rh-negative mothers was Group O which was (42.4%), followed by A, B and AB groups which were 27.6%, 26.7% and 3.7%, respectively. The highest percentage of Anti-D positive antibody was in (36-40) age category which was 44%, followed by 22% in both (26-30) and (31-35) and 11% in (over 40) Age category, and there was not any positive case inside (20-25) Age category. Haemolytic illness brought on by ABO incompatibility and other alloantibodies have thus become the main cause of hemolytic disease of the fetus and newborn. It is important to provide this data to prevent negative effects on newborns and to provide antigen-negative blood urgently.

Key Word: Hemolytic disease, ABO, Anti-D antibody, Du test.

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SYMBOLS and ABBREVIATIONS

%	Percent
°C	Centigrade
ABO	Blood Group System
AHG	Anti-Human Globulin
cDNA	Clone Complementary DNA
FMH	Fetal Maternal-Hemorrhage
HDN	Hemolytic Disease of the Newborn
HTR	Haemolytic Transfusion Reaction
IgG	Immunoglobulin G
IgM	Immunoglobulin M
µl	Microliter
mm	Milliliter
RBC	Red Blood Cell
RhD	Rhesus D antigen
mg	Milligram

1. INTRODUCTION

Erythroblastosis fetalis, often known as hemolytic disease of the newborn (HDN), is a hemolytic illness that mostly affects fetuses with rhesus positive blood and newborns with rhesus negative mothers. Due to rhesus or ABO incompatibility between the maternal and fetal blood, alloimmunization occurs, which causes maternal antibodies to assault fetal red blood cells. Prior to the development of immunoprophylactic medications, HDN was previously known to result in fetal mortality in 1% of all pregnancies, but if detected early, the disease may now be reasonably successfully controlled with fewer difficulties (Pegoraro et al., 2020: Myle and Al-Khattabi, 2021).

HDN was a significant contributor to prenatal and neonatal morbidity and death in the middle of the 20th century (Liumbruno et al., 2010; Pegoraro et al., 2020).

When a baby's red blood cells are damaged, HDN can happen (Chilcott et al., 2002). When HDN is present, it can cause severe newborn hyperbilirubinemia, fetal anemia, and kernicterus, all of which have elevated risks for fetal mortality (De Haas et al., 2014; Tugcu et al., 2019).

Clinical and laboratory findings associated with hemolysis caused by minor blood incompatibilities include moderate anemia, reticulocytosis, newborn hyperbilirubinemia, and significant fetal anemia and hydropic alterations (Eder, 2006). Since it has been demonstrated that intravenous immunoglobulin reduces the requirement for red blood cell transfusion, it has been employed as an alternate therapy strategy for HDN (Alcock and Liley, 2002).

Maternal antibodies, which are circulating proteins made by the immune system in reaction to the presence of a foreign material, are created against fetal blood cells in alloimmune HDN. Fetal anemia (a lack of red blood cells in the unborn fetus) is caused

by these antibodies, which cross the placenta to destroy red blood cells (Zwiers et al., 2018).

33 blood group systems have about 400 red blood cell antigens (Mbalibulha et al., 2015). Of these, more than 50 distinct antigens can result in fetal hemolytic illness and maternal alloimmunization. Because Rh hemolytic illness can develop, Rhesus D antigen (RhD) alloimmunization has long been of interest to obstetricians and hematologists. From one race to another, RhD negative is more or less common. RhD negativity affects 15% of Caucasians, compared to 5-8% of African Americans, 1-2% of Asians, and 1% of Native Americans. Nearly all native Africans are RhD positive (Aventand Reid, 2000; Al-Dughaishi et al., 2016).

When the first child inherits the paternal D antigen, whose inheritance has been shown to follow an autosomal dominant pattern, and an event occurs that results in mixing of maternal and fetal blood, the mother typically starts producing anti-D antibodies through a process known as alloimmunization. This is because she lacks the D antigen (Gupta et al., 2020).

IgM antibodies, which cannot pass the placental barrier, is the first antibody secreted immunologically. Isotype switching subsequently occurs, resulting in the production of IgG antibodies. IgG antibodies may breach the placental barrier, and they do so during the second and/or subsequent pregnancies. They assault the fetal RBCs, resulting in hemolysis and its accompanying consequences, such as Hydrops fetalis and jaundice (Alaqeel, 2019). Antibodies known as IgG, however, can also reach the fetal circulation through fetomaternal bleeding (Myle and Al-Khattabi, 2021).

Morbidity and mortality due to Rh(D)-related HDN in several countries substantially decreased from 12–13 to 1-2% after successful adoption of prophylaxis, changes in birth order, and improved medical care standards (Velati, C., 2007; Varghese et al., 2013). Other abnormal antibodies that were discovered to cause HDN,

primarily anti-c, anti-E, and antibodies to antigens of Kell, Kidd, Duffy, and MNS blood group systems, also rose to prominence at the same time (Moise, 2000).

In this study, it was aimed to design a geographic data profile for mothers who are Rh(D) negative and produced Anti-D antibody at Erbil-city. Studying and detecting of factors or defects in the management of Mothers who are Rh(D) negative. Finding the solution of factors or defects to prevent producing Anti-D from Mothers immune system. To make a standard procedure, and finding the best way for managing pregnant women during gestational period, by using Rh0 (D) immunoglobulin at right time, and performing Du test for all New-borns.

2. LITERATURE REVIEW

2.1. Definition and history of Rh hemolytic disease

Erythroblastosis fetalis, another name for HDFN, is a hemolytic condition that typically affects neonates and fetuses with the Rhesus positive (Rh+) and Rhesus negative (Rh-) racial or ethnic markers. The condition was initially recorded by a French midwife in 1609, but the fundamental reason wasn't discovered until the 1950s (Seto, 2008 ; Jackson and Baker, 2021).

According to estimates, 3 to 8 people out of every 100,000 suffer with HDN each year. Prior to the creation of anti-D prophylaxis, it caused fetal loss in 1% of all deliveries (Routray et al., 2021).

Although the incidence of HDN is shown to vary with ethnicity, it is closely connected with the female inheritance pattern that leads in the lack of the Rhesus (D) antigen. Table 2.1 shows that, for instance, whites have the highest incidence while Asians and American Indians have the lowest. Additionally, the D antigen is the most immunogenic Rh antigen that is currently recognized. Rh incompatibility affects roughly 10% of pregnant white women, according to estimates (Myle and Al-Khattabi, 2021).

Table 2. 1. Various ethnic groups' HDN prevalence rates

Ethnicity	Prevalence
Africans	4%
African-Americans	8%
Whites	15–16%
Eurasians	2.4%
Asians	<1%
Basque (Spain/France)	30–35%

2.1.1. Clinical manifestation

Maternal alloantibodies cause HDFN, which is the immune-mediated death of fetal RBCs and their erythroid progenitors. Clinical HDFN signs and symptoms include:

- fetal anaemia, including low haemoglobin levels
- hyperbilirubinemia (high bilirubin levels) which may lead to jaundice or kernicterus as bilirubin is released from lysed RBCs
- cardiomegaly (enlarged heart) from the compensatory hyperdynamic circulation
- fetal hydrops when oedema accumulates in the serous cavities and skin The presence and specificity of maternal alloantibodies implicated in HDFN can be identified using RBC serology (Daniels et al., 2002; De Haas et al., 2015).

2.2. Characteristics of Rh group

2.2.1. History

In clinical practice, understanding blood group systems is crucial, particularly for haematological illnesses. The ABO and Rhesus systems are the two most common blood group systems in humans. The human blood types were discovered by Karl Landsteiner in 1904. He described them using the Landsteiner law, which asserts that the matching antibodies for each blood type antigen that is not present on the RBCs are present in the plasma (Cowan, 2017). With the rhesus antigen**-**antigen D, this is not evident. Normally, neither Rh+ nor Rh- persons have anti-D antibodies; nevertheless, when Rh- individuals are exposed to the D antigen, they begin secreting the appropriate antibodies. Therefore, RBC agglutination and hemolysis, which are the root causes of Rh incompatibility, can occur when a person has both D antigens and anti-D antibodies (Akkök and Seghatchian, 2018).

The similar pattern is followed by ABO incompatibility, hence caution must be used while doing tissue transplants and blood transfusions. When alloimmunization takes place and maternal antibodies begin attacking fetal RBCs, the underlying cause of virtually all cases of HDN is often Rh or ABO incompatibility between the mother and the fetus. When IgG antibodies penetrate the placenta after isotype switching and enter the fetal circulation, or through Foeto-maternal haemorrhage (FMH), maternal antibodies reach the fetus (Gupta et al., 2020; Wang et al., 2021).

2.2.2. Genetic and Structural Features

The Rh blood group system, which has at least 45 distinct antigens and is the most polymorphic of the human blood types, is second only to ABO in terms of clinical importance in transfusion therapy. Understanding the molecular underpinnings of some of the Rh antigens has been made possible by the ability to clone complementary DNA (cDNA) and sequence the genes encoding the Rh proteins. An important source of suitable blood samples for molecular studies is the serologic identification of polymorphic blood group antigens and phenotypes (Avent and Reid, 2000).

Over 40 quite antigenic antigens are part of the Rh blood group system. It ranks exactly near to the ABO system in terms of immunological hematological relevance. It is a member of the family of trans-membrane proteins. It basically defines three Rh epitopes: the Rho or D antigen, which can be present or absent and indicates either a Rh positive (D+) or Rh negative (D- or d) phenotype.

The RHD and RHCE genes, which are located close to the Rh locus on chromosome 1 (1p34.3-p36.13), are responsible for producing the Rh protein. RHD and RHCE are inherited from both parents by D-positive people, whereas RHCE is inherited from one parent by D-negative people. The RHCE gene is used in a generic sense to identify the Rh genes that are unable to create an antigen D (Velkova, 2015).

RHCE, RHCe, RHcE, and RHce are four frequent genes that create various combinations of C, E, c, and e. In addition to the Rh genes, chromosome 6 (6p11-p21.1) also contains a gene called RHAG (Rh-associated glycoprotein), which is linked to the Rh protein at the level of the red blood cell membrane (Ridgwellet al., 1992).

The Rh phenotype of the examined red blood cells can be serologically determined by taking into account the available Rh antiserums (anti-D, anti-C, anti-c, anti-E, anti-e). As a result, the most likely Rh genotype may be determined using the phenotypic and gen frequency of the examined population.

The greatest method for determining the proper phenotype is still family research. An immunological response will almost certainly be triggered if D-negative adults are exposed to D-positive red blood cells through transfusion or pregnancy. About 80% of unintentionally D-negative patients who get a transfusion of 250 ml of D positive red blood cells produce anti-D (Mollisonet al., 1997). In 50% of the D-negative receivers, a much smaller volume of red blood cells (about 1ml) will also promote the formation of anti-D. IgG, often IgG1, IgG3, or a mix of these two subclasses, makes up the majority of Rh antibodies. Pregnancy or transfusion are the two main causes of the development of Rh antibodies (Velkova, 2015).

2.3. ABO antigens

Red blood cell antigens are divided into 38 blood group systems, totaling more than 340 RBC antigens, according to the International Society of Blood Transfusion. Of these, ABO, Rh, Kell, Duffy, Kidd, Lutheran, and MNS are clinically the most significant for transfusion, pregnancy, and transplantation.

