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ORIGINAL ARTICLE

Rh alloimmunization awareness among pregnant women in the north of Jordan: a cross sectional study

Short title: Rh Alloimmunization Awareness

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ABSTRACT

Objective. Rh alloimmunization is a condition where antibodies develop against fetal red blood cell antigens; this can lead to severe complications. However, studies on the awareness among pregnant women about this condition remain limited. This cross-sectional study aims to assess the awareness of Rh alloimmunization among pregnant women in the north of Jordan as a preliminary step toward enhancing the quality of healthcare services provided.

Materials and Methods. In this study, a total of 403 pregnant women were enrolled. Data were collected from pregnant women attending antenatal clinics at King Abdullah University Hospital and Princess Badea'a Hospital using a validated questionnaire. The questionnaire covered various aspects including demographics, awareness of blood type, current pregnancy history, anti-D immunoglobulin administration, and knowledge of Rh alloimmunization. Data analysis was conducted using Statistical Package for Social Sciences software, version 26, focusing on the awareness level of the participants and making comparisons between different categories. Kruskal-Wallis and Mann-Whitney U tests were employed to examine potential variations in knowledge scores.

Results. Based on our results, 39.5% of the participants were aware about Rh alloimmunization, its associated complications, and anti-D immunoglobulin. In addition, the analysis revealed a significant association between a higher level of knowledge about Rh alloimmunization and higher educational level, better socioeconomic status, first pregnancy, negative Rh status, exposure to a sensitizing event during pregnancy, history of indirect Coombs test and anti-D immunoglobulin administration (P-values < 0.001).

Conclusions. The study concluded that there was poor knowledge regarding Rh alloimmunization, its associated complications, and anti-D immunoglobulins.

Key words

Blood group incompatibility; maternal health; histocompatibility; maternal-fetal.

Introduction

Rh alloimmunization describes the development of antibodies against D antigens when a negative blood type (the mother) is exposed to positive red blood cells (from the fetus) [1, 2].

Surface antigens expressed on the surface of red blood cells (RBCs) include C, c, E, kell, and D antigens, which are known as rhesus factors (Rh factors). Among them, the D antigen appears to be the most important as it is highly immunogenic compared to other types of Rh factors [1, 2].

The IgG antibodies against D antigens persist in the maternal circulation and can cross the placenta freely during the pregnancy into the fetal circulation leading to formation of antigen-antibody complexes with the fetal RBCs and consequently the destruction of fetal RBCs. This leads to fetal anemia, hyperbilirubinemia, jaundice, kernicterus, neurological sequelae, erythroblastosis fetalis, and fetal demise. Therefore, the prevention of Rh alloimmunization is essential to avoid such complications [1, 2].

Rh alloimmunization may be prevented by the administration of anti-D prophylactic immunoglobulins [3, 4]. They are administered to Rh-negative females who have been exposed to Rh-positive blood. It is assumed that the anti-D immunoglobulins bind to the receptors on the surface of D antigen in Rh-positive RBCs [3]. This binding prevents the immune system of the Rh-negative person from recognizing these foreign antigens, thus preventing the immune response and the formation of antibodies against them. The rate of Rh alloimmunization without prophylactic anti-D immunoglobulin accounts for 16% [5].

Since 1977, a single dose of 300 micrograms of anti-D immunoglobulin during the 28th week of pregnancy and another dose after delivery has been recommended, which made a marked decrease in Rh alloimmunization rates to less than 0.2% in the United States. Nowadays, Rh alloimmunization prevalence is estimated to be between 0.4-2.7% worldwide [4, 6, 7]. In addition, the administration of prophylactic anti-D immunoglobulin, both antenatally and postnatally, has successfully decreased the rates of Rh alloimmunization to less than 1%. When using only postnatal injection, the rate decreases to 2% [5].

