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ORIGINAL ARTICLE

Spectrum of Maternal Red Cell Alloantibodies Identified in Pregnant Mothers in Perak State Hospital and the Neonatal Outcomes.

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Abstract

Red cell alloimmunization during pregnancy results from exposure to foreign red cell antigens due to fetomaternal hemorrhage or blood transfusions. Such alloantibodies can lead to hemolytic disease of the fetus and newborn (HDFN). This study sought to explore the prevalence and range of red cell alloantibodies among pregnant mothers at Hospital Raja Permaisuri Bainun (HRPB) and assess the perinatal outcomes associated with these pregnancies. Conducted over a period spanning from January 2016 to June 2020, this retrospective cross-sectional study was situated within the Transfusion Medicine Department (TMD) of HRPB. Data were sourced from TMD's database and the Hospital Information System (HIS), encompassing all registered pregnant women and their corresponding blood samples. Every antenatal mother underwent routine ABO and RhD typing, as well as the 3-screen red cell panel test. Additionally, antibody identification was performed on the blood samples of patients and referrals that tested positive for alloantibodies.The study findings disclosed that, out of the 467 mothers examined, 1.2% exhibited red cell alloantibodies. The majority of these mothers were of Malay ethnicity, followed by Indian mothers. Among them, 88.9% had a single antibody, while 11.1% had multiple alloantibodies. Clinically significant antibodies were observed in 58% of cases, with anti-D (31.7%), anti-E (11.3%), and anti-c (2.1%) being the most prevalent. Anti-Mi^{a} was the most frequently occurring non-clinically significant antibody, at 12.6%. Out of the 467 pregnancies, 363 had available outcomes, with 68.0% of newborns displaying jaundice, while 32.0% did not. Newborns born to mothers with clinically significant antibodies had an elevated risk of pathological jaundice (43.6%). Severe and lethal hemolysis cases were predominantly associated with RhD blood antigens, especially anti-D and anti-c.In conclusion, this research highlights the importance of early antenatal screening for alloantibodies, where prompt identification should be conducted to mitigate the risks associated with these antibodies.

Keywords: *haemolytic disease of foetus and newborn, neonatal outcome, perinatal outcome, red cell alloimmunization, spectrum of alloantibodies.*

Introduction

Alloimmunization of red blood cell (RBC) antigen in pregnancy may occur following blood transfusion or from fetomaternal haemorrhage during pregnancy or during delivery of the baby. When a mother is exposed to non-self RBC antigens, she may develop antibodies against that antigen, and this will pose a challenge to the obstetrician as the foetus will be at risk of developing haemolytic disease of foetus and newborn (HDFN). Risk of alloimmunization is dependent on the immunogenicity of the red cell antigen. Currently, there are 43 recognized blood group systems with 345 RBC antigens recognized by the International Society of Blood Transfusion Committee (ISBT) [1]. Hypothetically, the probability of a person develops antibody to red cells antigens should be high but in reality, rates of alloimmunization are low and it varies based on the study design. It can be as low as 0.74 [2]to 1- 1.2% in retrospective analysis [3][4] and 3.4% in prospective analysis in pregnant women [5]. Alloantibodies in pregnancy has the potential to cause destruction of red cells from transfer of antibodies across the placenta, causing intrauterine haemolysis with severe anaemia, leading to a hydropic foetus which is incompatible to life.

The state of Perak in Malaysia has 2.51 million people with multiracial breakdown of 59.5% Malays, 28.7% Chinese, 11.4% Indians, 0.4% ethnics from Sabah, Sarawak, and Orang Asli (indigenous people in Peninsular) and 4% are non-Malaysians [6]. Public health care services encompass 34 hospitals and Hospital Raja Permaisuri Bainun (HRPB), Ipoh is the state hospital for Perak and is the reference centre for Transfusion Medicine services in Kinta District, except Hospital Taiping. Studies on maternal red cell alloantibodies were few in numbers and were carried out on a dominant race in Malaysia [4]. Studies on HDFN resulting from maternal alloantibodies were also limited [7].

A study on prevalence of maternal alloantibodies in HRPB, Ipoh may be reflective of Perak state's prevalence of maternal alloantibodies since there exists diversity in ethnics and races in Perak state. This study was carried out to determine the prevalence and the relative frequencies of red cell antibodies in pregnant mothers and evaluate the pregnancy, and perinatal outcome of these pregnancies.

Methodology

This was a retrospective cross-sectional study on pregnant women from HRPB, whose blood samples were tested positive with indirect anti-globulin test (IAT). These patients were registered in Darah Link, the transfusion database of Transfusion Medicine Department (TMD) of HRPB from January 1, 2016, to June 30, 2020. All pregnant women who sought antenatal care, experienced miscarriages, had ectopic pregnancies, suffered intrauterine deaths, gave birth, or were postpartum mothers of neonates with jaundice and a positive IAT result were included in the analysis.