Different antigen expressions in ABO subgroups, such as A1 A2, A3, Ax, A End, A M, Ael, B3, Bx, Bm, Bel, AxB, 1Bx, AmB, A1Bm, AelB, A1Bel, cis-A2B3, cisA2B, and cisA1B3, distinguish them from one another. Other ABO subgroups are

extremely uncommon and have very poor A or B antigen expression, with the exception of A1, A2, A1B, and A2B. If irregular natural anti-A1 antibodies, which are reactive at 37 °C, are present, they can be clinically significant. If so, an RBC with the A2 phenotype must be made available for transfusion. White people are more likely to belong to subgroups, whereas Japanese people are more likely to belong to B subgroups (Hosoi, 2008).

Destructions or inactivating mutations in the genes encoding the Rh, Kell, and Kx blood group systems have resulted in the occurrence of unusual abnormalities (Rh-null, McLeod). The absence of the Kx antigen is linked to stomatocytosis, acanthocytosis, and hemolytic anemia. The Kx gene encodes the RBC membrane structural proteins (Wagneret al., 2005).

The blood group antigens for the ABO, Rh, and Kell blood types are the most important clinically. Natural anti-A and anti-B antibodies that are specific to the ABO system are what cause severe hemolytic transfusion responses when ABO incompatible blood is transfused. However, Kell (K, k) and Rh (D, E, C, c, e) antigens are immunogenic and are the most common cause of RBC alloimmunization linked to pregnancy and RBC transfusion. RBC alloimmunization occurs between 1 to 10% of the time generally, but in select groups of individuals who have received many transfusions, it can reach 60% (Ristovska et al., 2022).

The majority of alloimmunizations and subsequent hemolytic transfusion responses are prevented by RBC compatibility in the ABO, Rh, and Kell systems; these reactions have a frequency of 1:4000–12000 blood transfusions (Klein and Anstee, 2005).

Blood group antigens from the ABO, Rh, and Kell blood group systems are more or less common in people with diverse ethnic ancestries. Low-frequency antigens are those with an incidence of less than 1%, and high-frequency antigens are those with a frequency of greater than 90% (Daniels et al., 2004). The following criteria define a "rare blood group": lack of a high-frequency antigen in the general population (Vel-,

k-, or Lub-), lack of multiple frequent antigens within a single blood group system (D-c- or D+C+E+c-e-), or lack of multiple frequent antigens within different blood systems (e-, k-, Fyb-, Jka-, or s-) (Ristovska et al., 2022). Out of 340 blood group antigens, 160 have high frequency, such as the Cellano (k) antigen, which has a 99.8% frequency (Dean and Dean, 2005). Given the lack of unusual blood types in the donor registry, it is particularly challenging to furnish homozygous (K+k) red blood cells for patients with anti-k antibodies.

More than 70.000 registered blood donors with their determined ABO, Rh (D, C, c, E, e), and Kell (K, k) antigens are listed in the information system of the Institute for Transfusion Medicine in Skopje. Since 60% of the discovered antibodies are directed against the aforementioned antigens, most alloimmunized individuals may be treated with this blood type technique, table 2.2. (Makarovska-Bojadzieva et al., 2009).

Table 2. 2. Blood group systems (Mitra et al., 2014)

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

2.3.1. H-antigen

The ABO blood group antigens' predecessor is the H-antigen. It exists in all RBCs, regardless of the ABO system. Homozygous for the H gene (HH), those with the uncommon Bombay phenotype do not express H-antigen on their RBCs. Since H-antigen serves as a precursor for antigens A and B, its lack signifies their absence.

However, the people create isoantibodies to antigens A and B as well as H-antigen (Rahorst et al,.2019).

2.3.2. Rhesus system

After ABO, the rhesus-system is the most significant blood group system (Westhoff, 2004). Currently, only five of the 50 known blood group antigens that make up the Rh-system are significant. Rh factor and immunogenic D-antigen may or may not be present on a person's RBC surface. Either Rh-positive (D-antigen present) or Rh-negative (D-antigen lacking) status is therefore indicated. In contrast to the ABO system, people with D-negative RBCs often do not have anti-Rh antibodies in their blood until their circulatory systems have been exposed to D-positive RBCs. Because they are IgG in origin, these immunological antibodies can cross the placenta. When pregnant Rh-negative women who have given birth to Rh-positive children get anti-D Ig prophylaxis against the Rh immunization (Shahata et al., 2012).

2.3.4. Kell system

These erythrocyte antigens, which are distinguished by the immunological antibody anti-K, are the third most effective immunogenic antigens after ABO and Rh system. It was initially discovered in Mrs. Kellacher's serum. She had hemolytic responses after reacting to her newborn baby's erythrocytes. 25 Kell antigens have now been identified. Serious HDFN and hemolytic transfusion reactions (HTR) are brought on by anti-K antibodies (Kumariet al., 2018).

2.3.5. Duffy system

The first person with Duffy-antigen was a patient named Duffy who had hemophilia. It is located on the surface of RBCs and is also referred to as Fy glycoprotein. It functions as a non-specific receptor for a number of chemokines and

as a receptor for the Plamodium vivax human malaria parasite. The Duffy glycoprotein antigens Fya and Fyb can produce one of four phenotypes: Fy (a+b), Fy (a+b+), Fy (ab+), or Fy (ab). The IgG subtype antibodies can result in HTR (Langhi and Orlando Bordin, 2006).

2.4. RBC serology

As the maternal antibodies which cross the placenta are IgG in structure, the anti-human globulin (AHG) reagent (also known as the Coombs reagent) is required to observe RBC agglutination caused by the IgG antibodies in the maternal sera (McGowan, 2021) With the AHG reagent, the antiglobulin test can be performed for a case of HDFN as below:

1) Direct antiglobulin test (DAT)

• Detects sensitisation when RBC agglutination is observed upon addition of AHG reagent to umbilical cord RBCs (Dinesh, 2005; McGowan, 2021).

2) Indirect antiglobulin test (IAT; also known as the "Coombs test")

• Detect if the maternal sera reacts with paternal RBCs

• Identify specificity of the maternal alloantibody

• Identify the phenotype of fetal, maternal and paternal RBCs when using monoclonal IgG typing reagent (Hadley, A. and Soothill, 2002).

• Identify compatible RBC units for transfusion in a "crossmatch", where the patient (e.g fetal or maternal) sera is tested against donor RBCs.

In the event where RBC serology cannot identify the specificity of the maternal alloantibody, blood group genotyping can be performed to identify genetic differences between the mother, father and child to help resolve the specificity of the maternal alloantibodies.

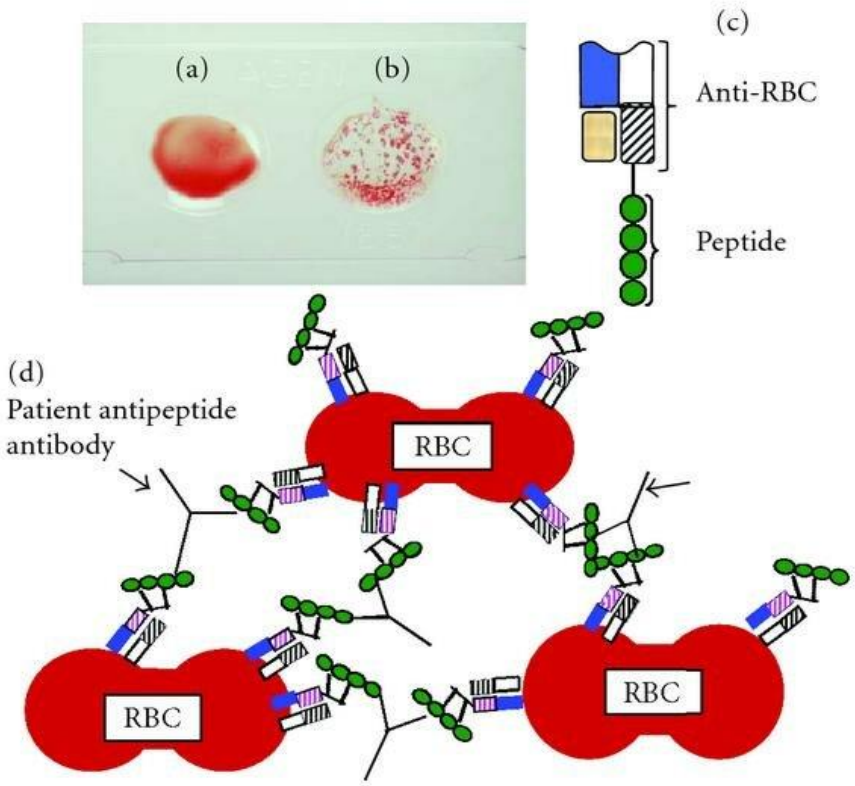

Figure 2. 1.Autologous agglutination assay (Hobson-Peters, 2012).

2.5. Blood group genotyping

In complex serological investigations, blood group gene sequencing offers the advantage of identifying feto-maternal blood group incompatibles from the DNA sequence (McBeanet al., 2015; McBean et al., 2016). Blood group incompatibilities are identified in an inheritance model when there is a segregation of the antigenpositive phenotype with a gene variant. This approach has led to the classification of the 'orphan' SARA antigen into the MNS blood group system. It has also provided further evidence for a HDFN case with a maternal antibody against the Ata antigen in the Augustine blood group system (McBeanet al., 2016).

The Ata antigen (AUG2) is a high-frequency antigen that occurs in more than 99% of the general population, which makes compatible units difficult to find for those with anti-Ata antibodies (Daniels et al., 2015; Storry et al., 2019).

2.6. Clinically significant antibodies

Human antibodies to blood group antigens can be IgM or IgG in structure. The antibodies, which have been reported to cause adverse events, are referred to as "clinically significant" and are associated with their specificity. The most clinically significant antibodies remain to be those against antigens in the ABO blood group system (Poole and Daniels, 2007). This is due to the universal presence of naturally occurring IgM antibodies in plasma, possibly due to an exposure to environmental factors (Poole, J. and Daniels, 2007; Daniels, 2008

In ABO incompatibility, the immediate haemolytic transfusion reaction (HTR) which follows can be fatal and adverse reactions include chills and haemoglobinuria 21. In contrast, the antibodies against antigens of the Lewis blood group system (e.g anti-Lea , anti-Leb and anti-Leab), which are IgM, are generally considered non-clinically significant as they are usually not haemolytic and are non-reactive when tested at 37°C (Velliquette and Westhoff, 2019).

Blood group IgG antibodies are produced as part of the adaptive immune system response, known as alloimmunisation21. After the initial exposure to the foreign blood group antigen, the IgG antibodies are then present in the plasma. Re-exposure to the foreign blood group antigen from a transfusion can result in an immediate (within a few hours) or delayed extravascular HTR (~2 to 10 days). The symptoms of extravascular HTR are similar to intravascular HTRs but are less severe, including fever, chills, jaundice and, to a less common extent, renal failure (McGowan, 2021).

2.6.1. Anti-D antibodies

The RhD blood group antigen in the Rh blood group system is routinely typed alongside ABO for patients and blood donors in many countries, highlighting the importance of preventing RhD immunisation by providing RhD compatible units or appropriate antenatal care (Malomgré and Neumeister, 2009).

The lack of the RhD protein in the RBC membrane results in the RhD-negative phenotype (Daniels, 2008). It is most commonly encoded by a homozygous RHD gene deletion in Caucasian populations but may also be a result of an inactive RHD gene or RHD gene variants (Adeyemi, A.S. and Bello-Ajao, H.T., 2016; McGowan et al., 2017).