During the early stages of pregnancy, it is recommended to test the blood type of both partners and to assess the presence of anti-D antibodies for Rh-negative women [8]. Also, prophylactic anti-D immunoglobulins are recommended for all Rh-negative non-sensitized pregnant women

at 28 and 34 weeks of gestation and within 72 hours after delivery [2, 5, 9]. However, for Rh-negative pregnant women who have been exposed to a sensitizing event an anti-D injection should be offered within 72 hours of the event [5]. During pregnancy, the fetus and the mother have separate blood circulations. However, mixing of the two circulations can occur [4]. This can happen in many situations like giving birth, miscarriage, abdominal trauma, ectopic pregnancy, antepartum hemorrhage, intrauterine procedures, or external cephalic version [2, 4].

A literature review which was conducted across different countries examined the practice of anti-D immunoglobulin administration antenatally and postnatally and found that antenatal anti-D prophylaxis was given 80-90% of the time compared to 95-100% for postnatal anti-D injection [10]. In contrast, once Rh alloimmunization has occurred and the level of anti-D antibodies has reached a critical point, anti-D injections are not effective in managing the situation. Instead, alternative options should be considered such as non-invasive monitoring of fetal anemia, intrauterine transfusion when done properly, or fetal delivery [2, 11]. In case of erythroblastosis fetalis or hydrops fetalis that is characterized by polyhydramnios, pleural effusion, and pericardial effusion, a fetal echocardiography is recommended [2, 12].

The choice of intervention depends on the severity of anemia and gestational age of the fetus [2]. In the absence of preventive measures, approximately 14% of susceptible women will develop anti-D antibodies within 6 months after delivery or during subsequent pregnancies [13].

Several cross-sectional studies conducted in Iraq, Nigeria, and Saudi Arabia aimed to assess population awareness of Rh status and Rh alloimmunization [14–17]. In the Iraqi study, newly married couples were targeted, with 1,136 couples participating. The findings revealed that 42 couples have Rh alloimmunization, and 64.3% of the male partners for the 42 couples had no prior knowledge of their wives' blood group [15]. Moreover, 83.3% of the male partners of the 42 couples had no prior knowledge about the importance or complications of Rh alloimmunization [15]. The Nigerian study was conducted among 215 pregnant women who attended antenatal clinics at Babcock University Teaching Hospital. The study concluded that they were aware of Rh alloimmunization but not its associated complications [17]. Another Nigerian study enrolled 927 secondary school girls, which concluded that there was poor knowledge and awareness of Rh alloimmunization and their own Rh status [14]. However, the Saudi Arabia cross-sectional study recruited pregnant women attending a routine antenatal clinic to measure their awareness of Rh alloimmunization. A total of 108 pregnant women participated in the study, of which 41.7% were aware of Rh alloimmunization and their Rh status [16].

The primary aim of this study is to assess the level of awareness among pregnant women in the north of Jordan regarding Rh alloimmunization, a crucial issue that demands immediate attention due to its significant impact on maternal and fetal health. Additionally, this study aims to assess the various factors that influence this awareness among the target population.

This study provides valuable insights that can help in the implementation of awareness programs for pregnant women, especially those at higher risk of developing Rh alloimmunization. In addition, this study's insights will help to enhance the quality of primary healthcare services offered to pregnant women in Jordan, which could aid in reducing preventable fetal and maternal complications, thereby lowering the overall healthcare costs associated with managing this condition.

To our knowledge, this study is the first of its kind in Jordan, which adds to its uniqueness and provides valuable insights for future research in this vital area of maternal and fetal health.

Materials and Methods

In this cross-sectional study, we targeted pregnant women in the north of Jordan. Data were gathered through face-to-face interviews and self-reported questionnaires. The sample was recruited from the two major tertiary hospitals in the north of Jordan: King Abdullah University Hospital (KAUH) and Princess Badea'a Hospital (PBH). A total of 403 pregnant women aged 18 to 50 attending antenatal care (ANC) clinics at KAUH and PBH were enrolled in the study and in the final data analysis. The number of participants the approximate monthly attendance of pregnant women at the ANC clinics, which is around 1,000 at KAUH and 2,000 at PBH. With a confidence interval of 95% and a margin of error of 5%, a sample size of 340 was requested in total from both hospitals. The study was approved by Yarmouk University institutional review boards. This work was done in adherence to the declaration of Helsinki, and all participants' data were kept confidential.

