COLLEGE OF HEALTH SCIENCES, SCHOOL OF MEDICINE, DEPARTMENT OF OBSTETRICS AND GYNECOLOGY POSTGRADUATE PROGRAM

Principal investigator: Melat Benti(MD) (Resident in Department of Obstetrics and Gynecology, School of Medicine, College of Health Sciences, Addis Ababa University)

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PREVALENCE AND DETERMINANT OF RH ALLOIMMUNIZATION IN RH NEGATIVE WOMEN IN THREE TEACHING HOSPITALS, ADDIS ABABA, ETHIOPIA, 2021.

Advisors:

- Dr. Kiflom Tesfaye (MD) (Associate Professor of Obstetrics and Gynecology, Department of Obstetrics and Gynecology; School of Medicine, College of Health Sciences, Addis Ababa University)

- Dr. Esayas Berhanu (MD) (Associate Professor of Obstetrics and Gynecology; School of Medicine, College of Health Sciences, Addis Ababa University)

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Addis Ababa, Ethiopia

APPROVAL SHEET
ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES, SCHOOL OF MEDICINE, DEPARTMENT OF OBSTETRICS AND GYNECOLOGY, POSTGRADUATE PROGRAM

I, Dr. Melat Benti, hereby declare that this thesis entitled "**Prevalence and determinant of Rh alloimmunization in Rh negative women in three teaching hospitals, Addis Ababa, Ethiopia, 2021**" in line with the requirement of graduate studies was fully undertaken by me under the guidance of my advisors and that I have, to the best of my knowledge and effort, avoided plagiarism or duplication of materials unless and otherwise cited and/or acknowledged and that it has not been so far submitted for any form of publication or consideration before the final approval.

Melat Benti (MD) _____ _____

Principal investigator Signature Date

We hereby certify that we have read and evaluated this research thesis relating to **Prevalence and determinant of Rh alloimmunization in Rh negative women in three teaching hospitals, Addis Ababa, Ethiopia, 2021** under our guidance from its inception up to in its current format and that it can be submitted for final approval in partial fulfillment to the Degree of Specialty in Obstetrics and Gynecology..

_____ _____ _____

1. Advisor Signature Date

_____ _____ _____

2. Advisor Signature Date

As a member of the MSc research open defense examination, I certify that I have read and evaluated the thesis prepared by Melat Benti (MD), and examined the candidate. I recommend that the thesis be accepted as fulfilling the thesis requirements for the Degree of Specialty in Obstetrics and Gynecology.

_____ _____ _____

Examiner	Signature	Date

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LIST OF ABBREVIATIONS

AAU: - Addis Ababa University

CHS: - College of health science

Rh D: - Rhesus

Ab: - antibody

Ag: - antigen

HDFN: - Hemolytic disease of fetus and newborn

RBCs: - Red blood cells

FMH: - Feto–maternal hemorrhage

RAADP: - Routine antenatal anti-D prophylaxis

IgG: - Immunoglobulin G

IgM: - Immunoglobulin M

ZMH: - Zewditu Memorial Hospital

GMH: - Gandi Memorial Hospital

TASH: - Tikur Anbesa Specialized Hospital

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Abstract

Background: Although the risks of adverse pregnancy outcomes associated with anti-D antibodies are reduced after the implementation of immunization to the D antigen, it's still a concern for most Sub-Saharan African countries, where there is poor and sometimes no Alloimmunization prevention following potentially sensitizing events. This may result in the compromise of the woman's obstetric care and increase perinatal morbidity and mortality. In Ethiopia the weight of the problem is less known and there is no study done in the study area. This study intended to fill this knowledge gap.

Objective: To study prevalence and determinant of Rh Alloimmunization in Rh negative mother in three teaching hospitals (TAH, GMH and ZMH) Addis Ababa, Ethiopia.

