

# A Real-world Prospective Study to Evaluate the Geographical Distribution, Isoimmunization Rate, and Utilization of Prophylactic Treatment of Rh-negative Pregnant Women in India (RhYTHM Study)

Pratik Shah<sup>1</sup>, Supriya Hiranman Pawar<sup>2</sup>, Shilpa Nandakumar Naik<sup>3</sup>, Tumati Sivjyothi<sup>4</sup>, Ashwin Rao<sup>5</sup>, Ashwin Kakkar<sup>6</sup>

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## ABSTRACT

**Aim and background:** There is a lack of comprehensive and recent data on rhesus (Rh)-negative pregnancies in India. The aim of this study is to examine the demographics, isoimmunization status, usage of prophylactic treatment, and complications associated with Rh-negative pregnancies in India.

**Materials and methods:** This is an interim analysis of an ongoing real-world observational study targeting the recruitment of 20,000 Rh-negative pregnant women throughout India. This article presents data from 1,421 participants who were followed prospectively for 3 months. Participants' demographics, obstetrics history, usage of prophylactic treatment, and pregnancy-related complications were recorded. Data were analyzed using descriptive statistics for all the outcome variables.

**Results:** The maximum number of participants belonged to the West region (47.4%). The mean gestational age of the participants was 37.7 weeks. There was an equal representation of primigravida and multigravida participants in the study population. The available indirect Coombs test data showed that most of the participants (116 of 125) were negative for circulating antiglobulin in the current pregnancy. However, isoimmunization during the current pregnancy occurred in 9 participants (9 of 125; 7.2%). Overall, 25.7% of the participants received anti-D prophylaxis during their current pregnancy.

**Conclusion:** This real-world evidence study demonstrates that most Rh-negative women carrying Rh-positive fetus are at risk of becoming sensitized to the Rh antigen. Despite the availability of prophylactic treatment, there are women who are getting sensitized. Hence, there is a need to create awareness among the Indian population about the Rh-disease-associated risks and available preventive measures to reduce the mortality rates.

**Clinical significance:** Anti-D prophylaxis is the most effective method to prevent sensitization in Rh-negative pregnant women. Anti-Rh (O) D immunoglobulin is a must administered drug both in antenatal and postnatal phases of pregnancy to prevent Rh-negative pregnant women from getting sensitized. Thus, further reducing the burden of Rh-sensitization in India and having healthy motherhood.

**Keywords:** Fetal complications, Pregnancy, Rh isoimmunization, Rh-negative.

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## INTRODUCTION

Owing to the highly immunogenic nature of the rhesus D (Rh-D) antigen, maternal–fetal Rh factor incompatibility is a serious issue that may lead to maternal Rh isoimmunization.<sup>1–6</sup> Rh isoimmunization can result in serious conditions, for example, the hemolytic disease of the fetus and newborn (HDFN), fetal or neonatal anemia, kernicterus, and early miscarriage due to hydropic changes (hydrops fetalis) in subsequent pregnancies.<sup>1,3,4,7</sup> The most adopted and effective prophylactic strategy to reduce the incidence of Rh isoimmunization is the administration of anti-Rh IgG.<sup>1,3,4,8,9</sup> This has decreased the rate of Rh isoimmunization from 16 to 1.5–2%,<sup>9,10</sup> and further reduced to ~0.5% upon the additional antepartum administration of anti-Rh IgG.<sup>9</sup> Many Rh-negative women, however, remain sensitized due to not being aware of the effects of Rh incompatibility and its effect on future pregnancies.