The questionnaire was designed based on previous similar articles and a literature search [12-15] using Google Forms, questionnaire link: https://docs.google.com/forms/d/e/1FAIpQLScYo7wnVBibbozXbill3sPGhhuSqxgIUl5UCPzqV8oBuc9iDA/viewform?usp=sharing). Before distributing the questionnaires, a pilot study was conducted involving 33 pregnant women, and its validity was checked by qualified specialists. The questionnaire was translated from English into Arabic by a professional medical team and was able to be retranslated. Data collection was done over a two-month period.

The questionnaire included four main sections. Demographics were gathered in the first section. The second section focused on assessing participants' pregnancy history, including past and current pregnancies, history of anti-D immunoglobulin administration, vaginal bleeding, abdominal trauma, miscarriage, indirect Coombs test (ICT), and the participants' awareness of their blood type and their partners' blood type. The third section incorporated multiple questions aimed at assessing participants' awareness of Rh alloimmunization, its associated complications, and its sensitizing events, as explained in Table 1. In addition, there were specific questions assessing the awareness of anti-D injection timing and its importance, as explained in ***Table 1***. The last section was about the sources of information.

Due to the absence of a universally accepted questionnaire measuring Rh alloimmunization, we developed a scoring system to calculate a total score that represented each participants' awareness. However, we were unable to define a specific cut point to categorize participants into those with good knowledge and others without. Consequently, the mean was used as a cut point, where those who scored above the mean were considered to have good knowledge while those below it were considered to have poor knowledge. One point was granted for each correct answer as shown on ***Table 1.***

The information was taken from Google Forms and converted to an Excel spreadsheet before being entered into the Statistical Package for Social Sciences (SPSS) version 26. The knowledge score was compared according to age, area of residence, educational level, occupation, economic status, Rh status, blood type, history of vaginal bleeding during pregnancy, and number of pregnancies. Descriptive analysis was used to display categorical variables as percentages and frequencies while presenting numerical variables as a mean and standard deviation to evaluate the data quantitatively. Normality was tested using the Shapiro-Wilk test. The Mann-Whitney U and Kruskal-Wallis tests were conducted to assess potential

differences in means among variables. A p-value less than 0.05 was considered statistically significant.

Results

A total of 403 women participated in the study, where 391 were within the 18-30 age range. ***Table 2*** demonstrates the demographic characteristics.

Regarding maternal blood group, 90.07% were familiar with their blood type and 67.7% were familiar with their husband's blood type. Considering maternal Rh status, 78.7% were Rh-positive while only 11.4% were Rh-negative and 9.9% were unaware of their Rh status.

On the other hand, 59.8% of the husbands were Rh-positive and only 7.9% were Rh-negative while 32.2% of the participants were unaware of their partners' blood type. However, 3.23% of the couples were Rh incompatible.

A small number reported exposure to sensitizing events during current pregnancy where 11.9% experienced vaginal bleeding, 4.2% underwent surgical or non-surgical gynecological procedures, and only 3.2% had abdominal trauma during current pregnancy. ***Table 3*** demonstrates the pregnancy and maternal health characteristics.

Regarding Rh alloimmunization knowledge questions, 28.78% were aware that Rh alloimmunization occurs in a Rh-negative mother with Rh-positive father and 20.74% were aware of Rh alloimmunization complications. Moreover, 31.76% were aware that Rh alloimmunization could result in fetal death and it was the most frequently reported complication, followed by fetal anemia and fetal jaundice, 21.84%, and 18.11%, respectively.

