Journal of Hematology and Blood Disorders
Volume 9 | Issue 1
ISSN: 2455-7641

Research Article Open Access

Prevalence of Alloimmunizations in Moroccan Pregnant Women

S Lagzoum[1,2*], H Anibat[2], S Achargui[3], A Belhachemi[4], N El Amraoui[5], H Abdelghaffar[1] and N Habti[2,5]

[1]Hassan II University of Casablanca, Faculty of Sciences and Techniques of Mohammedia, Morocco
[2]Hassan II University of Casablanca, Faculty of Medicine and Pharmacy of Casablanca, Morocco
[3]Regional Center for Blood Transfusion, Rabat, Morocco
[4]Blood Bank of Kenitra, Morocco
[5]National Center for Blood Transfusion and Hematology, Rabat, Morocco

[*]**Corresponding Author:** S Lagzoum, Hassan II University of Casablanca, Faculty of Sciences and Techniques of Mohammedia, Morocco. Tel: +212634798601 E-mail: soukainalagzoum@gmail.com

Citation: S Lagzoum, H Anibat, S Achargui, A Belhachemi, N El Amraoui, H, et al. (2023) Prevalence of alloimmunizations in Moroccan Pregnant Women. J Hematol Blood Disord 9(1): 101

Abstract

Introduction: Erythrocyte fetomaternal incompatibility is defined by the binding of erythrocyte alloantibodies of a pregnant woman to fetal antigens, inherited from the father. Alloantibodies are produced by the immune system after prior contact with these same antigens during transfusion, transplant or previous pregnancy. In Morocco, data concerning fetomaternal incompatibilities are insufficient despite the severe cases of hemolytic disease in newborns recorded. Our aim is to determine the prevalence of anti-erythrocytes alloimmunizations in Moroccan pregnant women.

Patients and Methods: A population of parturients with no obstetrical history was randomly recruited to the Blood Bank of Kenitra. A second population was formed by pregnant women referred to the Regional Blood Transfusion Center of Rabat for reasons of obstetrical and/or immunohaematological history. The tests were carried out using the gel cards and 2 panels of 11 red blood cells. Antibody titration was performed by indirect Coombs technique.

Results: Immunohematological tests and analysis of clinical-biological data made it possible to detect and confirm alloimmunization in 16 RhD negative and positive women. The prevalence of alloimmunization in RhD negative women is 2.46 % and the prevalence of alloimmunization in both RhD-/+ parturients was 1,06 %. In parturients with an obstetric history, the frequencies of alloantibodies are respectively 35.48% (11/31) and 100% (3/3) directed against 7 antigenic specificities: D, C, E, c, e, Jka and s. None of the parturients had received timely anti-D prophylaxis or prenatal RhD genotyping.

Conclusion: The discussion of our results highlights the problem of the management of pregnant women at risk of alloimmunization, putting Morocco in front of a challenge of setting up a health policy, including above all the training of health professionals and the accessibility at the national level to the biological examinations necessary for the follow-up of the pregnant women to reduce the prevalence of the HDFN.

Keywords: Alloantibodies; Immunized Multiparous Women; Identification of Antibodies; Feotomaternal Incompatibility

Introduction

Erythrocyte feotomaternal incompatibility (EFMI) is defined by the binding of anti- erythrocytes alloantibodies of a pregnant woman to fetal antigens, inherited from the father. These alloantibodies could also be produced by the immune system transfusion, transplant or previous pregnancy. Their placental transfer and binding to fetal erythrocyte antigenic targets cause hemolytic disease in fetus and newborn (HDFN). The clinical forms are varied, ranging from anemia with moderate neonatal hyperbilirubinemia to major fetal damage with in-utero death by hydrops fetalis (Bricca P. et al, 2011, Hamani F. et al, 2017).