Methods and Materials: Hospital based cross-sectional study was conducted from 328 Rh negative pregnant women who booked for antenatal and delivery service utilization in TASH, GMH and ZMH from October 2020 to May 2021 to assess prevalence and determinant of Rh Alloimmunization in Rh negative women. The data were collected by means of structured questionnaires. The data were entered, coded and analyzed using Statistical Package for Social Science (SPSS) version 25. Descriptive and logistic regression analyses were conducted. Statistical tests were done at a level of odds ratio with 95% confidence intervals and significance of $p < 0.05$. Variables with P value < 0.25 during the bivariate analysis were included in the multivariate analysis to see the effect of confounding factors

Results and conclusion: The study revealed that prevalence of Rh D negative and Rh Alloimmunization in the study area were 2.1% and 17.1% respectively. Being unemployed, not using prophylaxis's anti-D, having previous sensitizing event and having two or more pregnancy were statistically significant with Rh alloimmunized ($p<0.05$).

Recommendation: Improvement of access and affordability of anti-D medication. As a nation there is no data that assess the determining factor of Rh Alloimmunization so research must be done with a large sample size to improve the management. In addition assessment of knowledge and practice of the health professional also important.

1 INTRODUCTION

1.1. Background

Alloimmunization is the development of alloantibody within an individual in order to protect against antigen derived from a different member of the same species (1). Of these most important one is antibodies produced against different blood groups. This antibody production may be due to blood transfusion or because of pregnancy (2). In human the ABO system and the Rhesus (Rh) system remain the most clinically significant blood group antigens on the red cell membranes (3). The Rh system consists of two discrete genes RhD and RhCE, which expressed the D and CE antigen respectively (4). Among this the most imperative is the Rhesus D (Rh D) antigen (3). People who have the D antigen on their RBCs are said to be Rh D-positive, while those who do not are alleged to be Rh D-negative (3).

During gestation, if the mother is Rh D-negative and the fetus Rh D-positive, fetal cells can enter the blood stream of the mother due to trauma, invasive procedures or spontaneously in the third trimester. The mother produces antibody (Ab) against these foreign antigen (Ag),a process known as Rh D sensitization (5).This sensibilization is followed by hepatosplenic uptake and destruction of the RBC by the macrophage system of the fetus (5).Although sensitization is unlikely to affect the current fetus during the first pregnancy, it result in the production of immunological memory in the mother and for future pregnancies, if baby is again RhD +ve, then this may results in the boosting of Rh antibodies and can result in erythroblastosis foetalis or haemolytic disease of foetus and new born (3).

The risk of sensitization depends largely upon the following three factors: volume of transplacental hemorrhage, extent of the maternal immune response and concurrent presence of ABO incompatibility (6).

Hemolytic disease of the fetus and newborn (HDFN) is a clinical condition that occurs as a result of hemolysis of fetal or neonatal red blood cells (RBCs) due to maternal red cell IgG antibodies that can cross the placenta (7). HDFN is characterized by fetal or neonatal anemia, hyperbilirubinemia and sometimes even fetal hydropsfetalis (7). ABO incompatibility also can

result in HDFN. When O group mother bears a foetus of A, B or AB blood group, but the severity will be reasonably less. Kell, Kidd, Duffy, M, N, S and s blood group also rarely cause HDN (13).

Implementation of programs for antenatal and postnatal Rh D immunoglobulin prophylaxis has lead to a significant reduction in the frequency of maternal Rh D alloimmunization and associated fetal and neonatal complications (14).

The distribution of Rh D negative varies widely, while the prevalence of greater than 14% is recorded among Caucasians, the prevalence among different ethnic group of sub- Saharan Africa range between 2.4%-4.5%(3).There is a limited number of studies conducted in Ethiopia.