Moving to anti-D immunoglobulin awareness questions, 32% of the participants were aware that anti-D immunoglobulin administration decreases the risk of complications in future pregnancies and 29.78% knew that anti-D immunoglobulin prevent anti-Rh antibodies formation. Concerning the knowledge of anti-D timing, 32% were aware that anti-D immunoglobulin could be given in each pregnancy, and 22.58% were aware that anti-D immunoglobulin is given in the first three days after delivery. However, only 12.6% and 9.68% were aware that anti-D immunoglobulin is given in the 28^{th} and 34^{th} gestational weeks, respectively.

The knowledge score exhibited an average of 4.34 ± 5.13 (mean ± SD), with a median score of 2 (ranging from 0 to 21). Over one third (38.7%) had a score of zero and 18.86% had a score of more than 10. After using the mean as a cut-off point, 159 (39.5%) scored above the mean and were considered to have good knowledge while 244 (60.5%) scored below the mean and were categorized to have poor knowledge.

The Mann-Whitney U and Kruskal-Wallis tests were employed to examine potential variations in knowledge scores within our sample. We found that participants living in urban areas, with university-level education, who were employed, with a monthly income exceeding 1,000 Jordanian dinars, with negative Rh status, who experienced their first pregnancy, who reported vaginal bleeding during their current pregnancy, who received the anti-D immunoglobulin during the current pregnancy or in previous pregnancies, and who did the ICT at the beginning of pregnancy had significantly higher knowledge scores with all p-values < 0.001. ***Table 4 and 5***

demonstrate the relationship between knowledge scores and demographics, pregnancy, and maternal health.

Regarding sources of knowledge, 35% of the participants reported that their knowledge was from other sources than those mentioned in the figure. ***Figure 1*** demonstrates the information sources.

Discussion

Main findings

This cross-sectional study sought to assess the awareness of Rh alloimmunization among 403 pregnant women attending ANC clinics at KAUH and PBH in the north of Jordan. Our results revealed in general a poor knowledge of Rh alloimmunization as 39.5% of the participants exhibited good knowledge about Rh alloimmunization and prophylactic anti-D immunoglobulin. Only 29.78% knew when Rh incompatibility occurs, and 31.76% were aware that it could cause fetal death and it was the highest percentage regarding Rh related complications. In addition, a humble percentage 27.85% reported that anti-D immunoglobulin can prevent anti-Rh antibodies formation, while the most known timing for administration was in the first three days after delivery with 22.58%.

There are highly statistically significant associations between the level of knowledge about Rh alloimmunization and negative Rh status, higher educational level, better socioeconomic status, and history of anti-D immunoglobulin administration in the current and previous pregnancies, and indirect Coombs testing in current pregnancy (p-values < 0.001), along with vaginal bleeding during the current pregnancy (p-values = 0.012).

We assume that participants who have negative Rh status were more aware of Rh alloimmunization because they received information about their condition, underwent necessary testing (indirect Coombs test), and were given anti-D immunoglobulin by healthcare providers. Additionally, they are keen on acquiring knowledge about this disease, which could threaten the lives of their babies. In case of sensitizing events, such as vaginal bleeding, the medical team must follow a stepwise approach regarding the patient's condition and then offer an anti-D immunoglobulin within 72 hours of the sensitizing event [8]. This in turn raises awareness regarding the necessity of receiving an anti-D immunoglobulin injection when needed, in addition to informing the patient about potential complications that may arise.

Considering anti-D timing, the most frequently known time was during the first three days after delivery (22.58%); this might be explained by the more commonly practiced approach as explained in the literature review by (Fyfe) [10]. Postnatal anti-D prophylaxis was given in 95-100% of the cases and antenatal prophylaxis was given in 80-90% of the cases [8]. The standard dose of anti-D immunoglobulins covering 30 ml of fetal whole blood is 300 micrograms [18].

There are some minor differences between guidelines on when to give anti-D immunoglobulins prophylaxis. ***Table 6*** provides a summary of the following societies and organizations guidelines: American College of Obstetricians and Gynecologists (ACOG) guidelines, British Committee for Standards in Hematology (BCSH), National Institute for Health and Care

Excellence (NICE), and Royal Australian College of Obstetricians and Gynecologists (RANZCOG) [18–21].