The frequency of the RhD-negative phenotype is the highest in Caucasian populations at 15-17% (Westhoff, 2007). Of the antibodies which have been reported to cause HDFN, those recognising the RhD blood group antigen are among the most common to cause moderate to severe HDFN in Caucasian populations (Daniels et al., 2002). In contrast, the RhD-negative phenotype occurs in African populations at 3-5% (Omotade et al., 1999; Adeyemi and Bello-Ajao, 2016) , in an Indian population at 5%61 and are rare in East Asian populations at 3% or less (Liu et al., 2017). Although the frequency of the RhD-negative phenotype in these populations are lower than in Caucasian populations, anti-D antibodies have been reported to be among the most common antibodies implicated in HDFN in East/South East Asian , Indian and African patient groups (McGowanm, 2021).

Currently, the detection of clinically significant anti-D antibodies is considered of high importance to patient safety and is included in various guidelines, particularly for Caucasian patient populations (McGowan, 2021). Other clinically significant antibodies, such as those against low-frequency antigens (LFA), in a small percentage of pregnant women are not detected during routine testing and this poses risks of HDFN (Coghlan, 2009; Milkins et al., 2013).

2.7. Du test

Du is the D antigen's inadequate expression. Because some of these cells agglutinate after being added to antiglobulin sera, it is difficult to classify cells that are not initially agglutinated by anti-D sera as D negative. Du is the name given to this subpar reaction. If the recipient has already received an immunization, the Du cells may be killed. Du positive cells are likely to trigger an immunological response in D negative people. As a result, a receiver who tests negative for Du is considered positive for D.

2.7.1. Du Antigen:

When exposed to the D antigen during pregnancy or through blood transfusion, a significant fraction of persons whose RBCs lack D will produce anti-D. As a result, D- RBCs should be transfused to all D- patients, especially girls and women who may become pregnant. The Rh blood group system, the most polymorphic blood group system with 49 different antigens, contains the D antigen (Afshan, 2013).

A Du phenotype may result from one of three possible genetic circumstances.

a) An individual could inherit a gene that causes diminished quantitative expression of the D antigen.

b) The expression of the D antigen may change and become less strong as a result of gene interactions.

c) The whole composition of the antigen may not be encoded by a gene (Tayyab et al., 2000; Afshan, 2013).

2.8. Haemolytic disease of the foetus and newborn due to maternal-foetal ABO incompatibility

In fact, in 15-20% of pregnancies in the white population, there is incompatibility between a group O mother and a group A or B child; in 10% of these

pregnancies, HDFN develops as a result of destruction of the foetal red blood cells, caused by IgG class anti-A and/or anti-B antibodies in the maternal serum. HDFN is currently the most common neonatal hemolytic disease in the western world. A group O mother and a group A neonate are the mother-child serological pairs in which a clinically meaningful ABO HDFN develops most easily. However, the haemolytic condition only needs transfusion assistance in 1.5–2% of cases (Strauss, 2010; Basu et al., 2011).

The prevalent moderate clinical manifestation of HDFN caused by ABO incompatibility can be attributed to a number of factors, including:

- Low levels of A and B antigen expression are seen on neonatal and fetal red blood cells;
- Some of the maternal IgG that crosses the placenta is absorbed by the A and B substances, which are always present on endothelium and epithelial cells, including placental ones;
- Most anti-A and anti-B IgG are IgG2, a subtype of Ig with a lower ability to actively pass the placental barrier (Drabik-Clary et al., 2006; Dajak et al., 2011).

Anti-A and anti-B IgG testing during pregnancy is not very helpful in predicting the occurrence of ABO HDFN in the foetus. In actuality, the majority of pregnant women, especially those with group O blood, have anti-A and anti-B (and anti-A,B) IgG in their serum, but only a small percentage of newborns suffer from hemolytic illness, particularly clinically significant types (Bennardello et al., 2015).

2.8.1. Dose of anti-D immunoglobulin

The anti-D Ig dosage should correspond to the Fetal Maternal-Hemorrhage (FMH) dosage. When the FMH does not exceed 4 mL of foetal red blood cells (99% of FMH), a dosage of 625 IU (125 μg) is generally thought to be adequate to avoid active immunisation.

- A dosage of 625 IU (125 μg) of Ig* is deemed enough for prophylaxis following potentially immunizing events occurring up to 19^{+6} weeks of gestation (Warland, 2007; Bennardello et al., 2015).
- A minimum dosage of 625 IU (125 μg) of anti-D Ig should be delivered for all potentially immunizing events occurring after 20^{+0} weeks of pregnancy and after the delivery of a RhD-positive newborn, followed by an assessment of the FMH.
- A second dosage of anti-D Ig (125 IU per milliliter of RhD positive fetal red blood cells) must be delivered if measurement of FMH reveals that the amount of bleeding exceeds that covered by the dose (Bennardello et al., 2015).

The maximum dose that can be given in a 24-hour period is 10.000 IU (2.000 μg). Any extra doses must be given at intervals of 12 hours, with the maximum intravenous individual dosage not to exceed 4.500 IU (900 μg). This recommendation is based on the recognized risk of hemolysis linked to the intravenous anti-D Ig dosage used in regimens for treating idiopathic thrombocytopenic purpura that is RhD positive (Xie et al., 2020).

3. MATERIALS and METHODS

3.1. Materials

3.1.1. Materials used in this study

1. EDTA tube 2-5 ml.
2. Anti-A monoclonal, Anti-B monoclonal and Anti-D blend (IgG+IgM) sera reagents (Biorex, UK).
3. Pasteur pipettes.
4. Test tubes (12 X 75 mm).
5. Centrifuge or Cell washer for Test tubes (12 X 75mm), (Helmer , USA)
6. Isotonic saline 0.9%.
7. Bovine albumin (22%), (Biorex, UK).
8. Incubator (Heat block).
9. Anti-human globulin (polyclonal) reagent.
10. Anti-IgG
11. Gel card RH phenotype.
12. Check Cells (IgG-coated RBCs).

Screening cells: come in sets of 3 vials, each vial (donor) has been phenotyped for at least 18 antigens of the vials: D, C, E, c, e, M, N, S, s, P_1, Le^a, Le^b, K, k, Jk^a, Jk^b, Fy^a, Fy^b), (Ortho, UK)

Panel cell come in sets of 11 vials (each vial has been phenotyped for a several antigen, different from other vials partially, Figure 3.1.

A B

Figure 3. 2. A. Anti-A monoclonal, Anti-B monoclonal and Anti-D blend (IgG+IgM) sera reagents B. Anti-human globulin (polyclonal) reagent.

3.2. Method

3.2.1. Sample collection

In this study, 1028 blood samples were collected from Mothers (Rh-negative) and Fathers (Rh-positive), from April 2022 to April 2023, from Privates and Governmental Hospitals in Erbil city in Iraq.

3.2.2. ABO Typing test RhD

Performing ABO typing test (Anti-A, Anti-B) and RhD for all samples (Mothers and Fathers).

3.2.3. Du Test.

Performing Du test only for mother blood samples, at first by adding (100 µl) of Anti-D sera, then adding (50 µl) of 5% suspension of RBC's , after that Mixing and incubating for 15-30 minutes at 37^{o}C, then Washing the RBC's four times with normal saline, and discarding of supernatant, then adding (100 µl) of AHG (Mix and centrifuge for 30 seconds at 2000 rpm) and Gently re-suspending the cells and examine for agglutination, finally recording the result according to agglutination.

3.2.4. Antibody Screening.

Performing antibody screening for all mothers, for each sample we were going to add (100 µl) of patient serum to each tube then add (50 µl) of appropriate screening cells to each tube, then Centrifuge, then gently resuspending the cell button and read for agglutination or hemolysis then Record results. It should be noted that this step is optional because most significant antibodies are IgG and do not cause agglutination of saline-suspended RBCs.

The next step was adding (100 µl) of enhancement reagent to each tube (may vary with enhancement reagent used), then Incubation at 37°C for 15 to 30 minutes, and then during the incubation, antibody in the patient serum bind to antigens on the reagent RBC, which is this is called the sensitization phase then Centrifuging and gently resuspend the cell button and read for agglutination or hemolysis and Record results.

The last phase was washing (4 times), then remove any excess of supernatant. Then Adding (100 µl) of AHG to each tube (polyspecific or anti-IgG) after that Centrifuge, then gently resuspend the cell button and read for agglutination or hemolysis. Tests that are macroscopically negative are usually checked for microscopic agglutination. Finally Record results

For checking the test, adding 50 µl of Coombs control cells (or "check cells") to all negative tests then Centrifuge and reading the agglutination and the test should be repeated if agglutination is not observed.

3.2.5. Antibody identification.

It was performed for all Positive Antibody Screening in order to identify types of antibody. The procedure is the same as antibody screening at (section 3.2.4).

3.2.6. Anti-D antibody titter test.

Performing titration for all samples that have Anti-D antibody.

3.2.7. Statistical analysis

All statistical analyses are carry out using SPSS Software, version 20 (SPSS Inc., Chicago, IL, USA). Correlation and regression done at level ($p < 0.05$).

4. RESULTS and DISCUSSION

4.1. Results

4.1.1. Sample collection

In this study, 1028 blood samples have been taken aseptically from 514 family and both parents (Rh-Negative Mother and Rh-positive father), alongside with their history of Delivery, Abortion and Blood transfusion times from both private and governmental hospitals in Erbil city, and then all the samples have been passed through the process of Blood group identification, Antibody screening and Antibody identification especially Anti-D checkup antibody.

4.1.2. Antibody identification and blood analysis according to Age groups.

In our study, all the mothers' blood samples (514) have been divided into five different Age category (20-25; 26-30; 31-35; 36-40 and over 40), in which the highest number was 191 (37%) in (31-35) age category followed by 165 (32%), 112 (22%), 35 (7%) and 11 (2%) for (26-30, 36-40, 20-25 and over 40) Age groups, respectively.

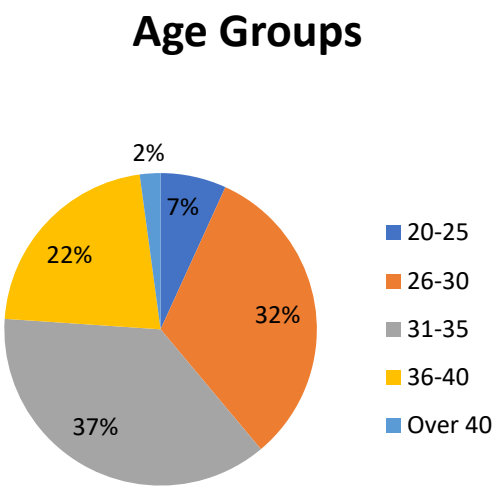

Figure 4. 1. Mother samples distribution according to different Age groups.

4.1.2.1. Antibody identification and blood analysis according to Ages of 20-25.

Out of 514 (100%) samples which have been tested, 35 (7%) of them were between the ages of (20-25). In regarding to delivery and abortion times, most of the mothers (65.7%) got birth to baby one time, followed by 31.4% of them two times and 2.9% of them four times. In contrast, only 22.8% of all the mothers in this age experienced baby abortion, in which 17.1% of them experienced one time abortion while 5.7% of them two times abortion.in term of blood transfusion, most of the mothers (82.9%) have not taken any blood transfusion while 5.7% of them have taken 1 time followed by 5.7%, 2.9% and 2.9% of them have taken blood transfusion 2,3 and 4 times, respectively. all of the data regarding to ages of (20-25) have been presented in table (4.1).

