ORIGINAL REPORT

Statistical model for prediction of ABO hemolytic disease of the fetus and newborn in India

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ABO incompatibility is the most common cause of immune hemolytic disease of the fetus and newborn (HDFN). The American Academy of Pediatrics lists blood group incompatibility as one of the major risk factors for severe hyperbilirubinemia in newborns. We have estimated the risk of ABO HDFN to determine the need for its routine screening. Blood group data from all blood donors who donated in the last 10 years were collected and analyzed. The population prevalence of ABO blood group genes using the phenotype data of blood donors was estimated. This information was further used to calculate an incidence of ABO HDFN requiring intervention in the population. ABO blood group typing was analyzed in 425,743 blood donors. The ABO phenotypes of A, B, O, and AB were 22.48, 36.73, 31.59, and 9.2 percent, respectively. The gene frequencies were 0.1733, 0.2647, and 0.5620 for *A, B,* and *O,* respectively. It was estimated that 13.84 percent of group O women would give birth to a non–group O baby and that approximately 2.77 percent of deliveries would likely have ABO HDFN in the study population. In India, the estimated risk of ABO HDFN is 2.9 percent, with a daily 2196 babies at risk of ABO HDFN requiring intervention. This analysis estimates the overall burden of ABO HDFN in the population, which could aid in the decision-making of policymakers, physicians, and community health practitioners to improve neonatal care. ***Immunohematology*** **2021;37:64–68. DOI:10.21307/immunohematology-2021-009.**

Key Words: ABO HDFN, *ABO* gene frequency, risk estimation

In recent years, clinical and public health efforts, such as red blood cell antibody screening of all pregnant women and administration of Rh immune globulin (RhIG) in developed countries, have reduced the burden of Rh hemolytic disease of the fetus and newborn (HDFN), ultimately resulting in ABO HDFN as the most common cause of immune HDFN. Approximately 15–25 percent of feto-maternal pairs are ABO incompatible (blood group O mother with non–group O neonate), of which 10–15 percent of neonates develop ABO HDFN.[1,2] ABO HDFN is usually managed successfully with phototherapy, although it is not always a benign condition. Severe cases having bilirubin-induced neurologic dysfunction and, rarely, antenatal hydrops fetalis have been described.[3] Ethnicity plays a critical role in the prevalence and severity of ABO HDFN. Toy et al.[4] highlighted that the prevalence of direct antiglobulin test positivity in a group A infant born to a group O mother was highest (50%) in Asians and lowest (31%) in Europeans.[4] The current scenario of ABO HDFN in India is unknown; unusually severe cases requiring active intervention such as exchange transfusions have been reported from India.[5,6] ABO blood group gene frequencies are useful to predict the risk of ABO HDFN in the population. Careful surveillance and early detection of infants at risk of bilirubin-related brain injury begins with recognition of an infant's risk for severe hyperbilirubinemia. It requires a systemic approach and awareness regarding significant risk factors for severe hyperbilirubinemia. This study was therefore planned to estimate the risk of ABO HDFN in our North Indian population and to assess the need for routine ABO HDFN screening.

Material and Methods

Blood group data from all blood donors who donated blood at a tertiary care hospital in Lucknow in the last 10 years (2010–2019) were collected from the hospital information system and analyzed. The prevalence of ABO blood group genes in our population using phenotype data of blood donors was estimated. It is assumed that gender does not influence *ABO* gene inheritance and blood group distribution; therefore, calculated gene frequencies can be applied to the female population. The data were further used to estimate the probability of feto-maternal ABO incompatibility and ABO HDFN requiring intervention.

Statistical Analysis

The frequency and percentage of each blood group (A, B, AB, and O) were calculated using descriptive statistics. Blood group allelic frequencies were calculated with the assumption of the Hardy-Weinberg equilibrium with Ceppellini

correction.[7] This iterative method yields maximum likelihood estimates. It was assumed that:

- ABO blood group system is determined by three alleles of a single gene: *A, B,* and *O*.
- *A* and *B* are co-dominant, and they are dominant over *O*.
- In the population, genes follow the Hardy-Weinberg principle.
- A random sample of blood donors from the population was studied.
- Static allele frequencies across generations assume that there is no mutation and migration, population size is infinite, and there is no selective pressure for or against any genotypes.