Method for ABO and Rhesus typing, screen and identification of red cell alloantibodies

All pregnant mothers had routine ABO and Rhesus typing as well as a 3-screen red cell panel test utilising one of these commercial brands: Serascan Diana 3, Diagnostic Grifols, Spain; Abtectcell^{TM} III, bioCSL Pty Ltd, Australia; and Bio-Rad ID-DiaCell I-II-III Asia, Japan. Cases with positive from the 3-screen red cell panel tests were then subjected to red cell antibody identification using one of the commercial 11 red cell panel brands: Identisera Diana/Extend, Diagnostic Grifols, Spain; Phenocell^{TM}, bioCSL Pty Ltd, Australia; and BioRad ID-DiaPanel 11, Japan. In cases involving several or complex antibodies, expanded red cell panels and further immuno-haematological investigations were carried out as necessary.

Mothers who were negative for Rhesus D were then tested for weak D using the direct haemagglutination tube method with anti-D IgM Diagast, France, and IAT tube method with anti-D IgG Novaclone®, Immucor Gamma, Dartmouth, Canada. Since the detected alloantibodies were only present in patients who were negative for the corresponding red cell antigen, genotyping of the antigen was carried out to confirm the identification of the alloantibody.

Red cell alloantibodies

The alloantibodies identified were categorised into clinically significant and not clinically significant[8]. Lawler and van Loghem [9] and MDJ Geiger [10] reported in 1947 and 1959, respectively, that anti-C^w is a clinically significant alloantibody causing HDFN and erythroblastosis fetalis. In this study, anti-C^w occurred together with anti-Le^a and is classified as clinically significant alloantibodies.

Mi^a, Mut, and Mur are hybrid glycophorins from the MNS blood group system, are encoded by the *GYP.Mur* gene. This hybrid gene's alloantibodies are classified as not clinically significant. Anti-Mi^a, anti-Mut, and anti-Mur detection was based on a positive reaction of the patient's serum with one of the three red cells in the 3-screen panel containing the Mi^a, Mut, and Mur antigens but a negative reaction with the other two red cells in the panel. Antibody identification tests using the 11 red cell panels were negative, implying that no other antibodies besides those directed against *GYP.Mur* were present in the patient samples. This is because the 11-cell panel lacked antigens for the *GYP.Mur* phenotype.

Data was extracted from TMD database, HIS of HRPB and hard copies of patients' results. All cases with complete and incomplete data were included.

Pregnancy, perinatal and neonatal outcome

Neonatal jaundice is defined as appearance of jaundice in newborns within the first week of life and with serum bilirubin levels (TSB) rises above 85 µmol/L (5 mg/dL)[11]. Physiological jaundice occurs from the inability of newborn's liver to clear bilirubin resulting in unconjugated hyperbilirubinaemia that appears after 24 hours of life (HOL) to 72 HOL and disappears after 14 days [11]. Pathological jaundice is clinical jaundice detectable in the first 24 HOL, and / or rapid increase in TSB >103 µmol/L/24 hours (6 mg/dL/hour). Pregnancy, perinatal and neonatal outcome were categorised into early fetal loss (miscarriage and ectopic pregnancy), intrauterine death (IUD), live newborns with no clinical jaundice, physiological jaundice, low-risk neonatal jaundice (NNJ), medium risk NNJ, and high-risk NNJ [11]. Risk assessment of NNJ is based on risk factors, which include isoimmune haemolytic disease, G6PD deficiency, weight loss >8%, previous history of sibling with jaundice, cephalhaematoma, and East Asian race [11]. Additionally, the level of TSB is mapped to the nomogram that designate risk at ≥ 36 weeks' gestational age with birth weight ≥ 2000 g or ≥ 35 weeks' gestational age with birth weight ≥ 2500g [11]. The live newborns were also categorised based on the type of treatment they received for jaundice: standard care without phototherapy, conventional phototherapy (CP), intensive phototherapy (IP), and a multitude of therapies like IP, exchange transfusions (ET), and/or immunoglobulin (IVIG).

Statistical analysis was performed using Statistical Package for Social Sciences software (SPSS) version 23. Categorical variables were expressed as percentages within groups and comparison across groups was analysed using Pearson's Chi-square test for independence of attributes. An alpha level of 5% was used, meaning that any p-value less than 0.05 was considered statistically significant.

Results

A total of 37,091 pregnant mothers' data was analysed from January 2016 to June 2020 and 467 mothers were identified to have red cell alloantibodies. This finding corresponds to a prevalence rate of 1.26%.

Demography of alloantibodies positive pregnant mothers

Malays made up the majority of these pregnant women who tested positive for red cell alloantibodies (60.7%), followed by Indians (24.6%), Chinese (5.4%), Orang Asli (4.5%), foreigners (3.0%), Sikhs (0.9%), and native Sabah women (0.9%) (Table1). Slightly more than half of these mothers (52.0%) were in the age group of 20 - 30 years (Table 2). The oldest pregnant women were 45 years, and the youngest was a 14-year-old teenager. The majority (63.2%; n=295) were multigravida, while 27.4% (n=128) were primigravida, according to Table 2. There were 22 (4.7%) pregnant teenagers (< 20 years), with 3 (0.6%) already in their second or third pregnancy ($p < 0.05$). The majority of primigravida (20.3%) were 20 - 30 years of age. There was almost equal distribution among the multigravida between the ages of 20 - 30 (30.4%) and 31 – 40 (30.0%). There were also young mothers aged 20-30 (1.3%; n=6) who were already grandmultipara.

