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Prevalence of blood donor women at risk of Hemolytic disease of the Fetus and Newborn in Southern Benin

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Abstract

Hemolytic Disease of the Fetus and Newborn (HDFN) is a genetic disorder caused by blood group incompatibility, particularly Rh (D) antigen, in the parental couple. This study aimed to determine the prevalence of blood donor women exposed to the risk of HDFN among 913 blood donors in the southern region of Benin. The study was conducted from March to June 2022 at the departmental blood transfusion centers in the Atlantic and Littoral regions. Samples were collected following the procedures of the National Blood Transfusion Agency (NBTA), using tube and microplate agglutination tests. Among the donors, 260 (28.5%) were women aged between 18 and 52 years. The O+ blood group donors constituted the largest proportion, accounting for 42% of the sample. Nearly one-third (33.3%) of the female blood donors were Rh (D) negative, putting them at risk of HDFN. Most of these women were of reproductive age, with a significant representation in the 20-30 age groups (42.1%). This study revealed that one-third of female blood donors in the Atlantic and Littoral regions were at risk of giving birth to an infant affected by HDFN. These findings underscore the importance of raising awareness and implementing preventive measures to mitigate the risks associated with this condition.

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Introduction

Hemolytic Disease of the Fetus and Newborn (HDFN) is a genetic disorder caused by blood group disparities, particularly the Rh(D) antigen of the Rh system (Rh) (Clarke *et al.*, 1968; Tsochandaridis *et al.*, 2016). It also results from an immune reaction stemming from antigen-antibody conflict between the mother and the fetus (Koelewijn *et al.*, 2008).

The process leading to this disease was elucidated by Levine in 1941 following the discovery of Rh(D) by Landsteiner and Wiener a year prior (Jm *et al.*, n.d.). HDFN stands as a major contributor to fetal and neonatal mortality, causing over 50,000 deaths annually, particularly in developing countries across Asia and sub-Saharan Africa (Agarwal *et al.*, 2014). For a woman to give birth to an infant with HDFN, the couple must be discordant in terms of ABO blood group and especially Rh(D) (the mother being Rh(D) negative and the father Rh(D) positive), attributed to the polymorphism of this antigen (Avent and Reid, 2000).

The development of this disease occurs in two stages: immunization of the mother during the first pregnancy with an Rh(D) positive fetus, followed by destruction of fetal red blood cells by immune antibodies produced in response to the initial immunization (Souabni *et al.*, 2021).

This scenario will recur with nearly all other Rh(D) positive fetuses of immunized Rh(D) negative mothers. Maternal immunization may occur during transplacental circulation of fetal blood cells to the maternal bloodstream (Zipursky *et al.*, 1959). It can manifest in circumstances such as miscarriage, missed miscarriage, abdominal trauma, ectopic pregnancy, induced abortion, amniocentesis, cordocentesis, ectopic pregnancy rupture, blood transfusion, or other rare pregnancy procedures. The destruction of fetal red blood cells leads to neonatal jaundice, fetal or neonatal anemia, and intrauterine fetal death (Castleman *et al.*, 2021). While various antibodies can trigger HDFN, the anti-D antibody predominates. Dziegiel *et al* reported that out of 100,000 antibodies implicated in the risk of this condition, only 328 were other than anti-D alloantibodies (Dziegiel *et al.*, 2021). Prevention of this disease relies on the administration of anti-D immunoglobulin (Woodrow *et al.*, 1971a; Jm *et al.*, 2015). This practice, introduced since 1969, has reduced the prevalence of HDFN from 16% to 2% (Blanco *et al.*, 2018). However, this approach has drawbacks such as reduced availability of anti-D immunoglobulin and potential virus transmission. The prevention of this disease can be enhanced through early injection of anti-D after fetal RHD genotyping in the mother (Woodrow *et al.*, 1971b).