The probability of a group O woman giving birth to a child with a non–group O phenotype was calculated. Maternal immunoglobulin (Ig)G anti-A and/or anti-B titer ≥512 predicts the risk of ABO HDFN and the need for invasive treatment.[8] Almost 20 percent of group O donors have IgG anti-A and/or anti-B titer ≥512.[9] Accordingly, we have estimated the risk of ABO HDFN in the population.

Results

ABO blood group data of 425,743 blood donors were analyzed, in which 17,881 (4.2%) were female and 407,862 (95.8%) were male. The details are summarized in Table 1. The study revealed that blood group B was the most prevalent (36.73%), closely followed by O at 31.59 percent, then A at 22.48, percent, and AB as the least frequent group, at 9.2 percent. The observed and expected phenotype frequencies are shown in Table 2. The χ^2 test between the observed and expected phenotype frequencies in case of ABO group was not statistically significant ($p > 0.0001$).

Calculation for gene frequency:

A, B, and *O* were represented by letters *p, q,* and *r*.

Calculation of *O* allele frequency (*r*):

The frequency of allele *O* is equal to the frequency of the recessive genotype (r^2).

r^2 = frequency of O phenotype ([number of group O individuals]/[total number of individuals])

$r^2 = (O/N)$

$r = \sqrt{(O/N)}$

$r = 0.5620$

Calculation of *A* allele frequency (*p*):

p = frequency of the group A phenotype + frequency of the group O phenotype.

The frequency of A + O = $p^2 + 2pr + r^2 = (p + r)^2$

$p + r = \sqrt{(A + O)}$

$p = \sqrt{(A + O)} - (r)$

$p = \sqrt{(0.2248 + 0.3159)} - 0.5620$

$p = 0.1733$

Calculation of *B* allele frequency (*q*):

Because $p + q + r = 1$

$q = 1 - (p + r)$

$q = 1 - (0.1733 + 0.5620)$

$q = 0.2647$

Thus, the calculated gene frequencies were 0.1733, 0.2647, and 0.5620 for the *A, B,* and *O* genes, respectively.

The calculation for the probability of non–group O child to group O mother:

The probability of a non–group O child to group O mother will be the probability of a group *OO* genotype woman mating with a genotype *AA, BB, AO, BO,* or *AB* man, resulting in an offspring with the non–group O gene. Using gene frequencies calculated above, the probability that an *OO* genotype woman mating with an *AA* genotype man is the product of frequency of *AA* genotype and frequency of *OO* genotype:

$AA = r^2 \times p^2 = 0.00948$

Table 1. Distribution of blood groups in the study population

Blood group	A		B		O		AB		
Rh(D) type	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Total
Number of donors (%)	91,027 (21.38)	4663 (1.10)	148,941 (34.98)	7454 (1.75)	127,680 (29.99)	6805 (1.60)	37,315 (8.76)	1858 (0.44)	
Total number (%)	95,690 (22.48)		156,395 (36.73)		134,485 (31.59)		39,173 (9.20)		425,743 (100)

Pos = positive; Neg = negative.

Table 2. Distribution of *ABO* allele frequencies

Blood group (phenotype)	Observed frequency	Genotype	Expected frequency		
A	0.2248	*AA*	p^2	0.0300	0.2247
		AO	$2pr$	0.1947	
B	0.3673	*BB*	q^2	0.0701	0.3677
		BO	$2qr$	0.2976	
O	0.3159	*OO*	r^2	0.3159	0.3159
AB	0.0920	*AB*	$2pq$	0.0917	0.0917
Total	**1**			**1**	**1**

p = *A* allele frequency; r = *O* allele frequency; q = *B* allele frequency.

Similarly,

$BB = r^2 \times q^2 = 0.02214$

The probability of an *OO* genotype woman mating with an *AO* genotype man is:

$AO = r^2 \times 2pr$

The probability of inheriting the *A* gene from the father is 50 percent. Therefore, the probability of an *AO* man and an *OO* woman having an *AO* child is:

$AO = (r^2 \times 2pr)/2 = r^2 \times pr$

$AO = 0.03076$

Similarly,

$BO = 0.04700$

The probability of an *OO* genotype woman mating with an *AB* genotype man and having a child with either the *A* or *B* gene is:

$AB = r^2 \times 2pr$

$AB = 0.02897$

The sum of all probabilities is 0.13837.