The most common blood group of these mothers were group O (36.6%), followed by group B (30.6%), group A (24.4%), and group AB (8.4%). Out of these, 66.6% (n=311) of these mothers were Rh-D positive, 33.0% (n=154) were Rh-D negative, and 2 (0.4%) were identified with weak D (Table 1).

Spectrum of alloantibodies identified

The majority of cases (88.9%; n = 415) had a single antibody while 11.1% (n = 52) had multiple alloantibodies. Clinically significant antibodies were present in more than half of these women (58%; n = 271). The most frequently occurring antibody was anti-D (31.7%; n = 148), followed by anti-E (11.3%; n = 53), and anti-c (2.1%; n = 10) (Table 3). Therefore, the biggest percentage of clinically significant alloantibodies (46.3%; n = 216) were caused by Rhesus antigens. Within the group of mothers with anti-D, the majority were multigravida (64.9%; n = 96) and of the 20–30 age group (54.7%; n = 81); ($p<0.05$) (Table 4). It may be concerning that Anti-D was found in primigravida (29.1%; n = 43), and 3.4% (n = 5) were teenagers (< 20 years of age), but this may be explained by the routine administration of Anti-D immunoglobulin (Rhogam) to Rhesus D negative mothers. The anti-D detected could be due to in vivo contamination with Rhogam, which could explain the high prevalence of anti-D and the presence of anti-D in primigravida. Within the group with anti-E, 69.8% (n = 37) were from the multigravida, whereas 15.1% (n = 8) were primigravida. Anti-E and anti-c (3.4%; n = 16) were the most frequent multiple and clinically significant antibodies, followed by anti-C and anti-e (0.9%; n = 4). The majority of anti-E and anti-c occurred in multigravida (87.5%; n = 14), with one case seen in a primip (6.3%; n = 1) ($p <0.05$). Table 4 shows the 5 most frequent clinically significant alloantibodies. Anti c and anti S, the majority occurs in multigravida, have no significant association reported.

Less than half of pregnant mothers (42%; n = 42) had clinically non-significant alloantibodies (Table 3). Anti-Mi^a was the highest single, not clinically significant antibody detected (12.6%; n = 59), followed by anti-M (10.5%; n = 49), whereas anti-Le^a and anti-Le^b (3.6%; n = 17) were the most frequent multiple, non-clinically significant antibodies.

Pregnancy, perinatal and neonatal outcome

94.2 % of these women gave birth to singleton. Among these 467 mothers, there were a total of 477 foetuses, including a set of triplets, 7 sets of twins, a set of twins with an IUD, 5 intrauterine deaths (including the IUD twin), 12 miscarriages, 2 ectopic pregnancies, and the rest were singletons. Out of the 477 foetuses and newborns, 23.9% (n = 114) had undetermined outcomes, leaving 363 foetuses and newborns with known fates. Fourteen of these resulted in early foetal loss (miscarriages and ectopic pregnancies), and 5 died in utero.

Of the 477 foetuses and newborns, 59.9% (n=206) of newborns were from mothers who had clinically significant alloantibodies and 40.1% had non-clinically significant antibodies (Table 5).

Sixty eight percent of neonates had some degree of jaundice, compared to 32.0% who had no clinical jaundice. The majority of newborns to mothers with clinically significant antibodies had medium risk NNJ (35.9%; n=74) and 9.3% (n=32) had high-risk NNJs. Most of the infants (52.7%; n=58) born to women with non-clinically significant antibodies did not exhibit clinical jaundice. There is almost equal prevalence of babies with no clinical jaundice, born to mothers with clinically significant antibodies and born to mothers without clinically significant antibodies (Table 5; p=0.000). However, mothers with clinically significant antibodies have significant associations with the development of pathological jaundice in this group of babies (43.6%; n = 150; p = 0.000).

Table 6 shows 43.9% (n=151) of these infants require routine postpartum care without phototherapy. These infants either lacked clinical jaundice or had TSB levels that were below the level for phototherapy. Slightly more than half of these infants (56.1%; n = 193) of these infants required treatment for the jaundice; 47.4% (n = 163) required conventional phototherapy (CP), 6.1% (n = 21) required intensive phototherapy (IP), and 2.6% (n = 9) required multitudes of therapies, which included IP, exchange transfusion, and/or intravenous immunoglobulin (IVIG). Majority of babies that require treatment for jaundice were from mothers with clinically significant alloantibodies (63.0%), which included babies that were severely jaundiced that require multitudes of therapies.