In Benin, the initial laboratory examination (ABO RhD blood typing) allowing the identification of at-risk women and enabling early disease prevention remains inadequately recognized. Some midwives and maternity nurses, especially in peripheral areas, often overlook this aspect. Between 2015 and 2019, an estimated 121 million unintended pregnancies occurred in Africa, further exacerbating the occurrence of HDFN in our countries (Bearak *et al.*, 2020, pp. 1990-2019). Understanding the extent of this issue can raise general awareness to curb the emergence of hemolytic disease of the fetus and newborn in Benin. This is why it appears timely to conduct this study on blood donor women at risk of giving birth to infants with HDFN.

Materials and methods

Design and study population

This study was a prospective investigation involving a total of 2,841 blood donors, of which 780 were women. The study was conducted from March to June 2022 at the Departmental Blood Transfusion Center of the Atlantic and Littoral regions. This facility within the Beninese transfusion system contributes to the collection and qualification of nearly one-third of the blood bags collected nationwide.

Sample collection and analysis

Sample collection adhered to the procedures of the National Blood Transfusion Agency (NBTA), involving the collection of two 5ml dry tubes of blood samples and one 5ml tube of blood in EDTA after medical pre-donation donor selection. Samples for blood typing and serological screening of blood

donations were aseptically collected from the satellite pouches of the blood bags during the blood collection for transfusion.

All samples from mobile collection teams and fixed sites were transported and stored on the same day at the biological qualification laboratory for blood donations. They were tested on the same day or the following day.

Two ABO RhD blood typings were performed by two different technicians. The first technician utilized *Séraclone*, a monoclonal reagent from Bio-Rad laboratory (Poincaré, Marnes-la-Coquette, France), following the tube agglutination technique in accordance with the Beth Vincent and Simonin methods. The second technician used DAGAST, a polyclonal reagent from the distributor Mediff (Mediff, Aubagne, France), using the agglutination technique on opaline plates.

This second technician also conducted the Beth Vincent test to identify ABO and RhD antigens present on the surface of erythrocytes and the Simonin test to identify any present antibodies in the serum. The two results were cross-matched to validate the donor's blood type. Any discrepancies led to a repeat blood typing until concordant results were obtained.

The research received approval from the Local Ethics Committee for Biomedical Research (CLERB-UP), under REF: 0578/CLERB-UP/P/SP/R/SA, and was conducted in strict compliance with applicable regulations. All participants gave their consent before being enrolled in the study.

Statistical analyses

Statistical analyses were performed using R version 4.2.0 software. The results are presented as proportions for qualitative variables. Pearson's chi-square test was used for comparing proportions. Figure was generated with Sigma Plot statistical analysis software 2014 (Systat Software, Inc. San Jose, CA, USA). A p-value <0.05 was considered statistically significant.

Results

Distribution of Donors by Gender

Fig. 1 displays the distribution of donors according to gender. The proportion of female subjects was significantly ($p<0.05$) higher than that of male subjects.

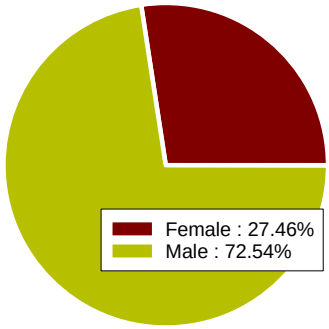

Fig. 1. Distribution of donors according to gender.

Distribution of Donors by Age Groups

Table 1 provides us with the distribution of donors according to age groups. The most represented age group in our study is 20 to 30 years, accounting for 40.1%.

Table 1. Distribution of Donors by Age Groups.

Age range	Workforce	Percentage(%)
]-;20[	275	9.7
[20;30[	1139	40.1
[30;40[	732	25.8
[40;50[	456	16.1
[50;+[	239	8.4
Total	2841	100

Frequency of Blood Group - RhD Phenotypes Among Donors

The findings presented in Table 2 indicated a higher prevalence of blood type O+ (42%), while blood type AB- was less common (0.2%). Moreover, considering the entire donor population in this study, blood type O emerged as the most frequent, constituting 46% of all cases.