The prevalence of the Rh-negative blood group varies among different populations. Therefore, there is a significant geographical variability in the incidence of Rh-negative pregnancies worldwide. The incidence of the Rh-negative blood group among various

<sup>1,2</sup>Bharat Serums and Vaccines Limited, Navi Mumbai, Maharashtra, India

<sup>3</sup>Department of Obstetrics & Gynecology, B.J. Medical College, Pune, Maharashtra, India

<sup>4</sup>Snehalatha Hospital, Anantapur, Andhra Pradesh, India

<sup>5</sup>Department of Reproductive Medicine and Endogynecology, Salem Polyclinic Private Limited, Salem, Tamil Nadu, India

<sup>6</sup>Vaishnavi Maternity Home, Kalyan, Maharashtra, India

**Corresponding Author:** Pratik Shah, Bharat Serums and Vaccines Limited, Navi Mumbai, Maharashtra, India, Phone: +91 9867363454, e-mail: drpratikin@gmail.com

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ethnicities is as follows: White populations (North Americans and Europeans): 15%; African populations: 4.8%; and Asian populations: 0.1–0.3%.<sup>3,4,11</sup> In a worldwide comparative study, the incidence of Rh-negative pregnancies in Indian women was reported to be low, varying from 3.0 to 5.7%.<sup>1,10</sup> The developed countries have reduced the incidence of Rh incompatibility-related fetal conditions, based on universal access to neonatal or fetal health services. However, an Rh incompatibility-related mortality rate of approximately 276 neonates or 100,000 live births has been reported for developing countries.<sup>3,4,12</sup> Although a few studies have been published describing the prevalence and outcomes of Rh-negative pregnancies in India.<sup>1,2,10,13</sup> They were conducted with a limited set of participants and/or for a specific region. Therefore, their findings cannot be generalized to the entire Indian population. Additionally, data on the utilization of prophylactic treatment among Rh-negative pregnant women, pregnancy complications, and Rh incompatibility-associated adverse events (AEs) or serious AEs are also lacking. The lack of such information might result in an inability to formulate strategies to meet real-world situations in eradicating Rh-negative pregnancy-related morbidities and mortalities in India.

On the basis of the above information, there is a need to identify gaps in the knowledge in this important area, and this large multicenter study can bridge those gaps. Thus, a real-world evidence study is being conducted in India to develop a database to collate all relevant data on Rh-negative pregnant women (3-month study period) from clinics and hospitals throughout India.

As part of the larger ongoing study, this interim analysis was conducted to evaluate the geographical distribution of pregnant Rh-negative women regarding demographics, isoimmunization status, usage of prophylactic treatment, pregnancy complications, delivery status, and pregnancy outcome.

## MATERIALS AND METHODS

### Study Design

This study is an interim analysis of an ongoing multicenter, prospective, observational, data collection study; the larger study plans to include approximately 20,000 Rh-negative pregnant women treated in clinics and hospitals throughout India. This article presents data from 1,421 participants. All the relevant medical records of enrolled pregnant Rh-negative women were retrieved by a designated person for the study and entered in an electronic case report form (eCRF). Participants' personal information was considered confidential. Written informed consent was obtained from all participants. The study protocol was approved by the appropriate ethics committees, and the study was conducted in accordance with the principles of the Declaration of Helsinki. This study was registered with the Clinical Trials Registry-India (CTRI) and the trial number is CTRI/2022/01/039101.

### Subject Selection

Rh-negative pregnant women who were  $\geq 18$  years of age and provided a signed informed consent form (ICF) were recruited in the study. These were identified from the Investigator's established patient population.

Study-specific procedures were performed only after obtaining a signed ICF from the subjects. In case of any withdrawals from participation, the investigator recorded the date and reason for early discontinuation in the eCRF.

### Visit Schedule and Study Procedures

There was no specified number of study visits; participants were followed up prospectively for 3 months after enrolment. Participants' demographics, including age, weight, height, the highest level of education, current employment, residential, and socio-economic status, were recorded. In addition, their relevant medical history, Indirect Coombs Test (ICT) results, anti-D administration, history of previous pregnancies, delivery details, and mode of delivery were also recorded, if available.

### Statistical Analysis

The geographical distribution of the enrolled participants, the incidence of isoimmunization, demographic parameters, anti-D administration, and AEs/complications were summarized using descriptive statistics.

If a participant withdrew from the study before the end of the study period (with valid consent for data usage), the data collected till the point of withdrawal were analyzed. The data entered in the eCRF was systematically checked using a pre-defined computerized validation check and data review by the sponsor and/or the sponsor's representatives.