The EFMI includes a varied set of anti-erythrocyte antibodies, but the most important in terms of frequency/danger ratio is anti-D (RH1), anti-K (KEL1) and anti-c (RH4), which expose to the risk of severe anemia from the fetal stage. The risk of developing complications is lower with antigens from other erythrocyte blood group systems (JK, FY, MNS). Indeed, the immunogenicity of erythrocyte antigens varies from one blood group system to another, and from one antigen to another within the same blood group system (Larsen et al, 2002; Ait Sebouani et al, 2021). While global statistics on the incidence of HDFN in pre-prophylaxis, particularly in Morocco, are not available, there is also a lack of comprehensive information on the decrease in this pathology following the implementation of prophylaxis since the 1970s. Nevertheless, this practice has been sufficiently generalized in France to divide the risk of alloimmunization by 10 in 30 years, thus reducing the rate of cases of fetomaternal incompatibility identified from 6 to 10% of live births in 1970 to less than 1% in 2006 (0.5 to 2%) (Cortey, A. et al, 2006; Cortey, A. et al, 2012). Thus, in the US population, the incidence has declined from 43.3 per 1000 samples of women of childbearing age in 1967 to 2.6 per 1000 in 1996 (Geifman-Holtzman et al,1997; Queenan et al,1969). Overall, 1.1% of women in a U.S. study were identified as carrying an antibody previously reported to be associated with hemolytic disease of the fetus/newborn (Geifman-Holtzman et al, 1997).

In Morocco, despite its availability, gamma-anti-D prophylaxis is not yet generalized, given the lack of infrastructure and health care resources in rural areas and the fact that there is no legislation to make ABO-Rh blood grouping and complete Rh/Kell phenotyping mandatory for pregnant women. There are also no recommendations for testing for irregular agglutinin in the management of parturients during pregnancy to detect and monitor maternal immunization. In addition, the data concerning the prevalence of alloimmunizations and anti-erythrocytes antibodies remain insufficient since the studies of EFMI are few, or even non-existent, thence, our aim is to determine the prevalence of anti-erythrocytes alloimmunizations in Moroccan pregnant women.

Patients and methods

Patients

The study concerned RhD negative and RhD positive parturients referred by the Maternity services and by private sector doctors in Kenitra and Rabat, within the framework of immunohaematological monitoring of pregnancies and diagnosis of EFMI. The parturients received at the Blood Bank of Kenitra from February 2020 to July 2022, were randomly recruited without any obstetrical and/or immunohaematological history. Blood samples were taken in 5 ml tubes with ethylenediaminetetraacetic acid (EDTA), 2 to 3 weeks before delivery. The percentages of antigenic specificities recognized by the alloantibodies were determined in pregnant women referred to the CRTS of Rabat because of obstetrical and/or immunohaematological history. Blood samples were taken in 5 ml tubes with EDTA, from March 2021 to July 2022 throughout the pregnancy. The data collected from the medical files of all the parturients concerned the obstetric, transfusion or transplantation history as well as the gestational age.

Immunohaematological tests

All the practical part was carried out in the laboratory of immunohematology of the CRTS of Rabat. Tests were performed using ID-Card-DiaClon gel cards (Biorad) according to the supplier's instructions: ABO/D+Reverse Grouping for ABO-Rh blood grouping, Rh- Subgroups+K for Rhesus phenotyping and Liss-Coombs polyspecific for irregular agglutinin test (IAT). When the latter was positive, the antibodies were identified on polyspecific gel cards using 2 panels of 11 red blood cells (Biorad): Id-DiaPanel and ID-DiaPanel-P. Their titration was carried out in glass tubes by the indirect Coombs technique with the polyvalent antiglobulin (A.H.G Elite Lorne).

Ethics requirement

The study was authorized by the health establishments. All parturients signed an informed consent form to participate in the study.

Results

A total of 221 parturients meeting the inclusion criteria consented to participate in our work. Random recruitment at the Blood bank of Kenitra involved 187 parturients. A number of 106 of whom were RhD positive vs 81 RhD negative. Alloimmunization involved only 2 RhD negative women among 81 (2.46%). At the CRTS of Rabat, among the 34 pregnant women (31 RhD negative vs 3 RhD positive), 14 (41.17%) had one or more alloantibodies: 7 different antigenic specificities. The analysis of the data from the medical files made it possible to impute the 16 alloimmunizations exclusively to the feotomaternal erythrocyte incompatibilities (table n°I). The prevalence of alloimmunized women and the percentages of alloantibodies identified in RhD – and RhD+ parturients at the CRTS of Rabat and the Blood Bank of Kenitra are presented in table n°II.