Strengths and Limitations

The major motive for this study was the lack of similar studies in Jordan, so our study is considered the first of its kind in Jordan. Based on our results in which the sample was recruited from two major tertiary hospitals in the north of Jordan, we found that there was a lack of sufficient awareness about this issue. Furthermore, our face-to-face interviews revealed a noteworthy observation: participants whose relatives received an anti-D immunoglobulin injection had better knowledge than those whose relatives did not receive the injection. However, this relation needs to be studied further.

This study faced some limitations including its cross-sectional nature, so we cannot establish a causal relationship. In addition, because this study was limited to pregnant women in the north of Jordan, the results cannot be generalized to the Jordanian population. In addition, we could not compare the awareness and knowledge between Rh incompatible and Rh compatible couples because the Rh incompatible couples group was very small compared to Rh compatible groups. Finally, our ability to compare our studies with international studies was hindered by the scarcity of globally conducted studies focused on assessing Rh alloimmunization.

Interpretation and Comparison with Other Literature

Several cross-sectional studies have been conducted to assess population awareness of Rh alloimmunization [14–17]. A Nigerian study, which enrolled 140 pregnant women attending ANC clinics, concluded that there was knowledge about Rh alloimmunization but poor awareness about the complications [17], which coincides with our results regarding awareness about complications but not about the Rh alloimmunization.

Furthermore, a cross sectional study was conducted in Saudi Arabia which recruited pregnant women attending a routine antenatal clinic to measure their awareness of Rh alloimmunization. A total of 108 pregnant women participated in the study, of which 41.7% were aware of Rh alloimmunization [16] while 28.78% were aware about Rh alloimmunization in our study. In comparison to our study, the Saudi study found a stronger association between awareness and the previous administration of anti-D immunoglobulin as well as vaginal bleeding in early pregnancy (p-value = 0.000) [14]. As opposed to the Saudi study that reflected an increased awareness among older women, our study showed no association between the age and the level of Rh alloimmunization awareness. Moreover, they found that parity had no association with awareness while in our study we found that first-time pregnancy had a considerable association [16]. This could be related to age of marriage, as the mean age of first marriage in Jordan is 24.6 years while it is 26.6 years in Saudi Arabia according to the World Bank [22].

An Iraqi study and another Nigerian study were conducted among different populations with newly married couples and undergraduate female students, respectively [14, 15]. In the Iraqi study, 1,136 couples were enrolled. There were 42 Rh incompatible couples of which 64.3% of them were unaware of their wife's blood group or Rh status and 83.3% were unaware of Rh alloimmunization consequences, compared to our results 79.26% were unaware of Rh alloimmunization consequences [15]. Nevertheless, the Iraqi study focused on newly married couples, while our study enrolled pregnant women.

In the Nigerian study, 23.85% were considered to have good knowledge, compared to our study, 39.5% were considered to have good knowledge. However, the population in the Nigerian study comprised of undergraduate females, while our study examined the knowledge of pregnant women [14].

Another study that was conducted among 1,800 Omani individuals to assess the awareness of their own blood type concluded that 95.2% were familiar with their blood type. This is in line with our study where 90.07% were familiar with their blood type [23]. However, this study did not examine the population awareness of Rh alloimmunization.

Conclusion

According to our findings, 39.5% of the pregnant women in this study exhibited good knowledge about Rh alloimmunization, its associated complications, anti-D timing, and its importance. Furthermore, knowledge of Rh alloimmunization showed a significant association with many factors including negative Rh status, higher educational level, better socioeconomic status, history of anti-D immunoglobulin administration, indirect Coombs testing, vaginal bleeding, and residing in urban areas. However, there was no significant association between knowledge of Rh alloimmunization and age, current trimester, previous surgical or non-surgical gynecological procedures, and previous miscarriage.

In conclusion, we recommend expanding the scope of the research to comprise the general population, raising awareness through publishing brochures, conducting seminars, and providing direct education to the patients, with a focus on rural areas. Also, we recommend including blood group testing as a part of pre-marital screenings and informing couples about the possibility of developing Rh alloimmunization and ways to prevent it.