1.2 STATEMENT OF THE PROBLEM

Despite the development and implementation of anti-D immune globulin prophylaxis, hemolytic disease of the fetus and newborn due to maternal Rh (D) alloimmunization continues to occur worldwide. Hemolytic disease of the fetus and newborn (HDFN) was an important cause of Perinatal morbidity and mortality until the 1960s. Today it is a rare, but potentially severe, complication of pregnancy (23). The fact that the prevalence of Rh-negative phenotype is significantly lower among Africans than Caucasians, Rh alloimmunization remains a major factor responsible for perinatal morbidity in Sub-Saharan Africa and may result in the compromise of the woman's obstetric care due to poor monitoring, accessibility and affordability of anti-D immunoglobulin (31). The knowledge of the distribution of Rh (D) blood group and determinant of Rh alloimmunization during pregnancy is essential for the effective management of pregnant mother during the antenatal and delivery period as well as treating complication due to Rh alloimmunization. Unfortunately, no study has so far has been conducted in the area to create sound understanding on prevalence of Rh negative and determinant of Rh alloimmunization during pregnancy. In the absence of sufficient understanding on the frequency Rh negativity and investigating the root cause that result in alloimmunization during pregnancy, our efforts to improve the health of mothers and children may become fruitless. Therefore, this study has been designed to fill the research gap.

1.3 Significance of the study

The insights of this will generate crucial findings regarding the prevalence of Rh negative, Socio-Demographic variables, factors that determine the risk of Rh sensitization in the context of the three teaching hospitals found in Addis Ababa. The study may further be used to know about the determinant of Rh sensitization in other areas of Ethiopia and other country with similar health care issues.

Further, the findings of the current study will provide policy makers, health practitioners, mothers and the community at large with useful insights regarding the prevalence and factor that determine the occurrence of Rh sensitization. Findings from the study will be published in international and local journals to disseminate knowledge and experience

2 LITERATURE REVIEW

2.1 Overview of RhD Negative pregnancy

Hemolytic disease of the newborn was first reported by a French midwife in a set of twins in 1609 (15). It's exact cause was determined by Levine after discovery of the Rh blood group system in 1940. The pathogenesis of Rh allo-immunization was confirmed in 1953 by Chown and coworkers (16). It's due to Rh positive fetal RBCs, inherited from the father, entering the maternal circulation and causing maternal isoimmunization (17). There is a dose-dependent relationship between the volume of Rh-positive RBCs to which the Rh-negative mother is exposed and the incidence of Rh immunization, with volumes as small as 0.1 mL resulting in antibody formation (18). In 1966, a combined study was carried out by two groups from the United States and United Kingdom in which they demonstrated that anti-D IgG prophylaxis soon after delivery prevented sensitization in Rh negative women (19). Women who are Rh negative require particular antenatal care and monitoring during pregnancy. If the fetus carried is Rhesus positive, there is a risk that mixing of fetal and maternal blood cells will lead to the woman becoming sensitized, which may cause fetuses in any subsequent pregnancies to suffer from HDFN (20). HDFN may result in jaundice, anemia, developmental problems, or intrauterine death (20).

2.2 Alloimmunization

Alloimmunization is the process by which the production of red blood cell antibody in response to exposure to foreign red cell antigen (24). It is well known that Rh antibodies develop in persons who are Rh-negative, either as a result of immunization during pregnancy or as a result of repeated transfusions when Rh-positive cells are given to Rh-negative recipients(4). Alloimmunization during pregnancy occur as a result of feto maternal hemorrhage. Although the fetal and maternal circulations are separate, there is often some antenatal mixing of fetal and maternal blood, even in asymptomatic women (4).

A fetomaternal hemorrhage (FMH) occurs in the antenatal period or, more commonly, at the time of delivery (24). Events such as miscarriage, ectopic pregnancy, antenatal bleeding, and

delivery, as well as procedures such as chorionic villous sampling, amniocentesis, pregnancy-related uterine curettage, and surgical treatment of ectopic pregnancy can lead to maternal exposure to fetal red blood cells and, consequently, Rh D alloimmunization(7). The volume of fetal–maternal hemorrhage leading to Rh D alloimmunization can be as small as 0.1 Ml (24). If a maternal ABO blood type incompatibility exists between the mother and her fetus, anti-A and/or anti-B antibodies lyses the fetal cells in the maternal circulation and destroy the RhD antigen. Even if this protective effect is not present, only 13% of deliveries of RhD positive fetuses result in RhD alloimmunization in RhD negative women who do not receive RhIG (24).