Table 1: Immuno-hematological and obstetrical data of alloimmunized parturients recruited in Rabat and Kenitra

ParturientN°/City	PhenotypeABO Rh K	Pregnancy N° / age	Ig anti-D A GN°	IUT N =	Ab/titer -1
1/Kenitra	B CcdeeK-	G3 / 6th month	No	0	Anti Jka / ND
2/Kenitra	O cde K-	G4 / 9th month	No	0	Anti-D/16Anti-C/ND
3/Rabat	B cde K-	G3/9th month	No	0	Anti-D/32Anti-C/ND
4/Rabat	A cde K-	G6 /7th month	No	1	Anti-D/64Anti-C/ND
5/Rabat	A cde K-	G6 /9th month	G4 andG5	0	Anti-D / month 4: 4 and month 9: 64
6/Rabat	A cde K-	G4 /5th month	G2 andG3	3	Anti-D/128
7/Rabat	O cde K-	G9 /1st month	No	0	Anti-D/1024
8/Rabat	B Ccdee K-	G2/8th month	No	0	Anti-D / month 3: 4 and month 8: 512
9/ Rabat	O cde K-	G2/9th month	No	0	Anti-D / month 3: 2 and month 9: 128

10/Rabat	O cde K-	G2/9th month	No	0	Anti-D / month 7:2 and month 9:16
11/Rabat	A cde K-	G5 /4th month	G2 andG3	5	Anti-D/128
12/Rabat	A cde K-	G4 /9th month	G3	1	Anti-D/256Anti-(C+E) / ND
13/Rabat	O cde K-	G8 /7th month	No	4	Anti-(D+C+s) / ND
14/Rabat	AB CCDee K-	G2 / 7th month	No	0	Anti-c/128
15/Rabat	O CCDee K-	G4 /4th month	No	2	Anti-c/128
16/Rabat	A ccDEE K-	G2 / 9th month	No	0	Anti-e / ND

Ab: antibodies, IUT N°: number of intrauterine transfusion, G: gestation, N°: Number of pregnancies, ND: not determined, Rabat: Regional center for blood transfusion, Kenitra: Blood bank of kenitra.

Table 2: Prevalence of alloantibodies identified in parturients in Rabat and Kenitra

RhD negative					RhD Positive			
Rabat N = 31 / Kenitra N = 81					Rabat N = 3 / Kenitra N = 106			
Antibodies	**D**	**C**	**E**	**s**	**Jka**	**c**	**e**	Total
Rabat : N / %	11 / 35,48	4 / 12,90	1/ 3,22	1/ 3,22	0	2 / 66,66	1/ 33,33	14/ 41,17
Kenitra : N / %	1 / 1,23	1 / 1,23	0	0	1 / 1,23	0	0	2 / 1,06

N / %: number/percentage of immunized parturients.

Discussion

In order to update the data concerning EFMI in Morocco, we determined the prevalence of alloimmunization in both RhD-/+ parturients (1.06%). Kenitra, is the metropolis of a region whose population (498,000 inhabitants) comes from all regions of Morocco because of its industrial and agricultural socio-economic attractiveness. The results obtained would roughly reflect those at the national level, to extend the study to other regions of the country. Thus, the prevalence of 1.06% would be comparable to those reported in different countries (table n° III).

Table 3: Comparison of alloimmunization prevalence in different countries

Country and/or Region (study)	**Prevalence of alloimmunization in RhD - /+ parturients**
Our present study	1.06%
Tunisia (A. Hachicha et al, 2021)	1%
Nigeria (Zazzheaus et al, 2011),	3.40%
Sudan, Khartoum (N. Abdelateif et al, 2022)	10.80%
Saudi Arabia (M. Abdelrahman et al, 2016)	1.40%
Iran (Shahverdi E et al, 2017)	4.50%

India, Delhi, (S. Pahuja et al, 2011)	1.48%
South India (J. Varghese et al, 2013)	1.25%
France (E. Maisonneuve et al, 2020)	1%

This difference in alloimmunization prevalence between these studies was expected, as antibody prevalence rates were known to vary between countries. It is worth mentioning that comparison of the results of the different studies between them is problematic, due to differences in health policies which are applied in each country and considered as the main issue. In contrast, the lack of financial support for the follow-up of pregnant women could be the second issue. As it seems that the recommended consigns do not appear to be respected when compared to other countries that tend to provide full support.

In our study, the alloantibodies identified in immunized parturients recognized 7 different antigenic specificities, we note a clear predominance of antibodies of the Rhesus system, in the first rank of which is the anti-D which represents 35.48% of all immunizations, followed by the Anti- C. Although our results of predominance of anti-RhD compared to other antibodies are consistent with the literature (Mannessier L,2009; Reem Ameen et al,2005; Pilar solves et al,2017; Cécile Toly-Ndour et al,2021).