IUDs and severe haemolysis

Table 7 displays the associations between the specificities of alloantibodies and the treatment received by the neonates. Anti-D was the most frequent alloantibodies. CP was the most frequent modality of treatment for 59 (17.2%) babies with maternal anti D, followed by 24 (7.0%) babies with anti E. Thirty-six babies with anti D presented with jaundice within first day of life. Within this group of babies with anti D, 28 were < 37 weeks at birth, 32 had birth weight < 2.5kg, 22 born to mothers affected by gestational diabetes or diabetic, 14 babies were presumed sepsis and 2 with G6PD deficiency. The severity of jaundice and the required treatment for jaundice were compounded by the other risk factors of NNJ. The 9 cases that required multitudes of treatment were mainly from antibody to Rhesus antigens, mainly from anti-D (1.5%; n=5), anti-c (0.6%; n=2), anti E and c (0.3%; n=1) and anti-D and C (0.3%; n=1).

Anti-Mi^a or low prevalence antigens, anti-M and anti-Le^a are the most common single non-clinically significant antibodies, with 58 (14.0%) of these babies treated with CP and 5 (1.4%) had IP. Other factors ie; maternal gestational diabetes, < 2.5g birth weight, prematurity, G6PD deficiency, and presumed sepsis contributed to the jaundiced state of these newborns. Two babies born to mother with Le^a alloantibodies had G6PD deficiency, while four newborns born to mothers with anti M, seven to anti Le^a and five to anti Mi^a were born before 37 weeks gestation (data not shown).

Cases of HDFN and rare maternal weak / variant D

One of the newborns with high-risk NNJ succumbed to severe haemolysis within the first 24 hours. This baby was born to a 38-year-old lady, G6P5, who underwent a Caesarean section. The MBG is O Rh D positive (R1R1 genotype). The baby was born with a good Apgar score but expired at 10 HOL from severe haemolysis. The baby's direct and indirect Coombs tests were 4+ and 3+, respectively. Anti-c was identified in both the mother's serum and baby's red cell elution test.

There were 2 cases of mothers with variant or weak D, with anti-D alloantibodies identified. The first case involved a 28-year-old Malay woman, G2P1, at 39 weeks and 4 days of gestation. Her blood group (MBG) was O, Rhesus weak / variant D, and genotype R1r. She had neurofibromatosis and group B streptococcus (GBS) positivity at 33 weeks, which were treated with IV ampicillin prior to delivery. Rhogam was administered at 34 weeks. Her husband's blood

group (HBG) was Rh D positive, and her first baby was Rh D negative with no history of severe NNJ or ET. For this pregnancy, she gave birth to a 2.44 kg baby girl, with cafe au lait spots indicating neurofibromatosis. The baby's blood group (BBG) was B Rh D positive and she had evidence of hemolysis at birth: retic counts of 10.7%, serum bilirubin levels of 75 umol/L, but her Coombs test came back negative and her haemoglobin level was 13.7 gm/dL. This neonate was categorised as high-risk NNJ and received 3 days of IP. The second case involved a 26-year-old Malay mother, G4 P3, at 38 weeks 5 day. The mother's blood group (MBG) was O weak D / variant D, R0R0 was the RhD genotype. Rhogam was given to her during pregnancy. The HBG and BBG were O Rh D positive. Baby's Coombs test was negative. Anti-D alloantibody was identified and presumably from the administration of Rhogam. This infant was classified as low risk, and no phototherapy was given. There was a case of severe Rhesus isoimmunization that required intrauterine transfusion in a 30-year-old Malay multigravida (G5P3+1) at 25 weeks 5 days of pregnancy. MBG was A negative and Anti-D was identified. Severe fetal anaemia with hemoglobin of 3.0 gm/dL was identified, and intrauterine transfusion was administered that increased the fetus's haemoglobin level to 13.5 gm/dL.

There were 1.4% (n=5) cases of IUD and with only one intrauterine death attributed to Rhesus isoimmunization. This case involved a primigravida who had received Rhogam at 28 weeks, but the IUD was detected on her antenatal visit at 30 weeks. The foetus was oedematous and histopathological examination showed abruptio placenta.

Discussion

This study reported a prevalence rate of 1.26% of red cell alloantibodies present in mothers in HRPB, which was higher than 0.74%, 0.99% and 1% reported by a study from a tertiary hospital in Southeast Michigan (USA)[2], study in Kelantan [4], and a study in Israel [17], respectively, but comparable with 1.2% reported by a study in Delhi [3]. Malays made up the dominant racial group in Perak, hence showing the highest prevalence of alloantibodies in this race. The Chinese were the second dominant racial group, but mothers of Indian descent have the second-highest occurrence of alloantibodies, while there was nearly equal occurrence of antibodies between the Chinese and the indigenous Orang Asli. There are no other studies across Southeast Asia that analyse the prevalence of alloantibodies among different ethnic groups. It is certainly interesting to note that the indigenous orang Asli has considerable occurrence of red cell alloantibodies. The study in Kelantan was conducted only among Malay mothers.

Exactly 63.2% of mothers with alloantibodies were multigravida, which can be best explained by the likelihood of exposure to non-self-antigens occuring in previous pregnancy. The exposure in a primigravida, and as young as 14-year-old teenager may be explained if these mothers had previous transfusion or experienced incidents of threatened abortion, leading to alloimmunization. Mixing of maternal and fetal blood during fetomaternal haemorrhage can occur throughout pregnancy with 3%, 12%, and 45% in first, second and third trimester respectively [17]. It is rare for alloantibody to be present in primigravida mothers, as alloimmunization typically occurs after delivery [17]. Unfortunately, this study did not capture the history of previous blood transfusion or potential triggering events that may cause the alloimmunization to occur.