Table 4. 1. Blood analysis and patient history according to ages of 20-25.

AGES 20-25	MOTHER	FATHER	TIMES	Delivery	Abortion	Blood Transfusion		POSITIVE
AB	2.9	2.9	0	0.0	77.1	82.9	Ab+	0
A	28.6	48.6	1	65.7	17.1	5.7		
B	31.4	25.7	2	31.4	5.7	5.7		
O	37.1	22.9	3	0.0	0.0	2.9		
			4	2.9	0.0	2.,9		
			5	0.0	0.0	0.,0		
			6	0.0	0.0	0.0		
			7	0.0	0.0	0.0		

On the other hand, After blood group test have been done, the most common blood type among Rh-negative mothers was O which was (37.1%), followed by A, B and AB groups which was 28.6, 31.4 and 2.9%, respectively. in contrast, the most common blood type among Rh-positive fathers was Group A which was 48.6%, followed by B,O and AB groups with the percentage of 25.7, 22.9 and 2.9, respectively. The blood group percentages bar chart of those around (20-25) ages has been illustrated in Figure (4.2)

Eventually, after antibody identification and Anti-D checkup tests, as a result, none of the mothers who were Rh-negative was positive to Anti-D antibody.

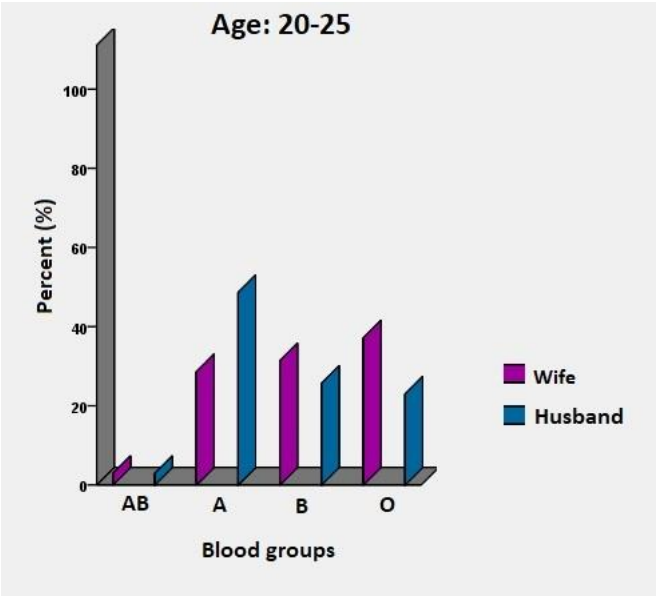

Figure 4. 2. Blood group profile of the parents around ages (20-25).

4.1.2.2. Antibody identification and blood analysis according to Ages of 26-30.

Out of 514 (100%) of Mother Samples which have been tested, 165 (32%) of them were between the ages of (26-30). In regarding to delivery and abortion times, most of the mothers (52.1%) got birth to baby two times, followed by 25.5% of them one time and 22.4% of them four times. In contrast, only approximately 33% of all the mothers in this age experienced baby abortion, in which 28% of them experienced one time abortion while 4.9 % of them two times abortion. In term of blood transfusion, most of the mothers (79.9%) have not taken any blood transfusion while 9.1% of them have taken 1 time followed by 4.9%, 2.4% and 2.4% of them have taken blood transfusion 2,3 and 4 times, respectively. all of the data regarding to ages of (26-30) have been presented in table (4.2).

Table 4. 2. Blood analysis and patient history according to ages of 26-30.

AGES 26-30	MOTHER	FATHER	TIMES	Delivery	Abortion	Blood Transfusion		POSITIVE
AB	4.2	0.6	0	0.0	66.5	79.9	Ab+	2
A	32.1	37.0	1	25.5	28.0	9.1		
B	27.9	38.2	2	52.1	4.9	4.9		
O	35.8	24.2	3	22.4	0.6	2.4		
			4	0.0	0.0	2.4		
			5	0.0	0.0	0.6		
			6	0.0	0.0	0.0		
			7	0.0	0.0	0.6		

On the other hand, After blood group test have been done, the most common blood type among Rh-negative mothers was Group O which was (35.8%), followed by A, B and AB groups which were 32.1%, 27.9% and 4.2%, respectively. in contrast, the most common blood type among Rh-positive fathers was Group B which was 38.2%, followed by A,O and AB groups with the percentage of 37, 24.2 and 0.6, respectively. The blood group percentages bar chart of those parents around ages of (26-30) has been illustrated in Figure (4.3)

Eventually, after antibody identification and Anti-D checkup tests, as a result, only two of the mothers who were Rh-negative were positive to Anti-D antibody.

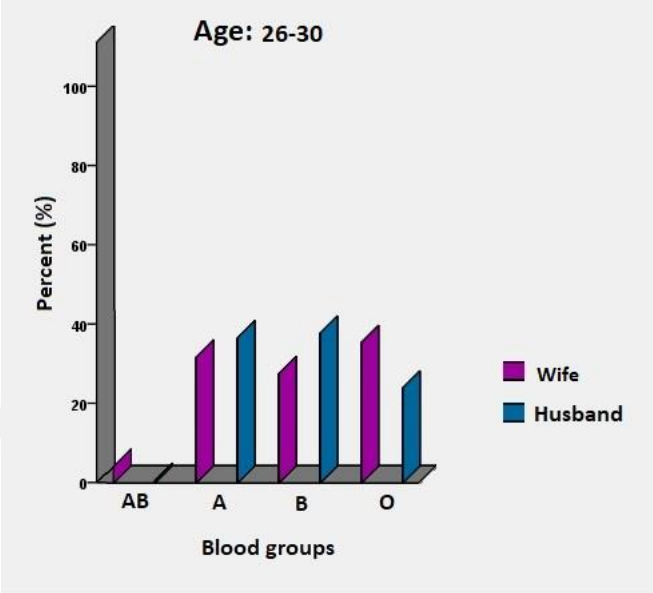

Figure 4. 3. Blood group profile of the parents around ages (26-30).

4.1.2.3. Antibody identification and blood analysis according to Ages of 31-35.

Out of 514 (100%) samples which have been tested, 191 (37%) of them were between the ages of (31-35). In regarding to delivery and abortion times, most of the mothers (41.5%) got birth to baby three times, followed by 35.8% of them two times and 17.6% of them four times. In contrast, approximately 40% of all the mothers in this age experienced baby abortion, in which 31.4% of them experienced one time abortion followed by 5.2% ,2.1 %and 1 % of them two, four and three times abortion, respectively. In term of blood transfusion, most of the mothers (77%) have not taken any blood transfusion while 13.1% of them have taken 1 times followed by 3.7%, 3.1% ,2.4%, 1%,1%, 1% of them have taken blood transfusion 3,2, 4,5 and 6 times, respectively. all of the data regarding to ages of (31-35) have been presented in table (4.3).

Table 4. 3. Blood analysis and patient history according to ages of 31-35.

AGES 31-35	MOTHER	FATHER	TIMES	Delivery	Abortion	Blood Transfusion		POSITIVE
AB	3.7	1.0	0	0.0	60.3	77.0	Ab+	2
A	27.2	42.9	1	2.6	31.4	13.1		
B	26.7	34.6	2	35.8	5.2	3.1		
O	42.4	21.5	3	41.5	1.0	3.7		
			4	17.6	2.1	1.0		
			5	2.6	0.0	1.0		
			6	0.0	0.0	1.0		
			7	0.0	0.0	0.0		

On the other hand, After blood group test have been done, the most common blood type among Rh-negative mothers was Group O which was (42.4%), followed by A,B and AB groups which were 27.6%, 26.7% and 3.7%, respectively. in contrast, the most common blood type among Rh-positive fathers was Group B which was 42.9%, followed by B,O and AB groups with the percentage of 34.6, 21.5 and 1, respectively. The blood group percentages bar chart of those parents around ages of (31-35) has been illustrated in Figure (4.4)

Eventually, after antibody identification and Anti-D checkup tests, as a result, only two of the mothers who were Rh-negative were positive to Anti-D antibody.

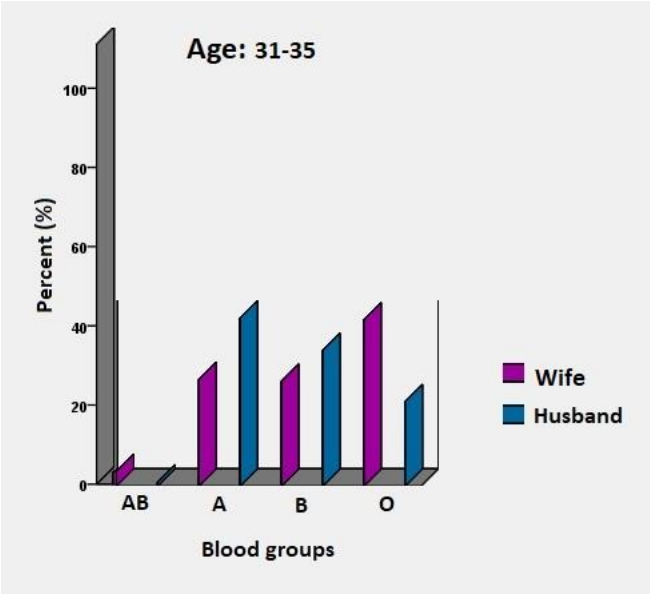

Figure 4. 4. Blood group profile of the parents around ages (31-35).

4.1.2.4. Antibody identification and blood analysis according to Ages of 36-40.

Out of 514 (100%) samples which have been tested, 112 (22%) of them were between the ages of (36-40). In regarding to delivery and abortion times, (35.7%) of the mothers got birth to baby four times, followed by 32.1% of them three times and 12.5% of them 2 and 5 times . In contrast, approximately 50 % of all the mothers in this age experienced baby abortion, in which 27.3 % of them experienced one time abortion followed by 14.5 % ,5.5 %and 1.8 % of them two, three and four times abortion, respectively. In term of blood transfusion, most of the mothers (66.4 %) have not taken any blood transfusion while 9.1 % of them have taken one and four times followed by 6.4%, 4.5% ,2.7 % and 1.8% of them have taken blood transfusion 3,2, 5 and 7 times, respectively. all of the data regarding to ages of (36-40) have been presented in table (4.4).

Table 4. 4. Blood analysis and patient history according to ages of 36-40.

AGES 36-40	MOTHER	FATHER	TIMES	Delivery	Abortion	Blood Transfusion		POSITIVE
AB	0.0	0.9	0	0.0	50.9	66.4	Ab+	4
A	31.3	52.7	1	0.9	27.3	9.1		
B	28.6	29.5	2	12.5	14.5	4.5		
O	40.2	17.0	3	32.1	5.5	6.4		
			4	35.7	1.8	9.1		
			5	12.5	0.0	2.7		
			6	4.5	0.0	0.0		
			7	1.8	0.0	1.8		

On the other hand, After blood group test have been done, the most common blood type among Rh-negative mothers was Group O which was (40.2%), followed by A and B groups which were 31.3 % and 28.6 % , respectively. meanwhile there was not any mother who has Blood group AB. in contrast, the most common blood type among Rh-positive fathers was Group A which was 52.7 %, followed by B,O and AB groups with the percentage of 29.5, 17 and 0.9, respectively. The blood group percentages bar chart of those parents around ages of (36-40) has been illustrated in Figure (4.5)

Eventually, after antibody identification and Anti-D checkup tests, as a result, only four of the mothers who were Rh-negative were positive to Anti-D antibody.