The presence of obstetrically significant anti-erythrocytic antibodies (Ab) in pregnant women requires an assessment of the hemolytic risk in utero by close immunohaematological monitoring including titration. The titration of Abs is a semi-quantitative method that makes it possible to monitor the evolution of the titer during pregnancy to detect a reactivity of the alloimmunization by the passage of the fetal red blood cells in the maternal circulation which can occur as early as the first trimester of pregnancy. The hemolytic potency of an alloantibody depends on its concentration and affinity (Hadef, R et al, 2016).

Titration is mandatory in any RhD negative pregnant woman with immune anti-D or other antibodies. The titers obtained in our study, varying from 4 to 1024, are similar to those reported in the literature (A. Hachicha et al, 2021; Loukil T et al, 2012; Mailloux A et al, 2011).

A study conducted by Rahman et al. Found high titers that could lead to HDFN in 42.85% of women in their 3rd trimester of pregnancy. In general, a titre of 16 to 32 is considered an important factor in the occurrence of HDFN (Rahman MM et al, 2011). However, antibody titers consistently below the critical laboratory titer throughout pregnancy predict an unaffected or mildly to moderately affected fetus (Harmening DM et al, 2008). Indeed, the identification and prenatal titrations of antibodies make it possible to predict the severity of HDFN. Thus, adapted management would save the fetus/newborn in utero through intrauterine transfusions and exchange transfusion.

Concerning alloimmunized RhD negative mothers, they must be followed serologically and management strategies for a safe delivery are necessary (Bowman et al, 1997). This requires good management, which begins with an investment in a comprehensive immunohaematological facility and expertise for intrauterine transfusions. The affected newborn could then benefit from intensive phototherapy and exchange transfusions (Gottstein & Cooke et al, 2003). An indicated in utero blood transfusion can be performed as early as the 18th week of amenorrhea until the beginning of the 9th month of pregnancy. It consists of an ultrasound-guided puncture of the umbilical vein with first confirmation of the anemia and then performing the transfusion of the fetus (Paul Maurice et al, 2021). In our study, in utero blood transfusion was performed in 6 fetuses whose mothers were multiparous and immunized by EFMI. None of them had a history of transfusions and none had received prophylactic γ-anti-D or late after being immunized by EFMI (Parturients n° 5, 6, 11 and 12). None was able to benefit from prenatal RhD genotyping before immunization, knowing that the latter is only available occasionally. These observations highlight the difficulties regarding the care of pregnant women in Morocco, which difficulties are increased for women from low-income families, unable to overcome the costs of biological tests and examinations.

Conclusion

Our results reflect the problem of the management of pregnant women at risk of alloimmunization in our country. The anti-erythrocyte alloimmunization remains a primary concern given its clinical consequences. A health policy including the awareness and training of health professionals as well as the establishment of a standard protocol for the screening of all pregnant women regardless of their Rhesus phenotype is necessary. It is also necessary to systematically ensure that prophylactic gamma anti-D injections are performed according to a reference protocol as well as perinatal immunohaematological tests for pregnant women and fetuses/newborns. The challenge for Morocco is that these tests, including prenatal Rh D genotyping, should be available nationwide in all health facilities, which would certainly lead to a decrease in the prevalence of HDFN.

References

1. Abdelateif NM, Mohamed AA (2022) Frequency and specificity of red blood cells alloantibodies among Sudanese multiparous women. Journal of Bioscience and Applied Research 89-92.

2. Abdelrahman M, Bolad A, Gader AA, Naem M (2016) Frequencies of Allo-antibodies In Pregnant Women.

3. Ameen, Reem (2005) Frequency of red blood cell alloantibody in Kuwaiti population. Medical Principles and Practice 14: 230-4.

4. Bowman J (1997) The management of hemolytic disease in the fetus and newborn. Semininars in Perinatology 21: 39-44.

5. Bricca P, Guinchard E, Guitton Bliem C (2011) Prise en charge des allo-immunisations fœto- maternelles antiérythrocytaires. Transfus Clin Biol 18: 269-76.

6. Cortey A (2006) Data from the French National College of Obstetrician Gynecologists (CNGOF). Prevention of fetomaternal Rhesus D alloimmunization. Full text and short text of the recommendations. Journal of Obstetrics Gynecology and Reproductive Biology. 35: S135□81.