Rhesus alloantibodies are the most common, with anti-D being the most prevalent. Severe and lethal haemolysis are seen with anti-D and anti-c (Table 7). According to a study conducted by the National Blood Centre in Kuala Lumpur, 97.5% of RhD-positive individuals in Malaysia are R1R1 (CDe/CDe) [18]. Therefore, any exposure to R2R2 (cDE/cDE) antigens, which is present in 4.7% of blood donors, may result in the production of alloanti-c and anti-E antibodies [18]. To avoid red cell hemolysis in the baby, it may be suggested that all pregnant women be Rh

genotyped and provide transfusion with the same Rh genotype. Monitoring levels of alloantibodies may be advantageous if red cell antibody screening is already being performed on a regular basis. ABO and Rhesus D typing are now routinely done in prenatal care. Only when the mother is Rh D negative, requires a blood transfusion or is undergoing a Caesarean section, Rhesus genotyping and alloantibody detection are performed.

Anti D detected in primigravida in our study was probably from the Rhogam administered to Rhesus D negative mothers. Iberahim S et al, reported a case of a primigravida with HDFN with multiple alloantibodies, involving anti Jk^a and anti-E [19]. In our study we found four cases with single Jk^a alloantibodies, with two cases showing no clinical jaundice in the babies. The other two cases involved a set of twins with moderate risk NNJ, prematurity, and birth weight <2.5kg who required conventional phototherapy. There was no obvious evidence of HDFN.

Anti-Mi^a was the highest among the not clinically significant antibody groups (12.6%), with a prevalence of 0.16% (59/37,091) among pregnant mothers. This finding is lower than a previous study in 2002, where the prevalence anti-Mi^a was 0.2% among 10,397 antenatal mothers in the study [18]. In our study, anti-Mi^a was predominantly found among Malay pregnant mothers (43/59; 72.9%).

Our findings showed that regardless of whether the mother's alloantibodies were clinically significant or not, 63% of babies from mothers with clinically significant alloantibodies and 45.6% of babies from mothers without clinically significant antibodies require treatment for jaundice. Being Southeast Asian was a risk factor present in all these infants. This is consistent with a study by MG Bentz et al., which found that Southeast Asians had a higher risk of jaundice readmission [20]. Nevertheless, mothers with clinically significant antibodies have a higher risk of babies with moderate to severe NNJ and require phototherapy in comparison to those without clinically significant antibodies.

Conclusion

In this 4½-year retrospective study, we successfully identified a prevalence rate of 1.26% of red cell alloantibodies in pregnant maternal population of Perak state hospital. Mothers of Malay and Indian descent, we believe, may have a higher likelihood of developing alloantibodies. Notably, clinically significant antibodies were more prevalent, with RhD antigens playing a prominent role. Anti-D and anti-c were identified as the most common alloantibodies responsible for severe and potentially life-threatening haemolysis in both unborn babies and newborns. It is crucial for antenatal mothers to undergo early screening for alloantibodies during pregnancy, and prompt identification of these antibodies in positive cases is essential.

Further research is warranted to determine whether RhD genotype testing should be incorporated into perinatal care practices and to assess the economic feasibility of exclusively providing R1R1 blood transfusions to R1R1 mothers.

Ethical approval

This study is registered National Medical Research Register (NMRR) with ID no: **NMRR-20-1231-55429** and obtained ethical approval from the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (MOH) with reference number: **KKM/NIHSEC/P20-1449**.

Conflict of Interest

No conflict of interest was declared by the authors.

Financial Disclosure

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Table 1. Characteristics of pregnant mothers with red cell alloantibodies.

		Frequency	Percentage
1.	**Race (Ethnicity)**	**(n= 467)**	**(%)**
	Malay	284	60.7
	Chinese	25	5.4
	India	115	24.6
	Orang Asli	21	4.5
	Sikh	4	0.9
	Foreigner	14	3.0
	Sabahan	4	0.9
2.	**Pregnancy**		
	Singleton	440	94.2
	Twins	7	1.5
	Triplets	1	0.2
	Miscarriage	12	2.6
	Intrauterine death	4	0.9
	Ectopic pregnancy	2	0.4
	Twin with single livebirth	1	0.2
	Total	467	
3.	**ABO Blood group**		
	A	114	24.4
	B	143	30.6
	AB	39	8.4
	O	171	36.6
4.	**Rhesus typing**		
	Positive	311	66.6
	Negative	154	33.0
	Weak or variant D	2	0.4

Table 2. Association between maternal age and parity

Age	**Primigravida** **n(%)**	**Multigravida (2-5)** **n(%)**	**Grandmultigravida (≥6)** **n(%)**	**Total** **n(%)**	**Significance** Pearson Chi - Square
< 20	19 (4.1)	3(0.6)	0 (0.0)	22(4.7)	p=0.000
20-30	95(20.3)	142(30.4)	6(1.3)	243(52.0)	
31-39	14(3.0)	140(30.0)	32(6.9)	186(39.8)	
>40	0 (0.0)	10(2.1)	6(1.3)	16(3.4)	
Total	128(27.4)	295(63.2)	44(9.4)	467(100)	

Table 3. Spectrum of RBC alloantibodies identified.