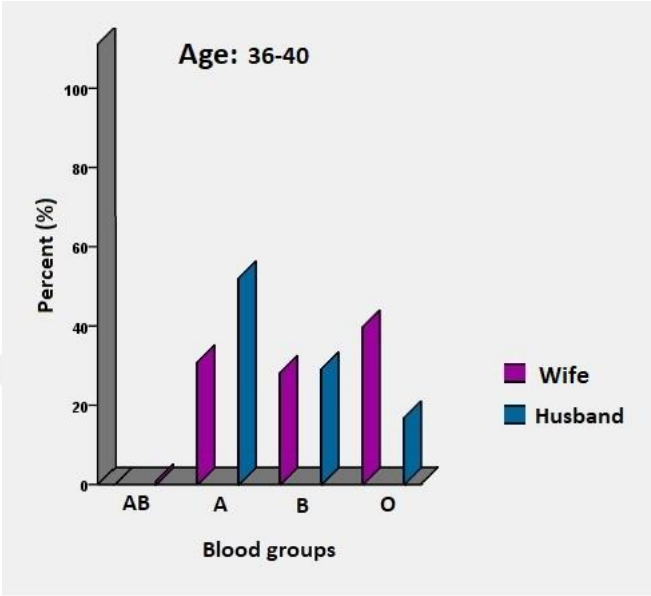

Figure 4. 5. Blood group profile of the parents around ages (36-40).

4.1.2.5. Antibody identification and blood analysis according to Ages over 40.

Out of 514 (100%) samples which have been tested, 11 (2%) of them were (over the ages of 40). In regarding to delivery and abortion times, most of the mothers (54.5 %) got birth to baby six times, followed by 27.3 % of them four times and 9.1 % of them three and five times. In contrast, approximately 54.6 % of all the mothers in this age experienced baby abortion, in which 27.3 % of them experienced two times abortion followed by 18.2 % and 9.1 % of them one and three times abortion, respectively. In term of blood transfusion, most of the mothers (81.8%) have not taken any blood transfusion while 9.1 % of them have taken one or seven times blood transfusion. all of the data regarding to ages of (over 40) have been presented in table (4.5).

Table 4. 5. Blood analysis and patient history according to ages over 41.

AGES OVER 41	MOTHER	FATHER	TIMES	Delivery	Abortion	Blood Transfusion		POSITIVE
AB	0.0	0.0	0	0.0	45.5	81.8	Ab+	1
A	0.0	45.5	1	0.0	18.2	9.1		
B	18.2	36.4	2	0.0	27.3	0.0		
O	81.8	18.2	3	9.1	9.1	0.0		
			4	27.3	0.0	0.0		
			5	9.1	0.0	0.0		
			6	54.5	0.0	0.0		
			7	0.0	0.0	9.1		

On the other hand, After blood group test have been done, the most common blood type among Rh-negative mothers was Group O which was (81.8 %), followed by Group B which was 18.2 % , while there was not any other blood groups has been detected. in contrast, the most common blood type among Rh-positive fathers was Group A which was 45.5 %, followed by B and O groups with the percentage of 36.4 and 18.2, respectively. The blood group percentages bar chart of those parents around ages of (over 40) has been illustrated in Figure (4.6)

Eventually, after antibody identification and Anti-D checkup tests, as a result, only one of the mothers who were Rh-negative was positive to Anti-D antibody.

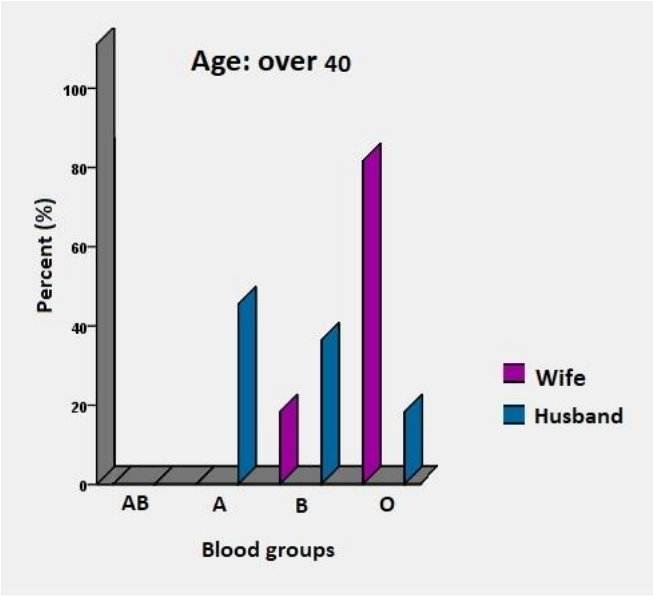

Figure 4. 6. Blood group profile of the parents around ages (over 40).

4.1.3. Blood group distribution among parents based on different Ages.

in our study, 1028 blood samples have been taken from 514 parents (Mother is Rh-negative and Father is Rh-positive), then all the samples have been separated and categorized according to various Age groups as can be seen in (Table 4.6), then blood group testing has been done in order to find their Blood types. In outcomes, in the first age category (20-25), the most common blood type among Rh-negative mothers was O which was (37.1%), and the least common BG was 2.9% for AB. in contrast, the most common blood type among Rh-positive fathers was Group A which was 48.6%, and the least common BG was AB group with the percentage of 2.9. In the second category (26-30), the most common blood type among mothers was Group O which was (35.8%), and the least common BG was 4.2 % for AB group. in contrast, the most common blood type among fathers was Group B which was 38.2%, and the least common BG was AB group with the percentage of 0.6. in the third category (31-35), the most common blood type among mothers was Group O which was (42.4%), and the least common BG was AB group which was 3.7%. in contrast, the most common blood type among fathers was Group B which was 42.9%, and the least common BG was AB group with the percentage of 1. In the fourth category (36-40), the most common blood type among mothers was Group O which was (40.2%), meanwhile there was not any mothers has Blood group AB. in contrast, the most common blood type among fathers was Group A which was 52.7 %, and the least common BG was AB group with the percentage of 0.9. finally, in the last age category (over than 40), the most common blood type among mothers was Group O which was (81.8 %), followed by Group B which was 18.2 % , while there was not any other blood groups has been detected. in contrast, the most common blood type among fathers was Group A which was 45.5 %, and the least common BG was O group with the percentage of 18.2, and there wasn’t any case of AB blood group.

Table 4. 6. Parental Blood group distribution among different Age groups.

Age	Blood Group							
	AB		A		B		O	
	Mother Rh(-)	Father Rh(+)	Mother Rh(-)	Father Rh(+)	Mother Rh(-)	Father Rh(+)	Mother Rh(-)	Father Rh(+)
20-25 N	2.9	2.9	28.6	48.6	31.4	25.7	37.1	22.9
25-30 N	4.2	0.6	32.1	37	27.9	38.2	35.8	24.2
30-35 N	3.7	1	27.2	42.9	26.7	34.6	42.4	21.5
35-40 N	0	0.9	31.3	52.7	28.6	29.5	40.2	17
45+ N	0	0	0	45.5	18.2	36.4	81.8	18.2

4.1.4. Anti-D antibody isolation and distribution among Age groups.

Out of 514 samples which have been screened for antibodies and especially anti-D antibody in mothers who have been pregnant by Rh-positive babies previously, it was found out that among all of the mothers and different Age groups only nine (9) of them being positive for Anti-D antibody isolation which was 2% of all the Mother samples, (Figure 4.7) illustrates the percentage of all the positive cases and their distribution among different Age groups. In outcomes, the highest percentage of Anti-D positive antibody was in (36-40) age category which was 44%, followed by 22% in both (26-30) and (31-35) and 11% in (over 40) Age category, and there was not any positive case inside (20-25) Age category.

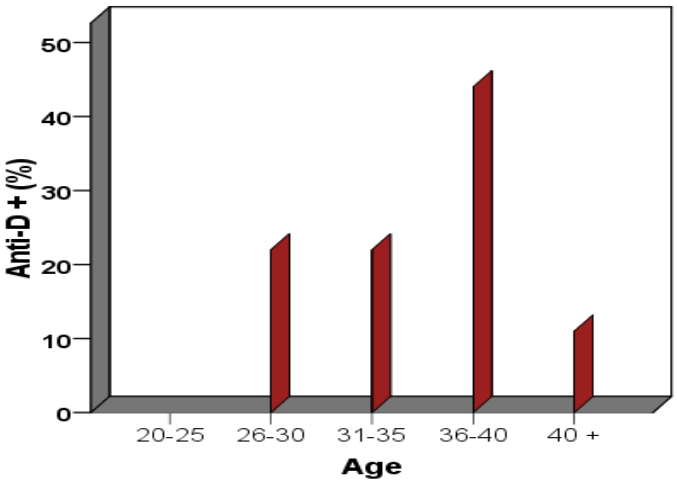

Figure 4. 7. Anti-D antibody distribution among age groups.

4.1.5. Delivery, Abortion and Blood transfusion backgrounds of mothers

This study is based on identification of hidden Anti-D antibody, so for this reason, in our study some of the important background factors such as (Delivery, Abortion and Blood transfusion) had been taken from age categorized mothers. All of these data are shown in Table (4.7; 4.8; 4.9) and Figure (4.8; 4.9; 4.10).

In regarding to delivery and abortion times, most of the mothers in first age category (20-25) which was (65.7%) got birth to baby one time, followed by 31.4% of them two times. In contrast, only 22.8% of all the mothers in this age experienced baby abortion, in which 17.1% of them experienced one time abortion while 5.7% of them two times abortion. In term of blood transfusion, most of the mothers (82.9%) have not taken any blood transfusion while 5.7% of them have taken 1 or 2 times blood transfusion.

Table 4. 7. Baby Delivery percentages among Rh-Negative mothers.

Age	Delivery						
	Times						
	1	2	3	4	5	6	7
20-25	65.7	31.4	0	2.9	0	0	0
N							
25-30	25.5	52.1	22.4	0	0	0	0

N							
30-35	2.6	35.8	41.5	17.6	2.6	0	0
N							
35-40	0.9	12.5	32.1	35.7	12.5	4.5	1.8
N							
45+	0	0	9.1	27.3	9.1	54.5	0
N							

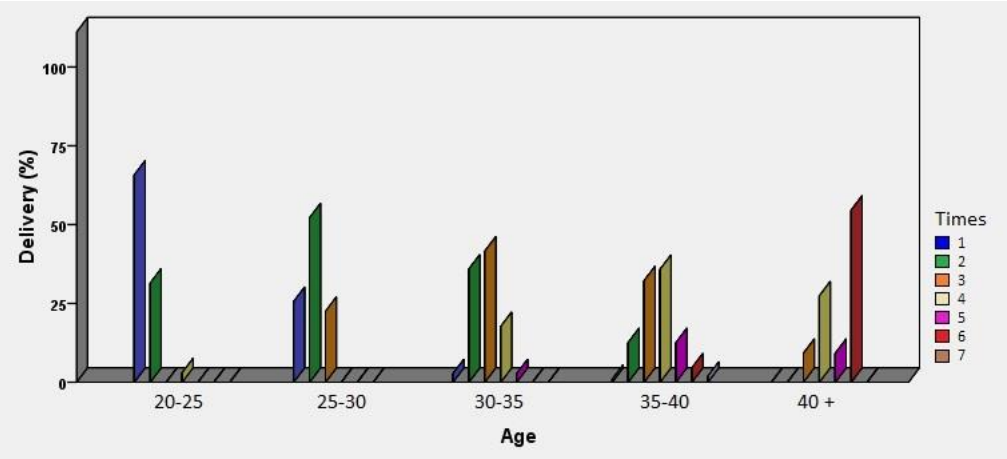

Figure 4. 8. Baby delivery percentage among different mother Age categories.