List of abbreviations

RBCs: red blood cells

KAUH: King Abdullah University Hospital

PBH: Princess Badea'a Hospital

ANC: antenatal care

ICT: indirect coombs test

SPSS: Statistical Package for Social Sciences

JD: Jordanian Dinar

ACOG: American College of Obstetricians and Gynecologists

BCSH**:** British Committee for Standards in Hematology

NICE: National Institute for Health and Care Excellence

RANZCOG: Royal Australian College of Obstetricians and Gynecologists.

COMPLIANCE WITH ETHICAL STANDARDS

Author contributions

Idea conceptualization: S.A[1], Y.A.

Data collection: S.A[2], M.H, A.A.A, Y.Q.A, Y.A, D.D.

Data analysis: J.F.A.

Organizing and mentoring: S.A[1], S.A[2]

Writing: S.A[2], M.H, M.N, A.A.A, Y.Q.A, Y.A, DD.

Review and editing: S.A[1], S.A[2], M.H, M.N

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Competing interests

The authors declare no competing interests, and all authors approved the final version.

Ethical approval and consent to participants

All ethical approvals needed for this study were obtained. The Institutional Review Board approval from Yarmouk University was obtained with the reference number IRB/2023/270.

Informed consent

A consent form was secured from each participant before filling the questionnaire and all data were kept confidential.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files], and they are available from the corresponding author on reasonable request.

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Table 1. Questionnaire Scores

Questions	**Participant's answers**	
	Yes	**No**
Do you know about Rh alloimmunization?	1 point	0 point
Who does Rh alloimmunization affect (you can choose one answer)?	**Participant's answers**	
Rh-negative mother and Rh-negative fetus	0 point	
Rh-negative mother and Rh-positive fetus	1 point	
Rh-positive mother and Rh-positive fetus	0 point	
Rh status does not matter	0 point	
I do not know	0 point	
Rh antibodies (choose the correct answer):	**Participant's answers**	
Affect the first pregnancy.	0 point	
Affect pregnancies after the first pregnancy	1 point	
Does not affect fetus	0 point	
I do not know	0 point	
Which one of following is/are complications of Rh alloimmunization (choose all correct answers)?	**Participant's answers**	
Rh alloimmunization causes anemia to the fetus	1 point	0 point
Rh alloimmunization causes death to the fetus	1 point	0 point
Rh alloimmunization causes hydrops to the fetus	1 point	0 point

Rh alloimmunization causes neurological complications to the fetus	1 point	0 point
Rh alloimmunization causes jaundice to the fetus	1 point	0 point
I do not know	0 point	0 point
Anti-D injections (choose all correct answers):	**Participant's answers**	
Anti-D injections are given in the first pregnancy only	0 point	1 point
Anti-D injections could be given in each pregnancy	1 point	0 point
Anti-D injections could be given multiple times in the same pregnancy	1 point	0 point
Anti-D injections could prevent complications of future pregnancies	1 point	0 point
Anti-D injections prevent anti Rh antibodies formation	1 point	0 point
I do not know	0 point	0 point
When should anti-D injections be offered:	**Participant's answers**	
28th gestational weeks	1 point	0 point
34th gestational weeks	1 point	0 point
In the first three days after delivery	1 point	0 point
Any time after delivery	0 point	1 point
Severe vaginal bleeding or recurrent vaginal bleeding during pregnancy	1 point	0 point
Trauma to the abdomen during pregnancy	1 point	0 point
After amniocentesis or chorionic villus sampling	1 point	0 point

After abortion	1 point	0 point
After ectopic pregnancy	1 point	0 point
I do not know	0 point	0 point
Total score	**22**	

Table 2. Demographic Characteristics (n = 403)