## RESULTS

In this ongoing study, pregnant women with Rh-negative status who visited various hospitals throughout India for antenatal care are being recruited. To date, data from 1421 women have been analyzed. The participant flow in a region-wise manner has been presented in Supplemental Table 1. The highest number of participants were recruited from the West region (674, 47.4%), followed by the North (308, 21.7%), the South (239, 16.8%), and the East region (200, 14.1%). A total of 392 of 1421 participants (27.6%) completed the 3-month follow-up.

Table 1 presents the demographic information of the participants. The mean age of the participants was 26.5 years (SD,  $\pm 4.4$  years). The average body mass index (BMI) of the participants was  $24.81 \pm 4.50$  kg/m<sup>2</sup>, showing that the participants belonged to the normal weight category (as per the Global World Health Organization definition of BMI-based normal status; 18.5–24.9 kg/m<sup>2</sup>; <https://www.who.int/europe/news-room/fact-sheets/item/a-healthy-lifestyle---who-recommendations>). The mean gestational age ( $37.7 \pm 2.9$  weeks) was comparable among the populations enrolled from different regions of the country. The distribution of ABO blood groups was A, 338 (23.8%); AB, 116 (8.2%); B, 488 (34.3%); and O, 479 (33.7%). The partner's Rh status was positive for a majority of the participants (988, 89.6%). More than half of the participant population was from lower-middle-class households.

Additional demographic data have been provided in Supplemental Table 2. A large proportion of the participants were well educated, with graduate or post-graduate degrees (453, 41.6%). However, most of the study participants were unemployed during the study period (763, 70.0%). Overall, rural and urban areas were comparably represented in the study (rural, 427, 39.2%; urban, 349, 32.0%).

The data on medical history were available for 1104 participants (Supplemental Table 3). A total of 68 medical conditions were reported among 56 participants. Mostly, the conditions were related to pregnancy and perinatal complications (28 events) followed by endocrine disorders (20 events). A total of 227 of 1,104 participants reported abortions. The maximum of these abortions was medically

**Table 1:** Demographic characteristics of the participants

| <i>No. of participants by region</i>                       | <i>East (N = 200)</i> | <i>North (N = 308)</i> | <i>South (N = 239)</i> | <i>West (N = 674)</i> | <i>Overall (N = 1,421)</i> |
|--|-----------------------|------------------------|------------------------|-----------------------|----------------------------|
| Age (mean ± SD), years                                     | 26.3 ± 4.6            | 27.7 ± 4.5             | 25.4 ± 4.3             | 26.4 ± 4.2            | 26.5 ± 4.4                 |
| Weight (mean ± SD), kg                                     | 62.98 ± 11.35         | 63.64 ± 11.54          | 61.24 ± 13.12          | 59.21 ± 10.47         | 61.14 ± 11.49              |
| Height (mean ± SD), cm                                     | 158.09 ± 6.62         | 159.09 ± 5.19          | 154.46 ± 5.66          | 156.52 ± 5.48         | 156.96 ± 5.85              |
| Body mass index (mean ± SD), kg/m <sup>2</sup>             | 25.23 ± 4.65          | 25.18 ± 4.58           | 25.63 ± 5.15           | 24.15 ± 4.02          | 24.81 ± 4.50               |
| Gestational age  |                       |                        |                        |                       |                            |
| Weeks (mean ± SD)  | 37.9 ± 2.0            | 37.5 ± 1.8             | 37.8 ± 3.9             | 37.5 ± 3.5            | 37.7 ± 2.9                 |
| No. of participants whose gestational age is available     | 79                    | 33                     | 27                     | 90                    | 229                        |
| No. of participants whose gestational age is not available | 121                   | 275                    | 212                    | 584                   | 1,192                      |
| Blood group, <i>n</i> (%)                                  |                       |                        |                        |                       |                            |
| A  | 46 (23.0)             | 70 (22.7)              | 39 (16.3)              | 183 (27.2)            | 338 (23.8)                 |
| AB   | 15 (7.5)              | 29 (9.4)               | 15 (6.3)               | 57 (8.5)              | 116 (8.2)                  |
| B  | 59 (29.5)             | 113 (36.7)             | 101 (42.3)             | 215 (31.9)            | 488 (34.3)                 |
| O  | 80 (40.0)             | 96 (31.2)              | 84 (35.1)              | 219 (32.5)            | 479 (33.7)                 |
| Rhesus factor, <i>n</i> (%)                                |                       |                        |                        |                       |                            |
| Rh-negative  | 200 (100.0)           | 308 (100.0)            | 239 (100.0)            | 674 (100.0)           | 1,421 (100.0)              |
| Partner's rhesus factor status                             |                       |                        |                        |                       |                            |
| Rh-positive  | 158 (89.8)            | 192 (81.7)             | 180 (92.8)             | 458 (92.0)            | 988 (89.6)                 |
| Rh-negative  | 18 (10.2)             | 43 (18.3)              | 14 (7.2)               | 40 (8.0)              | 115 (10.4)                 |
| Data not available   | 0                     | 0                      | 1                      | 0                     | 1                          |
| Socio-economic status, <i>n</i> (%)                        |                       |                        |                        |                       |                            |
| Lower socio-economic class                                 | 15 (7.5)              | 54 (17.5)              | 11 (4.6)               | 118 (17.5)            | 198 (13.9)                 |
| Lower-middle socio-economic class                          | 75 (37.5)             | 149 (48.4)             | 184 (77.0)             | 334 (49.6)            | 742 (52.2)                 |
| Upper-middle socio-economic class                          | 100 (50.0)            | 96 (31.2)              | 38 (15.9)              | 208 (30.9)            | 442 (31.1)                 |
| Upper socio-economic class                                 | 10 (5.0)              | 9 (2.9)                | 6 (2.5)                | 13 (1.9)              | 38 (2.7)                   |
| Socio-economic status details not available                | 0                     | 0                      | 0                      | 1                     | 1                          |