In regarding to delivery and abortion times in the second age category (26-30), most of the mothers (52.1%) got birth to baby two times, followed by 25.5% of them one time and 22.4% of them four times. In contrast, only approximately 33% of all the mothers in this age experienced baby abortion, in which 28% of them experienced one time abortion while 4.9 % of them two times abortion. In term of blood transfusion, most of the mothers (79.9%) have not taken any blood transfusion while 9.1% of them which is the highest percentage have taken only 1 time blood transfusion.

In regarding to delivery and abortion times in the third Age category (31-35), approximately (41.5%) of the mothers got birth to baby three times, followed by 35.8% of them two times and 17.6% of them four times. In contrast, approximately 40% of all the mothers in this age experienced baby abortion, in which 31.4% of them experienced one time abortion. In term of blood transfusion, most of the mothers (77%) have not taken any blood transfusion while 13.1% of them have taken 1 time blood transfusion.

Table 4. 8. Baby abortion percentages among Rh-Negative mothers.

Age	Abortus				
	Times				
	0	1	2	3	4
20-25 N	77.1	17.1	5.7	0	0
25-30 N	66.5	28	4.9	0.6	0
30-35 N	60.3	31.4	5.2	1	2.1
35-40 N	50.9	27.3	14.5	5.5	1.8
45+ N	45.5	18.2	27.3	9.1	0

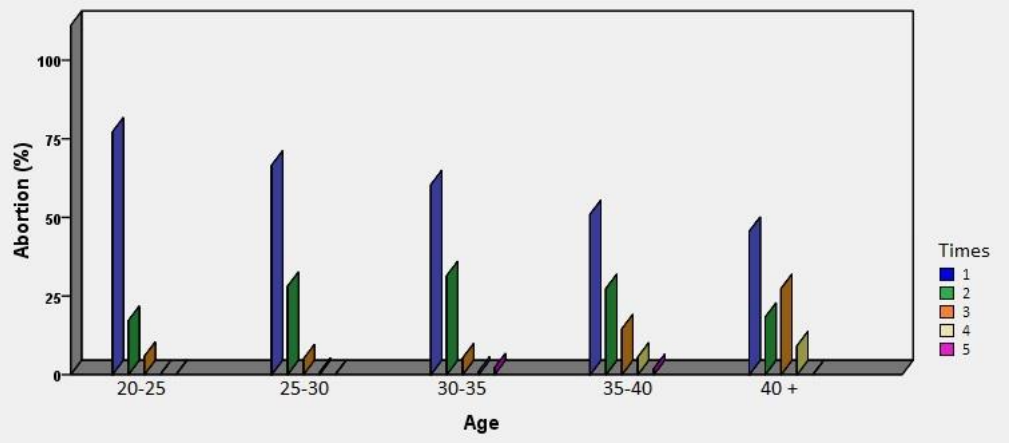

Figure 4. 9. Baby abortion percentages among different mother Age categories.

In regarding to delivery and abortion times in the fourth Age category (36-40), approximately (35.7%) of the mothers got birth to baby four times, followed by 32.1% of them three times and 12.5% of them 2 and 5 times . In contrast, approximately half of all the mothers in this age experienced baby abortion, in which 27.3 % of them experienced one time abortion followed by 14.5 % ,5.5 % and 1.8 % of them two, three and four times abortion, respectively. In term of blood transfusion, most of the mothers (66.4 %) have not taken any blood transfusion while 9.1 % of them have taken one and four times blood transfusion.

In regarding to delivery and abortion times in the final Age category (over 40 years), most of the mothers (54.5 %) got birth to baby six times, followed by 27.3 % of them four times. In contrast, approximately 54.6 % of all the mothers in this age experienced baby abortion, in which 27.3 % of them experienced two times abortion followed by 18.2 % and 9.1 % of them one and three times abortion, respectively. In term of blood transfusion, most of the mothers (81.8%) have not taken any blood transfusion while 9.1 % of them have taken one or seven times blood transfusion.

Table 4. 9. Blood transfusion percentages among Rh-Negative mothers.

Age	**Blood transfusion**						
	Times						
	1	**2**	**3**	**4**	**5**	**6**	**7**
20-25 N	82.9	5.7	5.7	2.9	2.9	0	0
25-30 N	79.9	91	4.9	2.4	2.4	0.6	0
30-35 N	77	13.1	3.1	3.7	1	1	1
35-40 N	66.4	9.1	4.5	6.4	9.1	2.7	0
45+ N	81.8	9.1	0	0	0	0	0

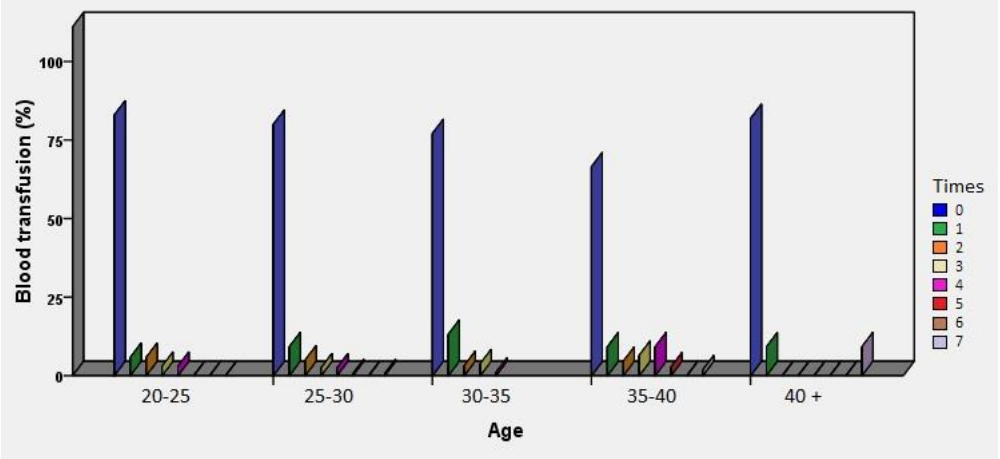

Figure 4. 10. Blood transfusion percentages among different Age categories.

4.2. Discussion

Maternal antibodies against fetal red cell antigens, which cross the placenta and result in hemolysis, are the cause of HDFN. Antibodies may be immunological or natural. In the latter scenario, exposure to the relevant antigen during a prior pregnancy or a transfusion is typically the sensitizing event. Alloanti-D antibodies develop in response to sensitizing events like pregnancy in which a RhD-negative woman conceives a RhD-positive fetus, transfusion of RhD-positive blood to RhD-negative women, and obstetric sensitizing events like abortion, ectopic pregnancy, and hemorrhagic episodes in RhD-positive women. This phenomenon has persisted as a problem leading to HDFN (Lubusky, 2010).

Other sensitizing variables, such a forgotten childhood blood transfusion, might account for the alloimmunization in young women. When it was discovered that a number of sensitizing variables, including parity, age, and other obstetric parameters, were connected to alloimmunization (Natukunda et al., 2011).

The blood type distribution among the 726 participants was as follows: O: 356 (49%); A: 190 (26%); B: 152 (21%); and AB: 28 (4%). RhD was not present in a total of 28 (3.86%) pregnant women. 88 (12.1%) of the subjects had anti-D alloantibodies, and 13 (14.8%) of them were RhD negative. Miscarriage, stillbirth, and postpartum hemorrhage were statistically significant risk factors for anti-D alloimmunization (Mbalibulha et al., 2015).

According to a research, out of 108 pregnant women, 45 (41.7%) were found to be aware of the anti-D immunoglobulin and blood group Rh(D)-negativity issues. 11.1% (12/108) of people had a negative blood group. Age, education, vaginal bleeding, blood types, and past injection of anti-D immunoglobulin were all shown to be substantially linked with awareness (Yahia et al., 2020).

(Cansu, 2018) studied that, Duration of gestation varied between 174 and 288 days (mean: 258,7 ± 19,2). 41.2% babies were preterm a 58.8% were male. Antenatal

transfusion was needed for 31.7% the patients. 8.8% patients were hydropic and 5.4% patients did not survive. 90.8% patients recieved IVIG and 9.2 % did not. 66.5% recieved phototherapy and 15.4% babies underwent exchange transfusion. 33.5% babies needed neither phototherapy nor phototherapy while 15.4% patients underwent exchange transfusion in addition to phototherapy. Patients with coexisting ABO incompatibility were found to have a milder course.

On 1251 pregnant women who were treated and delivered at the hospital, data was available. 7.3% of pregnant women tested negative for RhD. The most prevalent blood type was O, followed by A, B, and AB. 10% of individuals had RhD negative alloimmunization, with anti-D being the most frequently found antibody. There were no neonatal or stillbirth fatalities. For just one infant, postnatal transfusion was necessary (Al-Dughaishi et al., 2016).

In one research in Denizli, 89.9% of participants had positive Rh results, whereas 10.1% had negative Rh results. Blood types A, O, B, and AB were found to be more common, with respective frequencies of 42.6%, 33.3%, 16.8%, and 7.4%. comparable to other cities and regions in Turkey, Denizli has comparable blood type prevalence percentages (Balci et al., 2010).

5347 women underwent screening, and 339 (6.34%) of them tested negative for Rh. 79 women (1.48%; confidence range 1.17-1.84) had allosensitization. Alloantibodies could not be detected in 29 of these 79 (37%) women. 54 antibodies were described in the 50 remaining women. 36 women, including four Rh(D) positive individuals, were found to have a total of 40 clinically relevant antibody specificities. In 9.43% (confidence interval 6.55-13.06) of Rh(D) negative women and 0.08 percent (confidence interval.02-0.2) of Rh(D) positive women, allosensitization with clinically relevant antibodies was discovered. In 8.85 percent of Rh(D) negative women, anti D was the most prevalent antibody. Antibodies to the D and Rh systems made up 83.3 and 94.4 percent of the clinically relevant antibodies in women who were Rh(D) negative, respectively (Varghese et al., 2013).

In a Croatian research, clinically significant non-D antibodies resulted in HDFN in almost 55% of alloimmunized pregnancies, and severe HDFN, as measured by the need for perinatal transfusion or mortality, in around 25% (Dajak et al., 2011).

Researchers only looked at situations when the mother had the blood group O and the baby had the blood group A or B. Infants in groups A and B had maternal antibody-induced haemolysis equally often and severely in both cases. In comparison to cross-reactive antibodies (anti-A,B), monospecific antibodies (anti-B) were linked to a more severe haemolytic process in group B new-borns. There was no difference in the severity of the illness between monospecific antibodies (anti-A) and reciprocal antibodies (anti-A,B) in group A babies. Although there was no discernible difference in the afflicted babies' gender distribution, there were more boys in the more seriously affected group (Chan-Shu and Blair, 1979).

The relationship between laboratory measures and the frequency and seriousness of jaundice was investigated in a retrospective examination of 254 instances of ABO hemolytic illness of the newborn. Approximately 35% of control newborns or infants with ABO hemolytic disease of the newborn and negative direct antiglobulin test results did not have jaundice, but 65% of the infants with positive direct antiglobulin testing did. In comparison to control infants or infants with ABO hemolytic illness of the newborn and negative direct antiglobulin test findings, infants with ABO hemolytic disease of the newborn and positive direct antiglobulin test results also showed more severe jaundice (Dufour et al., 1980).