Item	Frequency (%)
Age in years	
18-30	206 (51.1)
30-40	185 (45.9)
40-50	12 (3.0)
Area of Residence	
Urban	211 (52.3)
Rural	168 (41.7)
Camp	24 (6.0)
Governorate	
Northern governorates	390 (96.8)
Central governorates	12 (3.0)
Southern governorates	1 (0.2)
Highest educational level achieved	
School	194 (48.1)
University	209 (51.9)
Occupation	
Employee	95 (23.5)
Housewife	308 (76.5)
Monthly family income	
Less than 500 JDs	281 (69.7)
500 - 1000 JDs	109 (27.0)
More than 1000 JDs	13 (3.3)

JDs: Jordanian Dinars

Table 3. Pregnancy and Maternal Health Characteristics (n = 403)

Item	Frequency (%)
First pregnancy	
Yes	60 (14.9)
No	343 (85.1)
Current trimester through this pregnancy	
First trimester (1-13 weeks)	34 (8.4)
Second trimester (14-27 weeks)	85 (21.1)
Third trimester (28-40 weeks)	284 (70.5)
Previous miscarriage	
Zero	180 (44.7)
Once	83 (20.6)
Twice	45 (11.2)
More than two	35 (8.7)
NA, since the first time to be pregnant	60 (14.9)
Number of children	
Zero	73 (18.1)
One	90 (22.3)
Two	90 (22.3)
More than two	150 (37.2)
Place of prenatal care *	
Government Hospital	207 (51.4)
University Hospital	173 (42.9)
Military Hospital	1 (0.2)
Health Center	14 (3.5)
Private Sector	93 (23.1)
Previous surgical or non-surgical gynecological procedures during the current pregnancy*	

Yes	17 (4.2)
No	386 (95.8)
Experienced any abdominal injuries during the current pregnancy	
Yes	13 (3.2)
No	390 (96.8)
Experienced vaginal bleeding during the current pregnancy	
Yes	48 (11.9)
No	355 (88.1)
Received the anti-D immunoglobulin during the current pregnancy	
Yes	14 (3.5)
No	205 (50.9)
Don’t know	39 (9.7)
NA	145 (36.0)
Received the anti-D immunoglobulin in the previous pregnancies if this is not the first pregnancy	
Yes	29 (7.2)
No	128 (31.8)
Don’t know if they received or not	186 (46.2)
NA	60 (14.8)
Timing of ICT for Rh-negative participants	
Start of pregnancy	10 (2.5)
Before receiving the immunization shot	7 (1.7)
During the seventh month of pregnancy - week 28	3 (0.7)
Did not undergo the test	25 (6.2)
Don't know if they performed or not	81 (20.1)
NA	277 (68.7)

ICT: Indirect Coombs Test, NA: Not Applicable, *: The question was multiple responses set.

* Surgical and non-surgical procedures: amniocentesis, chorionic villus sampling, and any other surgical procedures.

Table 4. The Relationship between Knowledge Scores and Demographics.

Item	Mean ± SD	Median (Max-min)	P-value
Age in years [A]			0.925
18-30	4.20 ± 5.07	2 (21-0)	
30-40	4.55 ± 5.31	2 (20-0)	
40-50	3.42 ± 3.37	3 (9-0)	
Area of residence [A]			**0.036**
Urban	4.64 ± 5.26	2 (21-0)	
Rural	4.31 ± 5.18	2 (21-0)	
Camp	1.87 ± 3.26	0 (12-0)	
Governorate [A]			0.390
Northern governorates	4.38 ± 5.15		
Central governorates	3.25 ± 4.83		
Southern governorates	-	-	
Highest educational level achieved [B]			**<0.001**
School	2.64 ± 3.91	1 (17-0)	
University	5.91 ± 5.62	5 (21-0)	
Occupation [B]			**<0.001**
Employee	6.82 ± 5.80	6 (21-0)	

Housewife	3.57 ± 4.68	1 (20-0)	
Monthly family income [A]			**<0.001**
Less than 500 JDs	3.61 ± 4.72	1 (21-0)	
500 - 1000 JDs	5.61 ± 5.28	5 (19-0)	
More than 1000 JDs	9.38 ± 7.53	6 (21-0)	

JDs: Jordanian Dinars, ICT: Indirect Coombs Test.