Hemolytic disease of the fetus and newborn (HDFN) is a clinical condition that occurs as a result of hemolysis of fetal or neonatal red blood cells (RBCs) due to maternal red cell IgG antibodies that can cross the placenta. HDFN is characterized by fetal or neonatal anemia, hyperbilirubinemia and sometimes even fatal hydrops fetalis (4). It can occur in case of antigenic difference between maternal and fetal RBCs. Clinically significant red cell alloantibodies are those that have the potential to cause hemolysis of red cells bearing the corresponding antigen. Maternal antibodies capable of causing HDFN could either be ABO antibodies or non-ABO alloantibodies that can develop in the mother due to sensitization following a blood transfusion or previous pregnancy with red cell antigenic differences (14).

2.3 Prevalence of Rh Negative Pregnancy

The prevalence of D-negativity varies in different ethnic groups with approximately 15% of Caucasians, 8% of Blacks and 1% of Asians being D-negative (25).A study from a tertiary care hospital in Southeast Michigan shows that out of the 4545 included females, 440 had a positive red cell antibody screen (14). Of these 440 females, 34 had clinically significant alloantibodies, giving an overall prevalence of 0.74% (14).A retrospective study done in Barcelona for two year illustrate that there is 39 active alloimmunization case from 3118 Rh negative pregnant women (1.25 in 100) (6).A cross- sectional study done in Uganda show that from a total of 2001 pregnant women; 3.6% of them being RhD-negative. Forty five women (2.2%; 95% CI: 1.6-2.9) were found to be alloimmunized to RBC antigens (26).Other study done in sub-Saharan Africa show that the frequency of RhD-negative phenotype in Nigeria 4.44%, 3.9% in Kenya, 4.06% in

Guinea, and 2.4% in Cameroon (27). Hospital based longitudinal study done in Jimma, Ethiopia; show that from a total population of 3679 maternity patients admitted in the Jimma University specialized hospital for a period from November 2013 to November 2014 representing both urban and rural Jimma population, 231 subjects were RhD negative. The RhD alloimmunization prevalence was 4.5% in urban and 9.8% in rural RhD negative population. Still birth among the study population was 12.0%. Among RhD alloimmunized mothers, still birth rate was 46.2% (28).

2.4 Determinant of Rh Sensitization

Rh D alloimmunization occurs when an Rh D-negative woman is exposed to red cells expressing the Rh D antigen. This risk starts when the Rh D negative women are married to Rh D positive husband. Screening for blood group and Rh is a standard antenatal procedure, with the goal of detecting maternal Rh D status as maternal alloimmunization to anti-Rhesus-D (anti-D) antibodyis recognized as a major contributor to fetal morbidity and mortality (27,29).

It has been reported that 1.5%–2% of pregnant women show atypical blood group sensitization (27). Opinion is divided as to the clinical importance of a repeat anti-D antibody screen at 28 weeks' gestation. Those in support of28 weeks' testing argue that there is the potential advantage to identify about 0.18% or fewer women particularly Rh-negative who become alloimmunized after their first antenatal screen possibly as a result of potential sensitizing event occurring after the first antenatal visit (27).The American Society of Clinical Pathology recommends that testing for unexpected antibody be carried out before antenatal anti-D is given to Rh-negative pregnant women and that repeat Rh testing be omitted if two documented test results confirming the Rh negative status of the woman are on her record (27).