7. Cortey A (2012) Incompatibilités fœtomaternelles érythrocytaires. EMC - Pédiatrie - Mal. Infect. 7: 1–22.

8. Emeline Maisonneuve (2020) Management of fetal-maternal erythrocyte alloimmunization.

9. Geifman-Holtzman O, Wojtowycz M, Kosmas E, Artal R (1997) Female alloimmunization with antibodies known to cause hemolytic disease. Obstet Gynecol 89: 272-5.

10. Gottstein R, Cooke RWI (2003) Systematic review of intravenous immunoglobulin in haemolytic disease of the newborn. Archives of Disease in Childhood-Fetal and Neonatal Edition 88: F6-10.

11. Hachicha A, Jannet JB, Salem SF, Benlakhal F, Elborgi W, et al. (2021) Anti-erythrocyte alloimmunization in obstetrics. Clinical and Biological Transfusion 28: S80.

12. Hadef R, Zahid H, Yahyaoui A, Labrini F, Uwingabiye J, et al. (2016) Immuno-pathological aspect of haemolytic disease in newborns linked to RH1 incompatibility. Moroccan Journal of Medical Sciences 20(3).

13. Hamani F, Mosbahi Z (2017) Knowledge-Attitudes survey of health professionals on the prevention of fetomaternal anti-RH1 alloimmunization. Harmening DM, 2008. Modern blood banking and transfusion practices. 5. New Delhi: Jaypee Brothers 387–389.

14. Larsen YBFPMM (2002) Prenatal diagnostics and follow-up of erythrocyte allo-immunizations. Sheets of Biology XXXXIII (n°245).

15. Loukil T, Amor I, Mnif H, Gargouri J (2012) Anti-RH1 antibody titration in pregnant women: Comparison of two techniques. J.I. Mr. Sfax No. 26: 35-9.

16. Mailloux A, Dufaux M, Zorayan V, Huguet-Jacquot S, Larsen M, et al. (2011) Attempt to correlate two techniques for titrating anti-erythrocyte antibodies in immunized pregnant women. Transfus Clin Biol 18: 370.

17. Mannessier L (2009) Immunohematological monitoring of pregnant women: new recommendations. Transfus Clin Biol 16: 195-200.

18. Maurice P (2021) Transfusion in utero: indications, modalities, specificities and issues. Clinical and Biological Transfusion 28: S22.

19. Pahuja S, Gupta SK, Pujani M, Jain M (2011) The prevalence of irregular erythrocyte antibodies among antenatal women in Delhi. Blood Transfus 9: 388-93.

20. Queenan JT, Smith BD, Haber JM, Jeffrey J, Gadow HC (1969) Irregular antibodies in the obstetric patient. Obstet Gynecol 34: 767-71.

21. Rahman MM, Mondal AG, Islam MA, et al. (2011) Study on detection of Rhesus-D Antibody and Titer of Chittagong Division Dinajpur. Med Col J 4: 80-2.

22.

23. Shahverdi E, Moghaddam M, Gorzin F (2017) Maternal red blood cell alloantibodies identified in blood samples obtained from Iranian pregnant women: the first population study in Iran. Transfusion 57: 97-101.

24. Solves P, Gómez-Seguí I, Guinot M, Saus A, Osorio J, et al. (2017) Prevalence of red blood cell alloantibodies in pregnant women and hemolytic disease of newborn in a tertiary care hospital. ARC J Gynecol Obs 2: 1-5.

25. Souabni SA, Habib BE, Oubahha I, Baqali JE, Aboulfalah A, et al. (2021) Severe neonatal thrombocytopenia due to maternal alloimmunization: case report and literature review. Pan Afr Med J. 38:67.

26. Toly-Ndour C, Petit H, Oubouzar N, Boennec ML, Guinchard E, et al. (2021) "Allo-immunisation foeto -maternal anti-erythrocyte": results of the survey on cases of poor fetal or neonatal care linked to a lack of immuno-haematological monitoring of pregnancy. Clinical and Biological Transfusion 28: S36.

27. Varghese J, Mary P, Chacko Rajaiah M, Daniel D (2013) Red cell alloimmunization among antenatal women attending a tertiary care hospital in south India. Indian J Res. 138: 68-71.

28. Zazzheaus A, Jeremiah, Augustina M, Teddy C (2011) Frequencies of maternal red blood cell alloantibodies in Port Harcourt, Nigeria. Asian J Transfusion Sci 5: 39-41.