**Table 2:** Obstetrics details of current pregnancy

| <i>No. of participants by region</i>                      | <i>East (N = 200)</i> | <i>North (N = 308)</i> | <i>South (N = 239)</i> | <i>West (N = 674)</i> | <i>Overall (N = 1,421)</i> |
|---|-----------------------|------------------------|------------------------|-----------------------|----------------------------|
| No. of participants who delivered during the study period | 79                    | 33                     | 27                     | 90                    | 229                        |
| Mode of delivery, <i>n</i> (%)                            |                       |                        |                        |                       |                            |
| No. of participants whose data are available              | 127                   | 131                    | 32                     | 199                   | 489                        |
| Assisted  | 0                     | 0                      | 0                      | 1 (1.1)               | 1 (0.4)                    |
| Cesarean  | 45 (57.0)             | 19 (57.6)              | 11 (40.7)              | 38 (42.2)             | 113 (49.3)                 |
| Vaginal   | 34 (43.0)             | 14 (42.4)              | 16 (59.3)              | 51 (56.7)             | 115 (50.3)                 |
| No. of participants whose data are not available          | 121                   | 275                    | 212                    | 584                   | 1,192                      |

induced (165 of 227, 72.7%), and the rest were surgical abortions (62 of 227, 27.3%). Of these, data on anti-D administrations were available for 206 of 227 participants and showed that 67 of 206 (32.5%) participants received anti-D administrations.

Table 2 shows the obstetrics details of the current pregnancy of the women who were part of this interim analysis. The available data showed that 229 of 489 participants (46.8%) delivered during the study period. An almost equal number of these deliveries were vaginal (115 of 229, 50.3%) and cesarean (113 of 229, 49.3%). One assisted delivery also occurred during the study. There was

an almost equal representation of primigravida and multigravida participants in the study (Supplemental Table 4).