A: Kruskal–Wallis, B: Mann–Whitney U test.

P value less than 0.05 was considered significant.

Table 5. Relationship between Knowledge Score and Maternal Health and Pregnancy

Item	Mean ± SD	Median (Max-min)	P-value
Maternal Rh status [B]			**<0.001**
Rh-Positive	4.09 ± 4.970	2 (21-0)	
Rh-Negative	8.09 ± 5.448	8 (21-0)	
First pregnancy [B]			**0.017**
Yes	5.83 ± 5.66	5 (21-0)	
No	4.08 ±5.00	2 (21-0)	
Current trimester through this pregnancy [A]			0.298
First trimester (1-13 weeks)	4.76 ± 4.94	2.5 (17-0)	
Second trimester (14-27 weeks)	4.99 ± 5.63	2 (21-0)	
Third trimester (28-40 weeks)	4.09 ±5.00	2 (21-0)	
Previous abortions [A]			0.302
Zero	3.69 ± 4.84	1 (19-0)	
Once	4.89 ± 5.47	3 (21-0)	
Twice	3.62 ± 4.35	2 (16-0)	
More than two	4.69 ± 5.31	2 (17-0)	
Number of children [A]			0.111
Zero	5.41 ± 5.85	3 (21-0)	
One	3.78 ± 4.51	2 (18-0)	
Two	5.03 ± 5.35	4 (20-0)	

More than two	3.73 ± 4.89	1 (18-0)	
Previous surgical or non-surgical gynecological procedures during the current pregnancy [B]			0.733
Yes	4.41 ± 5.86	3 (21-0)	
No	4.33 ± 5.11	2 (21-0)	
Experienced any abdominal injuries during the current pregnancy [B]			0.883
Yes	4.54 ± 5.94	1 (14-0)	
No	4.33 ± 5.11	2 (21-0)	
Experienced vaginal bleeding during the current pregnancy [B]			**0.012**
Yes	6.06 ± 5.78	4 (17-0)	
No	4.10 ± 5.00	2 (21-0)	
Received the anti-D immunoglobulin during the current pregnancy [B]			**<0.001**
Yes	10 ± 4.90	10 (18-0)	
No	3.89 ± 4.74	2 (21-0)	
Received the anti-D immunoglobulin in the previous pregnancies if this is not the first pregnancy [B]			**<0.001**
Yes	6.93 ± 4.91	7 (17-0)	
No	2.42 ± 4.04	0 (18-0)	

If the blood type is negative, when was the ICT performed [A]			**<0.001**
Start of pregnancy	8.50 ± 7.22	6.5 (21-0)	
Before receiving the immunization shot	9.14 ± 6.09	9 (18-1)	
During the seventh month of pregnancy - week 28	8.33 ± 3.06	9 (11-5)	

JDs: Jordanian Dinars, ICT: Indirect Coombs Test.

A: Kruskal–Wallis, B: Mann–Whitney U test.

P value less than 0.05 was considered significant

Table 6. Summary of Guidelines for Anti-D Prophylaxis Administration

Organization	Antenatal prophylaxis	Postnatal prophylaxis
ACOG	300 micrograms at 28 weeks	Within 72 hours of delivery
BCSH	28 weeks, 34 weeks 500 IU, or one dose 1500 at 28 weeks	Within 72 hours of delivery
NICE	Two doses 500 IU at 28 and 34 weeks Or two doses 1000-1650 IU 500 IU28 at 28 and 34 weeks Or one dose 1500 IU at 28 weeks or between 28-30 weeks	Within 72 hours of delivery
RANZCOG	Two doses 625 IU at 28 and 34 weeks	Within 72 hours of delivery

ACOG American College of Obstetricians and Gynecologists, BCSH British Committee for Standards in Hematology, NICE National Institute for Health and Care Excellence, RANZCOG Royal Australian College of Obstetricians and Gynecologists.