Table 2. Distribution of Donors by Blood Group - RhD Phenotypes.

Blood Group	RhD	Workforce		Frequency (%)		P-value
A	-	66	823	2.32	28.97	<0.001
	+	757		26.65		
B	-	35	568	1.23	19.99	
	+	533		18.76		
AB	-	6	146	0.21	5.14	
	+	140		4.93		
O	-	111	1304	3.91	45.9	
	+	1193		41.99		

Frequency of RhD-Negative Phenotype Among Donors

Table 3 displays the distribution of donors according to RhD and gender. This table shows that RhD-positive donors are more prevalent (92.32%) than RhD-negative donors (7.67%). Additionally, the majority of RhD-negative donors were male (5% compared to 2.67% for females).

Table 3. Distribution of donors according to RhD and gender.

Rhesus	Total		Female		Male		P-value
	Work force	%	Work force	%	Work force	%	
Negative	218	7.67	76	2.67	142	5.00	<0.001
Positive	2623	92.32	704	24.78	1919	67.55	

Frequency of RhD-Negative Phenotype among Donors, Adjusted by Gender

Table 4 illustrates the frequency of adjusted RhD-phenotype by gender. This table reveals that 9.7% of female blood donors in this study have RhD- blood type, indicating a risk of giving birth to infants affected by hemolytic disease of the newborn. Furthermore, the RhD- phenotype is more commonly observed in females than in males (p=0.013).

Table 4. Frequency of the adjusted RhD- phenotype according to gender.

	Workforce	%	p-value
Female	76	9.74	0.013
Male	142	6.89	

Discussion

The main objective of this study was to investigate the prevalence of women at risk of giving birth to infants with HDFN, addressing a critical data gap in this field. The study yielded intriguing results, indicating that nearly one in ten women (9.7%) in Benin faced the risk of having infants affected by HDFN. This finding stands out when compared to a similar study in Southern Ethiopia conducted by Kanko TK and Woldemariam MK, which reported a lower prevalence rate of 6.2% (Kanko and Woldemariam, 2021).

In order to provide a broader context for the prevalence of women at risk, particularly those with RhD-negative blood type, we compared our findings with data from the broader sub-region, encompassing populations of all genders. Interestingly, our results closely align with the prevalence observed among blood donors in Morocco with a rate of 9.85% (El Khabous, 2018). Similarly, studies in Algeria by Boulkadid *et al.* found a comparable prevalence rate of 10.26% among donors (Boulkadid *et al.*, 2016), and this trend was mirrored in blood donation samples and patients in central Morocco (10.13%) as reported by Khalloufi *et al.* (2017). However, it's noteworthy that our study's prevalence rate significantly exceeds that reported by Loua *et al.* among blood donors and patients in Guinea (4.06%) within the Guinean population (Loua *et al.*, 2007) and the figures found by Tagny *et al.* among blood donors and recipients in Cameroonian hospital settings (2.4% and 3.1% respectively among blood donors and recipients) (Tagny *et al.*, 2009).

Conversely, our study's prevalence rate of 9.7% is notably lower than the rate reported by Branger *et al.* in France, which stands at 15% (Branger and Winer, 2006). This marked difference can be attributed to the well-documented phenomenon observed among Caucasians, who consistently exhibit a higher percentage of RhD-negative individuals compared to Africans. Our findings underscore the significance of understanding regional variations in HDFN risk factors and highlight the importance of tailored healthcare interventions in populations with differing genetic profiles.

Conclusion

Hemolytic Disease of the Fetus and Newborn is a significant contributor to fetal and neonatal mortality worldwide. It remains a major cause of death in Africa and Benin. Awareness of the prevalence of women at risk of having infants with this disease is essential and can foster public awareness to prevent the disease effectively. Rigorous premarital and prenatal assessments, as well as diligent pregnancy monitoring, are imperative. This study revealed that nearly one in ten women is at risk of having infants affected by this disease in Benin. It also fills a gap in data availability regarding the prevalence of RhD-negative individuals in Benin.

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