Among these 229 participants, information on the baby was available for 220 mothers (Supplemental Table 4). Most of them delivered one baby (215 of 220, 97.7%), while a few participants delivered twins (5 of 220, 2.3%). Data on blood group were available for 207 babies; most exhibited blood type B, 84 (40.6%); followed by types O, 71 (34.3%); A, 40 (19.3%); and AB, 12 (5.8%). Most of the babies were Rh-positive (160, 77.3%). Follow-up data available for five babies born to the isoimmunized mothers showed that two

**Table 3:** Indirect Coombs test results

| Pregnancy status  | Indirect Coombs test (ICT) result  | East<br>(N = 200) | North<br>(N = 308) | South<br>(N = 239) | West<br>(N = 674) | Overall<br>(N = 1,421) |
|-------------------|--|-------------------|--------------------|--------------------|-------------------|------------------------|
| Past pregnancy    | Total no. of participants who underwent the ICT in the past pregnancy    | 23                | 13                 | 11                 | 40                | 87                     |
|                   | No. of participants with a negative ICT result, n (%)                    | 23 (100.0)        | 12 (92.3)          | 10 (90.9)          | 40 (100.0)        | 85 (97.7)              |
|                   | No. of participants with a positive ICT result, n (%)                    | 0                 | 1 (7.7)            | 1 (9.1)            | 0                 | 2 (2.3)                |
|                   | No. of participants whose data were not available                        | 177               | 295                | 228                | 634               | 1,334                  |
| Current pregnancy | Total no. of participants who underwent the ICT in the current pregnancy | 12                | 41                 | 35                 | 37                | 125                    |
|                   | No. of participants with a negative ICT result, n (%)                    | 11 (91.7)         | 37 (90.2)          | 31 (88.6)          | 37 (100.0)        | 116 (92.8)             |
|                   | No. of participants with a positive ICT result, n (%)                    | 1 (8.3)           | 4 (9.8)            | 4 (11.4)           | 0                 | 9 (7.2)                |
|                   | No. of participants whose data were not available                        | 188               | 267                | 204                | 637               | 1,296                  |

N, number of participants

**Table 4:** Complications during birth or adverse events

| Preferred term                                   | North (N = 308) |   | West (N = 674) |   | Overall (N = 1,421) |   |
|--|-----------------|---|----------------|---|---------------------|---|
|  | n (%)           | E | n (%)          | E | n (%)               | E |
| Any complications during birth or adverse events | 2 (0.6)         | 4 | 2 (0.3)        | 2 | 4 (0.3)             | 6 |
| Abdominal pain                                   | 1 (0.3)         | 1 | 0              | 0 | 1 (0.1)             | 1 |
| Swelling   | 1 (0.3)         | 1 | 0              | 0 | 1 (0.1)             | 1 |
| Abortion, missed                                 | 0               | 0 | 1 (0.1)        | 1 | 1 (0.1)             | 1 |
| Premature labor                                  | 0               | 0 | 1 (0.1)        | 1 | 1 (0.1)             | 1 |
| Vaginal hemorrhage                               | 1 (0.3)         | 1 | 0              | 0 | 1 (0.1)             | 1 |
| Hypertension                                     | 1 (0.3)         | 1 | 0              | 0 | 1 (0.1)             | 1 |

n, no. of participants who faced complications; E, no. of events that occurred

(40%) received intravenous (IV) fluid with antibiotic treatment, two (40%) were on ventilation, and one (20%) was on exchange transfusion.

Of the available obstetrics history details (Supplemental Table 5), 394 of 504 participants (78.2%) had successful delivery in past pregnancies. The majority of them had one child (345 of 394, 87.6%), some had twin children (47 of 394, 11.9%), and a few had delivered three children (2 of 394, 0.5%). The mean age of the children was  $4.7 \pm 2.9$  years. Of the 228 children with available data, most exhibited blood type B, 82 (36.0%); followed by types O, 72 (31.5%); A, 51 (22.4%); and AB, 23 (10.1%). Most of the children were Rh-positive (182, 79.8%).

The ICT results from past pregnancies were available for 87 of 1421 participants, with 85 of 87 (97.7%) negative and two (2.3%) positive results. Also, ICT was performed for 125 of 1,421 participants in their current pregnancy, with 116 of 125 (92.8%) negative and nine (7.2%) positive results (Table 3). These nine participants with positive ICT results represent the Rh-negative women who were sensitized in the current pregnancy.

A total of six complications/AEs were recorded among four participants: one case each of abdominal pain, swelling, vaginal hemorrhage, hypertension, missed abortion, and premature labor (Table 4). There were four fetal deaths reported.