Despite being widespread, ABO HDN is not a dangerous illness and does not require invasive prenatal screening. Patients who have undergone many transfusions may develop anti-Kell, and the fast progression of their hemolysis may prevent such systematic follow-up as in Rh HDN (Narang and Jain, 2001).

Seventy-seven of the 618 newborns exhibited parental ABO heterospecificity. In 20 instances (3.2%), maternal alloantibodies were discovered. Of the 86 full-term babies, 20 had jaundice, which was probably caused by ABO incompatibility. Six

patients required blood transfusions, while the remaining twelve required only phototherapy (Arévalo, 1992).

Four racial categories and six classes of the recipient's and her husband's relatives were calculated for. In general, there is a reasonably constant relationship between the donor's degree of relatedness to the recipient and husband and the likelihood of HDN. If the spouse contributed, the relative risk for developing HDN later on was as high as 4.1. The risk changed depending on the recipient's race and Rh(D) status. When employing directed donors, the danger of transfusing a woman of reproductive age with blood from her spouse or his family should be taken into account, however how tiny it is (Kanter and Hodge, 1989).

As of yet, neither a clinical nor a serological diagnosis can be made for infant ABO hemolytic illness. Babies from moms in groups A or B are virtually universally affected. The immunological results do not strongly correspond with the severity of the clinical course, in contrast to Rh hemolytic illness. Sometimes it is tough to distinguish between non-antibody caused hyperbilirubinemia and ABO hemolytic disorder. Discussions of pathogenetic elements that might clarify the distinctions between newborns with ABO and Rh hemolytic illness (Fischer, 1977).

5. CONCLUSIONS and RECOMMENDATIONS

Accordingly, the main causes of HDFN currently appear to be haemolytic illness brought on by ABO incompatibility and other alloantibodies. All pregnant women in many affluent countries are routinely screened, and some of them even have nationwide screening programs. This is required to prevent adverse effects on the infant and ensure the prompt supply of antigen-negative blood. Pregnant women in underdeveloped nations still frequently have anti-D antibodies in their bodies. In addition, the etiology of moderate-severe haemolytic illness has lately been linked to alloantibodies other than anti-D in several Asian nations. The fact that some of these have been reported in women who are Rh (D) positive is especially worrisome. ABO incompatibility is not always benign and may need to be actively managed, according to studies.

All the mothers' blood samples have been divided into five different Age category 20-25: 35 (7%), 26-30: 165 (32%), 31-35: 191 (37%), 36-40: 112 (22%), and over 40: 11 (2%), Age groups. The most common blood type among Rh-negative mothers was Group O which was (42.4%), followed by A, B and AB groups which were 27.6%, 26.7% and 3.7%, respectively. The highest percentage of Anti-D positive antibody was in (36-40) age category which was 44%, followed by 22% in both (26-30) and (31-35) and 11% in (over 40) Age category, and there was not any positive case inside (20-25) age category.

This study is based on identification of hidden Anti-D antibody, so for this reason, in our study some of the important background factors such as (Delivery, Abortion and Blood transfusion) had been taken from age categorized mothers.

In regarding to delivery and abortion times, most of the mothers in first age category (20-25) which was (65.7%) got birth to baby one time, followed by 31.4% of them two times. In contrast, only 22.8% of all the mothers in this age experienced baby abortion, in which 17.1% of them experienced one time abortion while 5.7% of them two times abortion. In term of blood transfusion, most of the mothers (82.9%) have

not taken any blood transfusion while 5.7% of them have taken 1 or 2 times blood transfusion. As a result of our study, most of the mothers who had miscarriages had different rates and at different ages (26–30, 31–35, 36–40 and above 40 years) (33%, 40%, 27.3% and 54.6%), respectively .

Given everything said above, widespread prenatal screening for all expectant women must be started since D-negative women develop alloantibodies. To find unusual antibodies, a vigilant watch must be kept throughout the pregnancy. Universal screening seems appropriate, but it would be extremely expensive and need extensive infrastructure. The necessity for universal prenatal screening should be taken into consideration while creating recommendations for underdeveloped and developing countries. Women and their children who have blood group incompatibilities will benefit from future developments in blood typing and non-invasive testing. Additionally, the racial and ethnic groups who are most at danger need to receive greater attention. Our hope is that with proper prophylaxis and education, Rh haemolytic disease will become one of the rarest diseases and even eradicate from the face of the earth.

REFERENCES

ADEYEMI, A.S. and BELLO-AJAO, H.T., 2016. Prevalence of Rhesus D-negative blood type and the challenges of Rhesus D immunoprophylaxis among obstetric population in Ogbomoso, Southwestern Nigeria. *Annals of Tropical Medicine and Public Health*| *Jan-Feb*, *9*(1).

AFSHAN, N., 2013. Detection of Du Antigen in Rh Negative Blood Group Individuals. *RADS Journal of Biological Research & Applied Sciences*, *4*(2), pp.16-19.

AKKÖK, Ç.A. and SEGHATCHIAN, J., 2018. Immunohematologic issues in ABO-incompatible allogeneic hematopoietic stem cell transplantation. *Transfusion and Apheresis Science*, *57*(6), pp.812-815.

ALAQEEL, A.A., 2019. Hyporegenerative anemia and other complications of rhesus hemolytic disease: to treat or not to treat is the question. *Pan African Medical Journal*, *32*(1).

ALCOCK, G.S. and LILEY, H., 2002. Immunoglobulin infusion for isoimmune haemolytic jaundice in neonates. *Cochrane database of systematic reviews*, (3).

AL-DUGHAISHI, T., AL HARRASI, Y., AL-DUHLI, M., AL-RUBKHI, I., AL-RIYAMI, N., AL-RIYAMI, A.Z., PATHARE, A.V. and GOWRI, V., 2016. Red cell alloimmunization to Rhesus antigen among pregnant women attending a tertiary care hospital in Oman. *Oman medical journal*, *31*(1), p.77.

AVENT, N.D. and REID, M.E., 2000. The Rhesus blood group system. *J Am Soc Haematol*, *95*(2), pp.375-387.

BALCI, Y.I., GULTEKIN, O.V.E.T., COVUT, I.E., GONCU, F. and YILMAZ, M., 2010. ABO and Rh blood groups frequency in Denizli province. *International Journal of Hematology and Oncology*, *32*(4), pp.103-105.

BASU, S., KAUR, R. and KAUR, G., 2011. Hemolytic disease of the fetus and newborn: Current trends and perspectives. *Asian journal of transfusion science*, *5*(1), p.3.

BENNARDELLO, F., COLUZZI, S., CURCIARELLO, G., TODROS, T. and VILLA, S., 2015. Recommendations for the prevention and treatment of haemolytic disease of the foetus and newborn. *Blood Transfusion*, *13*(1), p.109

CHAN-SHU SY, BLAIR O. 1979, ABO hemolytic disease of the newborn. Am J Clin Pathol. Jun;71(6):677-9. doi: 10.1093/ajcp/71.6.677. PMID: 453084.

CHILCOTT, J., JONES, M.L., WIGHT, J., FORMAN, K., WRAY, J., BEVERLEY, C. and TAPPENDEN, P., 2003. A review of the clinical effectiveness and cost-effectiveness of routine anti-D prophylaxis for pregnant women who are rhesus-negative. *NIHR Health Technology Assessment programme: Executive Summaries*.

COGHLAN, G., 2009. Antibodies to low-incidence antigens. *Transfusion and Apheresis Science*, *40*(3), pp.199-202.

COWAN, H., 2017. The discovery of human blood groups: Karl Otto Landsteiner. *British Journal of Cardiac Nursing*, *12*(6), pp.290-292.

DAJAK, S., STEFANOVIĆ, V. and ČAPKUN, V., 2011. Severe hemolytic disease of fetus and newborn caused by red blood cell antibodies undetected at first-trimester screening (CME). *Transfusion*, *51*(7), pp.1380-1388.

DANIELS, G., 2008. *Human blood groups*. John Wiley & Sons.

DANIELS, G., BALLIF, B.A., HELIAS, V., SAISON, C., GRIMSLEY, S., MANNESSIER, L., HUSTINX, H., LEE, E., CARTRON, J.P., PEYRARD, T. and ARNAUD, L., 2015. Lack of the nucleoside transporter ENT1 results in the Augustine-null blood type and ectopic mineralization. *Blood, The Journal of the American Society of Hematology*, *125*(23), pp.3651-3654.

DANIELS, G., HADLEY, A. and SOOTHILL, P., 2002. Blood group antibodies in haemolytic disease of the fetus and newborn. *Alloimmune disorders of pregnancy*, *1*, pp.21-40.

DANIELS, G.L., FLETCHER, A., GARRATTY, G., HENRY, S., JØRGENSEN, J., JUDD, W.J., LEVENE, C., LOMAS-FRANCIS, C., MOULDS, J.J., MOULDS, J.M. and MOULDS, M., 2004. Blood group terminology 2004: from the International Society of Blood Transfusion committee on terminology for red cell surface antigens.

DE HAAS, M., FINNING, K., MASSEY, E. and ROBERTS, D.J., 2014. Anti-D prophylaxis: past, present and future. *Transfusion Medicine*, *24*(1), pp.1-7.

DE HAAS, M., THURIK, F.F., KOELEWIJN, J.M. and VAN DER SCHOOT, C.E., 2015. Haemolytic disease of the fetus and newborn. *Vox sanguinis*, *109*(2), pp.99-113.

DEAN, L. and DEAN, L., 2005. *Blood groups and red cell antigens* (Vol. 2). Bethesda: NCBI.

DINESH, D., 2005. Review of positive direct antiglobulin tests found on cord blood sampling. *Journal of paediatrics and child health*, *41*(9-10), pp.504-507.

DRABIK-CLARY, K., REDDY, V.V., BENJAMIN, W.H. and BOCTOR, F.N., 2006. Severe hemolytic disease of the newborn in a group B African-American infant delivered by a group O mother. *Annals of Clinical & Laboratory Science*, *36*(2), pp.205-207.

DUFOUR DR, MONOGHAN WP. 1980 , ABO hemolytic disease of the newborn. A retrospective analysis of 254 cases. Am J Clin Pathol. Mar;73(3):369-73. doi: 10.1093/ajcp/73.3.369. PMID: 7189092.

EDER, A.F., 2006. Update on HDFN: new information on long-standing controversies. *IMMUNOHEMATOLOGY-WASHINGTON DC-*, *22*(4), p.188.

GUPTA, G.K., BALBUENA-MERLE, R., HENDRICKSON, J.E. and TORMEY, C.A., 2020. Immunohematologic aspects of alloimmunization and alloantibody detection: a focus on pregnancy and hemolytic disease of the fetus and newborn. *Transfusion and Apheresis Science*, *59*(5), p.102946.

HADLEY, A. and SOOTHILL, P. EDS., 2002. *Alloimmune disorders of pregnancy: anaemia, thrombocytopenia and neutropenia in the fetus and newborn*. Cambridge University Press.

HOBSON-PETERS, J., 2012. Approaches for the development of rapid serological assays for surveillance and diagnosis of infections caused by zoonotic flaviviruses of the Japanese encephalitis virus serocomplex. *Journal of Biomedicine and Biotechnology*, *2012*.