	RBC alloantibody		Frequency	(%)
1.	**Type of alloantibodies**			
	Clinically significant		271	58.0
	Not clinically significant		196	42.0
2.	**Number of antibodies**			
	Single		415	88.9
	Multiple ≥2		52	11.1
3.	**Antibody specificity**			
	Single and clinically significant			
	Rhesus	Anti-D	148	31.7
		Anti E	53	11.3
		Anti-e	2	0.4
		Anti c	10	2.1
		Anti C	3	0.6
	Kell	Anti K	2	0.4
	Kidd	Anti-Jk^{a}	6	1.3
		Anti-Jk^{b}	3	0.6
	MNSs	Anti-s	1	0.2
		Anti-S	7	1.5
	Duffy	Anti-Fy^{a}	1	0.2
		Anti-Fy^{b}	2	0.4
	Single and not clinically significant			
	MNS	Anti-M	49	10.5
		Anti-Mi^{a}	59	12.6
	Lewis	Anti-Le^{a}	51	10.9
		Anti-Le^{b}	14	3.0
	P1PK	Anti-P1	4	0.9
	Multiple and clinically significant			
		Anti-K, -Le^{a}	1	0.2
		Anti-E, -c	16	3.4
		Anti-C,-e	4	0.9
		Anti D, E	2	0.4
		Anti D and C	1	0.2
		Anti e, c , Fy^{a}	2	0.4
		*Anti Jk^{a}, Jk^{b}/ Anti Jk^{a}, Fy^{b}/ Anti E, anti Mi^{a}/ Anti-C, Anti-Le^{b} Anti-Mur/ Anti e, anti Jk^{a}/ Anti-M, anti Fy^{b}/ Anti Le^{a}, anti Cw	7	1.4
	Multiple and not clinically significant			
		Anti Le^{a}, Le^{b}	17	3.6
		**Anti-M, anti Le^{b}/ Anti Mut, Anti Mur	2	0.4

* This is a collective (n) of groups of multiple clinically significant antibodies which has only 1 patient per group.** This is a collective (n) of groups of multiple non clinically significant antibodies which has only 1 patient per group.

Table 4. Association between 5 most frequent clinically significant alloantibodies with age and parity.

Antibody	Age	Primigravida	Multigravida	Grandmultip	Total	Chi-square
Anti D (n=148;31.7%)		n (%)	n (%)	n (%)	n (%)	
	<20	5(3.4)	-	-	5(3.4)	P= 0.000
	20-30	33(22.3)	47(31.8)	1 (0.7)	81 (54.7)	
	31-39	5(3.4)	46 (31.1)	7 (4.7)	58 (39.2)	
	>40	-	3 (2.0)	1 (0.7)	4 (2.7)	
	Total	43 (29.10)	96 (64.9)	9 (6.1)	148 (100)	
Anti E	<20	1 (1.9)	-	-	1 (1.9)	P= 0.079
(n=53;11.3%)	20-30	5 (9.4)	14 (26.4)	1 (1.9)	20 (37.7)	
	31-39	2 (3.8)	21 (39.6)	6 (11.3)	29 (54.7)	
	>40	-	2 (3.8)	1 (1.9)	3 (5.7)	
	Total	8 (15.1)	37 (69.8)	8 (15.1)	53 (100)	
Anti E &	<20	1 (6.3)	-	-	1 (6.3)	P=0.002
Anti c	20-30	-	6 (37.5)	1 (6.3)	7 (43.8)	
(n=16;3.4%)	31-39	-	8 (50.0)	-	8 (50.0)	
	>40	-	-	-	-	
	Total	1 (6.3)	14 (87.5)	1 (6.3)	16 (100)	
Anti c	<20	-	-	-	-	P=0.053
(n=10; 2.1%)	20-30	-	6 (60.0)	-	6 (60.0)	
	31-39	-	2 (20.0)	2 (20.0)	4 (40.0)	
	>40	-	-	-	-	
	Total	-	8 (80.0)	2 (20.0)	10 (100)	
Anti S	<20	-	-	-	-	P=0.190
(n=7;1.5%)	20-30	-	2 (28.6)	-	2 (28.6)	
	31-39	-	3 (42.9)	1 (14.3)	4 (57.1)	
	>40	-	-	1 (14.3)	1 (14.3)	
	Total	-	5 (71.4)	2 (2.6)	7 (100)	
Anti Jk^a	<20	-	-	-	-	P=0.269
(n=6;1.3%)	20-30	1 (16.7)	3 (50.0)	-	4 (66.7)	
	31-39	-	1 (16.7)	1 (16.7)	2 (33.3)	
	>40	-	-	-	-	
	Total	1 (16.7)	4 (66.7)	1 (16.7)	6 (100)	

Table 5. Association between mothers with types of alloantibodies with outcome of live newborns.