Table 5 shows data on the utilization of anti-D immunoglobulin prophylaxis. Of the total 1,421 participants, 513 (36.1%) received a total of 606 anti-D administrations. Of these, 176 participants were administered anti-D in their past pregnancies, where 19 of 176 (10.8%) participants received antenatal, 154 (87.5%) received

postnatal, and 3 (1.7%) received both antenatal and postnatal anti-D immunoprophylactic treatment. A total of 365 participants were administered anti-D in their current pregnancy; of these, 312 (85.5%) received antenatal, 47 (12.9%) received postnatal, and 6 (1.6%) received both antenatal and postnatal anti-D immunoprophylaxis. The pregnancy trimester details were available for 259 of 312 participants who were administered antenatal prophylaxis. Most of the participants were in the third trimester (188 of 259), while a few were in the first trimester (38 of 259), or the second trimester (33 of 259) of pregnancy.

## DISCUSSION

In this interim analysis of a large multicenter study conducted throughout India, we analyzed data on the isoimmunization rate, maternal outcomes, and anti-D prophylaxis among Rh-negative pregnant women. In this study, the distribution of Rh-negative pregnant women was highest from the West region, followed by the North, the South, and the East region. Maternal/fetal Rh-factor incompatibility is caused when an Rh-negative woman carries an Rh-positive fetus.<sup>3,4,8,9</sup> Therefore, the determination of the Rh-blood group type of both parents is important. Guidelines issued by the Federation of Obstetric and Gynaecological Societies of India (FOGSI) as part of a “practice points” document recommend that the Rh-typing of the mother should be performed at the first antenatal visit (<https://www.fogsi.org/wp-content/uploads/tog/anti-d-immunoglobulin-2022.pdf>). All the participants in this study underwent Rh-typing and were included based on their

**Table 5:** Utilization of anti-D immunoglobulin prophylaxis by pregnancy status

| <i>No. of participants who received anti-D prophylaxis</i>                              | <i>East<br/>(N = 200)</i> | <i>North<br/>(N = 308)</i> | <i>South<br/>(N = 239)</i> | <i>West<br/>(N = 674)</i> | <i>Overall<br/>(N = 1,421)</i> |
|---|---------------------------|----------------------------|----------------------------|---------------------------|--------------------------------|
| Total no. of participants with available anti-D administration data, <i>n (%)</i>       | 73 (36.5)                 | 139 (45.1)                 | 67 (28.0)                  | 234 (34.7)                | 513 (36.1)                     |
| Total no. of anti-D administrations performed, <i>E</i>                                 | 79                        | 151                        | 72                         | 304                       | 606                            |
| During past pregnancy   |                           |                            |                            |                           |                                |
| Total no. of participants who received anti-D   | 25                        | 41                         | 30                         | 80                        | 176                            |
| No. of participants who received only antenatal prophylaxis, <i>n (%)</i>               | 5 (20.0)                  | 5 (12.2)                   | 1 (3.3)                    | 8 (10.0)                  | 19 (10.8)                      |
| No. of participants who received only postnatal prophylaxis, <i>n (%)</i>               | 20 (80.0)                 | 36 (87.8)                  | 28 (93.3)                  | 70 (87.5)                 | 154 (87.5)                     |
| No. of participants who received both antenatal and postnatal prophylaxis, <i>n (%)</i> | 0                         | 0                          | 1 (3.3)                    | 2 (2.5)                   | 3 (1.7)                        |
| During current pregnancy  |                           |                            |                            |                           |                                |
| Total no. of participants who received anti-D   | 52                        | 103                        | 39                         | 171                       | 365                            |
| No. of participants who received only antenatal prophylaxis, <i>n (%)</i>               | 47 (90.4)                 | 94 (91.3)                  | 39 (100.0)                 | 132 (77.2)                | 312 (85.5)                     |
| No. of participants who received only postnatal prophylaxis, <i>n (%)</i>               | 3 (5.8)                   | 9 (8.7)                    | 0                          | 35 (20.5)                 | 47 (12.9)                      |
| No. of participants who received both antenatal and postnatal prophylaxis, <i>n (%)</i> | 2 (3.8)                   | 0                          | 0                          | 4 (2.3)                   | 6 (1.6)                        |
| Before signing informed consent form (ICF) (during current pregnancy)                   |                           |                            |                            |                           |                                |
| Total no. of participants who received anti-D   | 20                        | 36                         | 23                         | 61                        | 140 <sup>a</sup>               |
| No. of participants who received only antenatal prophylaxis, <i>n (%)</i>               | 20 (100.0)                | 36 (100.0)                 | 23 (100.0)                 | 61 (100.0)                | 140 (100.0)                    |
| After signing ICF (during current pregnancy)  |                           |                            |                            |                           |                                |
| Total no. of participants who received anti-D   | 33                        | 67                         | 16                         | 116                       | 232 <sup>a</sup>               |
| No. of participants who received only antenatal prophylaxis, <i>n (%)</i>               | 28 (84.8)                 | 58 (86.6)                  | 16 (100.0)                 | 77 (66.4)                 | 179 (77.2)                     |
| No. of participants who received only postnatal prophylaxis, <i>n (%)</i>               | 4 (12.1)                  | 9 (13.4)                   | 0                          | 39 (33.6)                 | 52 (22.4)                      |
| No. of participants who received both antenatal and postnatal prophylaxis, <i>n (%)</i> | 1 (0)                     | 0                          | 0                          | 0                         | 1 (0.4)                        |