Therefore, 13.84 percent of deliveries will result in a non–group O child to group O mother. Because 20 percent of group O mothers will have IgG anti-A and/or anti-B titer ≥512, ultimately 2.77 percent of deliveries in our study population are at risk of ABO HDFN, requiring intervention.

Discussion

In India, diversity has been observed in the geographical distribution of blood groups. In the present study, group B was the most prevalent blood group, followed by group O, in a north Indian population; this observation is similar to that found in the study by Arora et al.,[10] although a multicentric study shows group O as the most common blood group, followed by group B, in India.[10,11]

Using *ABO* gene frequency, we calculated the probabilities of ABO incompatibility as 13.8 percent. Bhat and Kumar[12] found 17.3 percent ABO incompatibility, similar to our estimate. Akanmu et al.[13] did a similar study in Nigeria in which they estimated that 14.3 percent of deliveries would result in feto-maternal ABO incompatibility, again similar to our findings. They estimated that 4.3 percent of deliveries are likely to suffer ABO HDFN, which is quite high compared with our estimation. This finding is consistent with previous reports stating that the incidence of ABO HDFN is higher in individuals of African descent. This difference can be due to the very high cutoff value (≥512) for antibody titer used in the present study.[14,15]

The burden of neonatal jaundice is highest in South Asia, where it is the seventh leading cause of neonatal mortality.[16] Various studies from India reported neonatal jaundice as the most common cause of neonatal morbidity, varying from 7 to 16.7 percent.[17,18] Statistical estimates allow us to project the overall burden of ABO HDFN in the population. Therefore, we extended this observation to national data and found that 2.9 percent of neonates are at risk of ABO HDFN in India (Table 3). The birth rate in India is 20 per 1000 population, and currently the total population is 1,381,625,777.[19,20] Thus, approximately 2196 babies are born per day who are at risk of ABO HDFN, requiring intervention. Barring the limitations associated with statistical modeling of disease burden, our data suggest that neonatal jaundice due to ABO HDFN is an important cause of neonatal morbidity.

Phototherapy and exchange transfusions are the mainstay treatments for severe hyperbilirubinemia, but these therapies require adequate infrastructure. The need for exchange transfusion has been reduced with the introduction

Table 3. Comparison between Indian population and study population

Allele frequency	Our data	Indian data[11]
A	0.1733	0.1653
B	0.2647	0.2254
O	0.5620	0.6093
Probability		
Non–group O child to group O mother	0.13835	0.1450
ABO HDFN requiring intervention	2.77%	2.90%

HDFN = hemolytic disease of the fetus and newborn.

of phototherapy and intravenous immunoglobulin (IVIG) therapy. However, availability and cost of IVIG therapy are major limitations for its routine use in resource-poor nations. Immediate exchange transfusion is required when phototherapy fails to curtail the rate of bilirubin rise and the total serum bilirubin. Provision of exchange transfusion requires centers with fully equipped blood banks with component separation, such as a sterilized connecting device, leukocyte filtration, gamma irradiation, a dielectric tube sealer, laminar air-flow, and trained personnel. In a developing and resource-constrained nation such as India, infrastructure with facilities for special care, including monitoring and resuscitation capabilities, is most likely available at tertiary care hospitals. Findings of this study should help administrators plan for the provision of exchange transfusion when considering improvements in the Indian medical care infrastructure.

As per routine antenatal care, the blood group and Rh(D) type of the mother (ABO and Rh) is determined by a blood test. In resource-constrained areas, group O mothers should be tested for ABO antibody titers, and ABO-incompatible babies of high-risk group O mothers should be kept under vigilance. At present, D incompatibility is the only cause of HDFN for which screening is routinely done.[21] Routine screening for ABO HDFN may be considered as an additional step in risk assessment of HDFN. Timely diagnosis with the appropriate intervention will result in a better outcome in babies at risk.

A limitation of the study is that the numbers used to calculate the risk of severe ABO HDFN are from a donor base that is likely all male, although gender does not influence ABO blood group distribution.

Conclusions

The estimated risk of ABO HDFN is 2.77 percent of all deliveries in our study population. In India, the calculated risk is 2.9 percent, with a daily number of 2196 babies at risk of ABO HDFN. A policy for routine screening of these neonates is needed.

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