Types of Alloantibodies	No jaundice	Physiological jaundice	Low Risk NNJ	Medium Risk NNJ	High Risk NNJ	Total	Chi-square
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
Clinically significant	52 (15.1)	4 (1.2)	44 (12.8)	74 (21.5)	32 (9.3)	206 (59.9)	P= 0.000
Not clinically significant	58 (16.9)	9 (2.6)	31 (9.0)	32 (9.3)	8 (2.3)	138 (40.1)	
Total	110 (32.0)	13 (3.8)	75 (21.8)	106 (30.8)	40 (11.6)	344 (100)	

Table 6. Association between mothers with types of alloantibodies with treatment of live newborns

Types of Alloantibodies	No treatment	Conventional phototherapy	Intensive phototherapy	Multiple treatment	Total	Chi-square
	n (%)	n (%)	n (%)	n (%)	n (%)	
Clinically significant	76 (22.1)	106 (30.8)	15 (4.4)	9 (2.6)	206 (59.9)	P= 0.002
Not clinically significant	75 (21.8)	57 (16.6)	6 (1.7)	0 (0.0)	138 (40.1)	
Total	151 (43.9)	163 (47.4)	21 (6.1)	9 (2.6)	344 (100)	

Table 7. Association between mother's alloantibodies with treatment of live newborns

Specificity of antibodies		No treatment n (%)	Conventional phototherapy n (%)	Intensive phototherapy n (%)	Multiple treatment n (%)	Total n (%)
Rhesus	Anti D	41 (11.9)	59 (17.2)	9 (2.6)	5 (1.5)	114 (33.1)
	Anti E	17 (4.9)	24 (7.0)	3 (0.9)	0 (0.0)	44 (12.8)
	Anti e	1 (0.3)	1 (0.3)	0 (0.0)	0 (0.0)	2 (0.6)
	Anti C	1 (0.3)	0 (0.0)	1 (0.3)	0 (0.0)	2 (0.6)
	Anti c	4 (1.2)	1 (0.3)	0 (0.0)	2 (0.6)	7 (2.0)
Kell	Anti K	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
Kidd	Anti Jk^a	2 (0.6)	2 (0.6)	0 (0.0)	0 (0.0)	4 (1.2)
	Anti Jk^b	2 (0.6)	1 (0.3)	0 (0.0)	0 (0.0)	3 (0.9)
Ss	Anti S	0 (0.0)	4 (1.2)	0 (0.0)	0 (0.0)	4 (1.2)
	Anti-s	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
Duffy	Anti Fy^a	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
	Anti Fy^b	0 (0.0)	2 (0.6)	0 (0.0)	0 (0.0)	2 (0.6)
MN	Anti-Mi^a	30 (8.7)	15 (4.4)	2 (0.6)	0 (0.0)	47 (13.7)
	Anti M	18 (5.2)	16 (4.7)	3 (0.9)	0 (0.0)	37 (10.8)
Lewis	Anti Le^a	18 (5.0)	17 (4.7)	0 (0.0)	0 (0.0)	35 (10.2)
	Anti Le^b	4 (1.2)	2 (0.6)	0 (0.0)	0 (0.0)	6 (1.7)
P1PK	Anti P1	2 (0.6)	1 (0.3)	0 (0.0)	0 (0.0)	3 (0.9)
anti E, anti c		3 (0.9)	6 (1.7)	2 (0.6)	1 (0.3)	12 (3.5)
anti C, anti e		1 (0.3)	2 (0.6)	0 (0.0)	0 (0.0)	3 (0.9)
anti D, anti E		0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)	1 (03)
anti D, anti C		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	1 (0.3)
anti E, anti-Mi^a		0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.3)
anti e, anti c, anti Fy^a		0 (0.0)	1(0.3)	0 (0.0)	0 (0.0)	1 (0.3)
anti C, anti Le^b, anti Mur		0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.3)
anti Le^a, anti Cw		1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
anti Le^a, & Le^b		2 (0.6)	6 (1.7)	1 (0.3)	0 (0.0)	9 (2.6)
anti M, anti Le^b		1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
Total		151 (43.9)	163 (47.4)	21 (6.1)	9 (2.6)	344 (100)

References

[1]. International Society of Blood Transfusion Working Party on Red Cell Immunogenetics and Blood Group Terminology. https://www.isbtweb.org/isbt-working-parties/rcibgt.html (accessed on 20th September 2022)

[2]. Moinuddin I, Fletcher C, Millward P. Prevalence and specificity of clinically significant red cell alloantibodies in pregnant women - a study from a tertiary care hospital in Southeast Michigan. J Blood Med. 2019 Aug 20;10:283-289. doi: 10.2147/JBM.S214118.

[3]. Sangeeta Pahuja, Santosh Kumar Gupta, Mukta Pujani, Manjula Jain. The Prevalence of Irregular Erythrocte Antibodies among antenatal women in Delhi. Blood Transfusion (Oct 2011); 9(4): 388-393 doi:10.2450/2011.0050-10

[4]. Mohd Nazri Hassan, Noor Haslina Mohd Nor, Shah Reza Johan Noor, Salamah Ahmad Sukri, Rapiaah Mustafa. Red Blood Cell Alloimmunization among Malay Pregnant Women: A Tertiary Hospital Experience. International Medical Journal (June 2015) Vol 22,No. 3, pg 154-158.