<sup>a</sup>Seven participants received anti-D administration at both stages – before signing the ICF and after signing the ICF; these participants are included with both populations

Rh-negative status. The prevalence of the Rh-positive blood group among the spouses of the participants was very high (89.6%), and thus the risk of Rh disease due to possible incompatibility with fetal blood was also high in this population. Additionally, 45.6% of the participants were multigravida, and thus, the need for careful handling of their current pregnancies was high among the participants. Six delivery-related complications or AEs were noted among the participants, and six fetal complications were recorded in babies (five Rh-negative babies and one Rh-positive baby).

The 2022 FOGSI guidelines emphasize the importance of ICT in managing Rh-negative pregnancies and recommend that ICT should be performed at the first antenatal visit, and thereafter at 28 weeks (<https://www.fogsi.org/wp-content/uploads/tog/anti-d-immunoglobulin-2022.pdf>). Indirect Coombs test data were available for only 125 of 1421 participants in the current study. The incidence rate of ICT positivity was 7.2% (9 of 125 participants), which is in line with the isoimmunization rate of 10.7% (78.4% attributed to anti-D) reported by Pahuja et al.<sup>14</sup> in Rh-negative mothers attending antenatal clinics in Northern India. Notably, this figure (7.2%) is much higher than that reported from developed countries, which stands at 0.5%,<sup>15</sup> and underlines the need for implementing robust prophylaxis strategies in developing countries including India.

Anti-D prophylaxis is the most effective method to prevent Rh disease and has been recommended by various organizations worldwide.<sup>3,4,8-10,13</sup> The FOGSI 2022 guidelines specified “practice points” for Rh-D prophylaxis at various stages of pregnancy: routine antenatal anti-D prophylaxis (single dose of 300 µg intramuscular (IM) in women with negative ICT results at 28 weeks), antenatal prophylaxis after sensitization events (single IM dose; if before 20 weeks: 150 µg; if post-20 weeks: 300 µg), and postpartum anti-Rh prophylaxis (300 µg IM administered within 72 hours of delivery; <https://www.fogsi.org/wp-content/uploads/tog/anti-d-immunoglobulin-2022.pdf>). In this study, of those with data available, 71.1% received anti-D prophylaxis (365 of 513 participants) as IM doses. Most of these participants (85.5%) received only antenatal prophylaxis, while a small proportion (1.6%) received both antenatal and postnatal prophylaxis. Thus, the rate of anti-D utilization was moderately high (71.1%), though it needs to be improved further.

The Federation of Obstetric and Gynaecological Societies of India recommends that cord blood testing should be performed immediately after delivery and should guide the administration of postpartum immunoprophylaxis. In this study, blood typing of the baby was performed for 207 of 225 babies (92.0%) born to Rh-negative mothers. This shows that compliance with standard

guidelines for handling Rh-negative pregnancies was high. The incidence of the Rh-positive phenotype among the babies (and thus maternal–fetal Rh incompatibility) was 77.3% (160 of 207), underlining the importance of anti-D prophylaxis in this population.