HOSOI, E., 2008. Biological and clinical aspects of ABO blood group system. *The journal of medical investigation*, *55*(3, 4), pp.174-182.]

JACKSON, M.E. and BAKER, J.M., 2021. Hemolytic disease of the fetus and newborn: historical and current state. *Clinics in Laboratory Medicine*, *41*(1), pp.133-151.

KANTER MH, HODGE SE. 1989., Risk of hemolytic disease of the newborn as a result of directed donations from relatives. Transfusion. Sep;29(7):620-5. doi: 10.1046/j.1537-2995.1989.29789369681.x. PMID: 2773028.

KLEIN, H.G. and ANSTEE, D.J., 2005. Blood donors and the withdrawal of blood. *Mollison's Blood Transfusion in Clinical Medicine, 11th ed. Oxford UK: Blackwell Publishing*, pp.1-18.

KUMARI, A., GUPTA, S. and BHATEJA, A., Blood Grouping Systems: A REVIEW.

LANGHI, D.M. and ORLANDO BORDIN, J., 2006. Duffy blood group and malaria. *Hematology*, *11*(5-6), pp.389-398.

LIU, J., ZHANG, S., WANG, Q., SHEN, H., ZHANG, Y. and LIU, M., 2017. Frequencies and ethnic distribution of ABO and RhD blood groups in China: a population-based cross-sectional study. *BMJ open*, *7*(12), p.e018476.

LIUMBRUNO, G.M., D'ALESSANDRO, A., REA, F., PICCININI, V., CATALANO, L., CALIZZANI, G., PUPELLA, S. and GRAZZINI, G., 2010. The role of antenatal immunoprophylaxis in the prevention of maternal-foetal anti-Rh (D) alloimmunisation. *Blood transfusion*, *8*(1), p.8.

LUBUSKY, M., 2010. Prevention of RhD alloimmunization in RhD negative women. *Biomedical Papers of the Medical Faculty of Palacky University in Olomouc*, *154*(1).

MAKAROVSKA-BOJADZIEVA, T., BLAGOEVSKA, M., KOLEVSKI, P. and KOSTOVSKA, S., 2009. Optimal blood grouping and antibody screening for safe transfusion. *Prilozi*, *30*(1), pp.119-128.

MALOMGRÉ, W. and NEUMEISTER, B., 2009. Recent and future trends in blood group typing. *Analytical and bioanalytical chemistry*, *393*(5), pp.1443-1451.

MBALIBULHA, Y., MUWANGUZI, E., MUGYENYI, G.R. and NATUKUNDA, B., 2015. Occurrence of anti-D alloantibodies among pregnant women in Kasese District, Western Uganda. *Journal of Blood Medicine*, *6*, p.125.

McBean, R., Liew, Y.W., Wilson, B., Kupatawintu, P., Emthip, M., Hyland, C. and Flower, R., 2016. Genotyping confirms inheritance of the rare At (a−) type in a case of haemolytic disease of the newborn. *The Journal of Pathology: Clinical Research*, *2*(1), pp.53-55.

MCBEAN, R.S., HYLAND, C.A., HENDRY, J.L., SHABANI-RAD, M.T. and FLOWER, R.L., 2015. SARA: a "new" low-frequency MNS antigen (MNS47) provides further evidence of the extreme diversity of the MNS blood group system. *Transfusion*, *55*(6pt2), pp.1451-1456.

MCGOWAN, E., 2021. An investigation of human antibodies to blood group antigens for the management of haemolytic disease of the fetus and newborn.

MCGOWAN, E.C., FLOWER, R.L., O'BRIEN, H., MILLARD, G. and HYLAND, C.A., 2017. Non-invasive prenatal testing for sub-saharan Africa: Tailoring approaches for foetal RHD genotyping in RHD-negative pregnant women to manage African-associated RHD Alleles. *Africa Sanguine*, *19*(1), pp.18-29.

MILKINS, C., BERRYMAN, J., CANTWELL, C., ELLIOTT, C., HAGGAS, R., JONES, J., ROWLEY, M., WILLIAMS, M. and WIN, N., 2013. Guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories.

British Committee for Standards in Haematology, *Transfusion Medicine*, *23*(1), pp.3-35.

MITRA, R., MISHRA, N. and RATH, G.P., 2014. Blood groups systems. *Indian journal of anaesthesia*, *58*(5), p.524.

MOLLISON, P.L., ENGELFRIET, C.P. and CONTRERAS, M., 1997. Infection agent transmitted by transfusion. *Blood transfusion in clinical Medicine. 10th ed. Oxford, UK: Blackwell Science*, pp.509-70.

MYLE, A.K. and AL-KHATTABI, G.H., 2021. Hemolytic Disease of the Newborn: A Review of Current Trends and Prospects. *Pediatric Health, Medicine and Therapeutics*, *12*, p.491.

NARANG A, JAIN N. 2001., Haemolytic disease of newborn. Indian J Pediatr. Feb;68(2):167-72. doi: 10.1007/BF02722039. PMID: 11284186.

NATUKUNDA, B., MUGYENYI, G., BRAND, A. and SCHONEWILLE, H., 2011. Maternal red blood cell alloimmunisation in south western Uganda. *Transfusion Medicine*, *21*(4), pp.262-266.

OMOTADE, O.O., ADEYEMO, A.A., KAYODE, C.M., FALADE, S.L. and IKPEME, S., 1999. Gene frequencies of ABO and Rh (D) blood group alleles in a healthy infant population in Ibadan, Nigeria. *West African journal of medicine*, *18*(4), pp.294-297.

PEGORARO, V., URBINATI, D., VISSER, G.H., DI RENZO, G.C., ZIPURSKY, A., STOTLER, B.A. and SPITALNIK, S.L., 2020. Hemolytic disease of the fetus and newborn due to Rh (D) incompatibility: A preventable disease that still produces significant morbidity and mortality in children. *PloS one*, *15*(7), p.e0235807.

POOLE, J. and DANIELS, G., 2007. Blood group antibodies and their significance in transfusion medicine. *Transfusion medicine reviews*, *21*(1), pp.58-71.

RAHORST, L. and WESTHOFF, C.M., 2019. ABO and H Blood Group System. In *Transfusion Medicine and Hemostasis* (pp. 139-147). Elsevier.

RIDGWELL, K., SPURR, N.K., LAGUDA, B., MACGEOCH, C., AVENT, N.D. and TANNER, M.J.A., 1992. Isolation of cDNA clones for a 50 kDa glycoprotein of the human erythrocyte membrane associated with Rh (rhesus) blood-group antigen expression. *Biochemical Journal*, *287*(1), pp.223-228.

RISTOVSKA, E., BOJADJIEVA, T.M., VELKOVA, E., DIMCEVA, A.H., TODOROVSKI, B., TASHKOVSKA, M., RASTVORCEVA, R.G. and BOSEVSKI, M., 2022. Rare Blood Groups in ABO, Rh, Kell Systems–Biological and Clinical Significance. *prilozi*, *43*(2), pp.77-87.

ROUTRAY, S.S., BEHERA, R., MALLICK, B., ACHARYA, D., SAHOO, J.P., KANUNGO, G.N. and PATI, B., 2021. The spectrum of hemolytic disease of the newborn: evaluating the etiology of unconjugated hyperbilirubinemia among neonates pertinent to immunohematological workup. *Cureus*, *13*(8).

SETO, E., 2008. The Inhibitory Effect of Kell Blood Group Antibodies on Erythroid Progenitor Cell Growth (Doctoral dissertation).

SHAHATA, W.M., KHALIL, H.B., ABASS, A.E., ADAM, I. and HUSSIEN, S.M., 2012. Blood group and Rhesus antigens among Blood donors attending the Central Blood Bank, Sudan. *Sudan Journal of Medical Sciences*, *7*(4), pp.245-248.

STORRY, J.R., CLAUSEN, F.B., CASTILHO, L., CHEN, Q., DANIELS, G., DENOMME, G., FLEGEL, W.A., GASSNER, C., DE HAAS, M., HYLAND,

C. and YANLI, J., 2019. International society of blood transfusion working party on red cell immunogenetics and blood group terminology: report of the Dubai, Copenhagen and Toronto meetings. *Vox sanguinis*, *114*(1), pp.95-102.

STRAUSS, R.G., 2010. Hemolytic disease of the fetus/newborn: reflections on articles from TRANSFUSION Volume 1. *Transfusion*, *50*(4), pp.748-751.

TAYYAB, M., MALIK, A.R. and KHAN, A.S., 2000. D u PHENOTYPE-A REVIEW. *JAMC*, *12*(3).

TUGCU, A.U., INCE, D.A., TURAN, O., BELEN, B., OLCAY, L. and ECEVIT, A., 2019. Hemolytic anemia caused by non-D minor blood incompatibilities in a newborn. *The Pan African Medical Journal*, *33*.

VARGHESE, J., CHACKO, M.P., RAJAIAH, M. and DANIEL, D., 2013. Red cell alloimmunization among antenatal women attending a tertiary care hospital in south India. *Indian Journal of Medical Research*, *138*(1), pp.68-71.

VELATI, C., 2007. A survey of the current use of anti-D immunoprophylaxis and the incidence of haemolytic disease of the newborn in Italy. *Blood Transfusion*, *5*(1), p.7.

VELKOVA, E., 2015. Correlation between the amount of anti-D antibodies and IgG subclasses with severity of haemolytic disease of foetus and newborn. *Open Access Macedonian Journal of Medical Sciences*, *3*(2), p.293.

VELLIQUETTE, R. and WESTHOFF, C.M., 2019. Lewis, I, P1PK, FORS, and GLOB Blood Group Systems. In *Transfusion Medicine and Hemostasis* (pp. 169-175). Elsevier.

WAGNER, F.F., MOULDS, J.M. and FLEGEL, W.A., 2005. Genetic mechanisms of Rhesus box variation. *Transfusion*, *45*(3), pp.338-344.

WANG, R., LI, Y., TONG, Y. and SU, N., 2021. Hemolytic disease of the fetus and newborn caused by anti-group A IgG from a group B mother. *Journal of Pediatric Hematology/Oncology*, *43*(6), pp.e785-e787.

WARLAND, J.E., 2007. *Is maternal hypotension during pregnancy and/or posterior located placenta associated with increased risk of stillbirth? A case-control study* (Doctoral dissertation).

WESTHOFF, C.M., 2004. The Rh blood group system in review: a new face for the next decade. *Transfusion*, *44*(11), pp.1663-1673.

WESTHOFF, C.M., 2007, January. The structure and function of the Rh antigen complex. In *Seminars in hematology* (Vol. 44, No. 1, pp. 42-50). WB Saunders.

XIE, X., FU, Q., BAO, Z., ZHANG, Y. and ZHOU, D., 2020. Clinical value of different anti-D immunoglobulin strategies for preventing Rh hemolytic disease of the fetus and newborn: A network meta-analysis. *PLoS One*, *15*(3), p.e0230073.

YAHIA, A., MISKEEN, E., SOHAIL, S.K., ALGAK, T. and ALJADRAN, S., 2020. Blood Group Rhesus D-negativity and Awareness Toward Importance of Anti-D Immunoglobulin Among Pregnant Women in Bisha, Saudi Arabia. *Cureus*, *12*(2).

ZWIERS, C., SCHEFFER-RATH, M.E., LOPRIORE, E., DE HAAS, M. and LILEY, H.G., 2018. Immunoglobulin for alloimmune hemolytic disease in neonates. *Cochrane Database of Systematic Reviews*, (3).