[5]. Jeremiah ZA, Mordi A, Buseri FI, Adias TC. Frequencies of maternal red blood cell alloantibodies in Port Harcourt, Nigeria. Asian J Transfus Sci. 2011 Jan;5(1):39-41. doi: 10.4103/0973-6247.75987.

[6]. Department of Statistics. Pocket Stats Perak Quarter1, 2020, published on May 2020. Accessed on 20th September 2022 https://www.dosm.gov.my/v1/index.php?r=column/cone&menu_id=RTRycHhPcisweHpMdlVwKzhMY25XUT09

[7]. Mohd Nazri Hassan, Noor Haslina Mohd Nor, Shah Reza Johan Noor, Salamah Ahmad Sukri, Rapiaah Mustafa, Hans Van Rostenberghe Luc Aster.Haemolytic Disease of Fetus and Newborn due to Maternal Red Blood Cell Alloantibodies in the Malay population. Asian J Transfus Sci, Vol 8(2), 2014 pg 113-117. doi:10.4103/0973-6247.137449

[8]. Transfusion Practice Guidelines for Clinical and Laboratory personnel published by Ministry of Health Malaysia in 2016

[9]. Lawler SD, Van Loghem JJ, Jr. The rhesus antigen CW causing haemolytic disease of the newborn. Lancet 1947; 2: 545

[10]. MDJ Geiger. Erythroblastosis fetalis caused by sensitization to factor rhw (Cw),The Journal of Pediatrics, Volume 54, Issue 4,1959,pages 484-87,https://doi.org/10.1016/S0022-3476(59)80107-4.

[11]. Ismail, H.I.H.M., Ibrahim, H.M., Ng, H.P., Kesihatan, M.K. and Thomas, T. (2019) Paediatric Protocols for Malaysian Hospitals. 4th Edition, Ministry of Health, Putrajaya.

[12]. Hafizuddin A., Siti Mariam J., Nurul Adhiyah W.I., Zawiyah D., Determinants of

Neonatal Jaundice Among Newborns in Pasir Puteh District, Kelantan. International Journal of Public Health and Clinical Sciences; Vol 6: No 6: page 109-122; https://doi.org/10.32827/ijphcs.6.6.109.

[13]. Menachem Fisher. Acute RH Isoimmunization Following Abdominal Trauma Associated with Late Abruption Placenta. Acta Obstetricia et Gynecologica Scandinavia. Vol 68, Issue 7, 1989; pg657-659; https://doi.org/10.3109/00016348909013289.

[14]. May-Wewres J, Kaiser JR, Moore EK, et al. Severe neonatal analysis due to maternal antibody to low frequency Rh antigen Cw. Am J Perinatol. 2006;23:213-217.

[15]. Rahimi-Levene N, Chezar J, Yahalom V; Israeli HDFN Study Group Investigators. Red blood cell alloimmunization prevalence and hemolytic disease of the fetus and newborn in Israel: A retrospective study. Transfusion. 2020 Nov;60(11):2684-2690. doi: 10.1111/trf.15987. Epub 2020 Aug 8. Erratum in: Transfusion. 2022 Jul;62(7):1466. PMID: 32770778.

[16]. Prathiba R, Lopez CG, Usin FM. The prevalence of GP Mur and anti-"Mia" in a tertiary hospital in Peninsula Malaysia. Malays J Pathol. 2002 Dec;24(2):95-8. PMID: 12887167.

[17]. Delaney M, Matthews DC. Hemolytic disease of the fetus and newborn: managing the mother, fetus, and newborn. Hematology Am Soc Hematol Educ Program 2015;2015(1):146-151.

[18]. Musa RH, Ahmed SA, Hashim H, Ayob Y, Asidin NH, Choo PY, Al-Joudi FS. Red cell phenotyping of blood from donors at the National blood center of Malaysia. Asian J Transfus Sci. 2012 Jan;6(1):3-9. doi: 10.4103/0973-6247.95042. PMID: 22623834; PMCID: PMC3353626.

[19]. Iberahim S, Aizuddin MJ, Kadir NA, Rameli N, Adzahar S, Noor NHM, Abdullah WZ. Hemolytic Disease of Fetus and Newborn in a Primigravida with Multiple Alloantibodies Involving Anti-Jk^{a} and Anti-E: A Case Report. Oman Med J. 2020 Nov 30;35(6):e206. doi: 10.5001/omj.2020.135.

[20]. Bentz MG, Carmona N, Bhagwat MM, Thimmig LM, Saleh J, Eke U, Kokroko J, Dadasovich R, Rice B, Cabana MD. Beyond “Asian”: specific east and southeast Asian races or ethnicities associated with jaundice readmission. Hospital Pediatrics. 2018 May;8(5):269-73. https://doi.org/10.1542/hpeds.2017-0234