Patient education plays an important role in preventing Rh disease. In this study, most of the participants were from lower-middle-class to upper-middle-class households, and rural and urban areas were comparably represented. These data support the contention that most of the study participants may have relatively easy access to healthcare, and hence, it may be easy to increase awareness among them for Rh disease, and the associated risks to mother and baby.

In addition to patient education, proper pregnancy monitoring and adopting the recommended screening strategies are essential to prevent Rh disease. Phototherapy and exchange transfusions can be provided to newborns to reduce the chances of HDFN and other complications of Rh disease as part of routine monitoring and care.<sup>3,4,10</sup>

The main limitation of this study is the lack of availability of complete data for many participants. This can be attributed to the real-world nature of the study. Despite the missing data, the data presented here may contribute to and strengthen the limited large-scale data available in this field. Additionally, since the study is ongoing with many thousands of participants expected to be enrolled, future publications are expected to include more robust data from the participants, which will overcome these limitations.

Lack of sufficient recent data on the prevalence of the Rh-negative blood group among pregnant women is an important issue. Such data are important to guide public health policy with reference to promoting awareness and prophylaxis measures for Rh-negative pregnant women. This underlines the need for and importance of this study.

## CONCLUSION

In summary, our analysis shows that Rh-negative pregnancies need to be managed carefully, and the latest guidelines and recommendations should be applied in all cases. Antenatal screening and anti-D prophylaxis should be applied diligently to prevent maternal and fetal complications. Our study underlines the need for patient education about the risks associated with Rh-negative maternal–fetal incompatibility in both isoimmunized mothers as well as those who are at risk of isoimmunization. Additionally, mothers who are at risk of isoimmunization need to be educated and informed about the need and availability of anti-D prophylaxis. Further, this study provides a basis for developing a database containing pertinent data on this population, which can help to improve the management of high-risk Rh-negative pregnancies and ensure favorable clinical outcomes for mothers and babies.

## Clinical Significance

Rhesus D-negative women who carry an Rh-D-positive fetus are at risk of being sensitized to produce immune anti-D antibodies following a fetomaternal hemorrhage during pregnancy, leading to HDFN. India has an average 5% prevalence of Rh-negative status in pregnant women out of total pregnancies every year which is approximately 13 lakh Rh-negative pregnant women every year as per the National Family Health Survey-5 (2019–2020).

Even though there is an availability of prophylactic treatment, there are patients who are still getting sensitized just because they have not taken the antenatal and postnatal dose of

anti-Rh (O) D immunoglobulin. For patients, it is easy to deny taking these two dosages of anti-Rh (O) D immunoglobulin since they do not understand the cost of getting sensitized. However, the desire of becoming a mother or multigravida remains a dream because she gets sensitized subsequently as a result of a missed dosage of anti-Rh (O) D immunoglobulin post the previous pregnancy.

Nevertheless, there is still hope for such sensitized patients to continue with the current pregnancy and have a healthy fetus at least until delivery and/or beyond through a procedure called fetal blood transfusion *in utero*. However, this process is very expensive, and patients may be required to repeat the fetal blood transfusion *in utero* multiple times in the current pregnancy to have a healthy fetus to turn into a healthy newborn. Nonetheless, there are very minimal chances of the fetus surviving even after multiple transfusion procedures, which also induces transfusion-related financial burden vis-a-vis the cost of two dosages of anti-Rh (O) D immunoglobulin.

Thus, there is a need to enforce awareness among the Indian population about Rh-disease-associated risks including the financial burden and available preventive measures to reduce the mortality rates due to Rh-disease.

## SUPPLEMENTARY MATERIALS

All the supplementary tables from 1 to 5 are available online on the website [www.jsafog.com](http://www.jsafog.com).

## ORCID

Pratik Shah  <https://orcid.org/0009-0005-3214-488X>

Supriya Hiranman Pawar  <https://orcid.org/0009-0001-2132-2290>

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