Predisposing factors for fetomaternal hemorrhage include delivery, spontaneous or induced abortion, ectopic pregnancy, missed abortion, intrauterine fetal death, abdominal trauma, antepartum hemorrhage, amniocentesis, chorionic villous sampling, fetal blood sampling, embryo reduction, shunt insertion, external cephalic version, manual removal of the placenta and caesarean section (30). It is important to note that with no apparent predisposing factor, fetal red

cells have been detected in maternal blood in 6.7%, 15.9%, and 28.9% of women in the first, 2nd and 3rd trimesters respectively (30).

Prior to 1970, HDFN due to anti-D was a significant cause of morbidity and mortality. By 1990, a reduction in mortality from 1.2 per 1000 births to 0.02 per1000 births had been achieved in response to the introduction of immunoprophylax is with anti-D immunoglobulin (27). At that time the sensitization rate dropped to about 1.2%. A further reduction to between 0.17% and 0.28% was achieved by introducing prophylaxis during the third trimester of pregnancy (27).

Study also shows that Rh alloimmunization remains a major factor responsible for perinatal morbidity in Sub-Saharan Africa and may result in the compromise of the woman's obstetric care due to the unaffordability of anti-D immunoglobulin (31). Care management with anti-D prophylaxis in patients presenting with severe alloimmunization is difficult to access in Sub-Saharan Africa. In addition to poor access to anti-D prophylaxis, there is lack of alloimmunization in prevention during illegal abortions and poor documentation of adequate information in patients' medical notes (31).Similar study shows that in sub- Sahara Africa still there is higher prevalence of Rh Alloimmunization. The main reason is poor antenatal practices which fail to identify rhesus negative women, cost of procuring anti-D immunoglobulin, absence of a universal access program for all Rh-negative women, failure to recognize potential sensitizing events in pregnancy as such and to treat them appropriately and failure and absence of facilities to assess the extent of fetomaternal hemorrhage. Other factors include failure to offer Rh-negative pregnant women anti-D immunoglobulin following potentially sensitizing event during pregnancy and failure to comply with postpartum prophylaxis guidelines to offer further anti-D immunoglobulin to all Rh negative women delivered of Rh-positive babies (33, 35).

3 OBJECTIVES

3.1 General Objective

- To study prevalence and determinant of Rh alloimmunization in Rh negative mother in three teaching hospitals, Addis Ababa, Ethiopia.

3.2 Specific Objective

- To determine the proportion of women who are alloimmunized.
- To identify determinant of Rh alloimmunization.

4 METHODS AND MATERIAL

4.1 Study area

The study was conducted in the three teaching hospitals: Tikur Anbesa Specialized Hospital, Gandhi Memorial Hospital and Zewditu Memorial Hospitals which are found in Addis Ababa, Ethiopia. These hospitals serve as central referral teaching hospitals and all obstetric emergencies including high risk pregnancies are referred to these hospitals from Addis Ababa and its vicinity.

4.2 Population

Source Population: All pregnant women who visit the three teaching hospitals during the Study period.

Study population: All Rh D negative pregnant women in the three teaching hospitals at the time of data collection and fulfilling the inclusion criteria

4.3 Study design and period

Hospital based cross-sectional study design was used to assess the prevalence of Rh negative mother and associated factor of alloimmunization among pregnant women, who visit the three teaching hospitals from October,2020 to May, 2021.

4.4 Inclusion and Exclusion Criteria

Inclusion criteria

- All women who are pregnant.

Exclusion criteria

- Women who are not willing.

4.5 Sample size and sampling technique

4.5.1. Sample size

Women who came for antenatal and delivery service and Rh D negative which fulfilled the inclusion criteria are included in the study.

4.6 Data Collection Method

The study was conducted from pregnant women who booked for antenatal and delivery service utilization in TASH, GMH and ZMH from October 2020 to May 2021. Information was retrieved from patient antenatal and delivery registry book in order to know prevalence of Rh negative mother. Women who registered in the antenatal care visit labeling were made on the women chart in order to avoid registration on the delivery book, to avoid counting for the second time. Women who didn't have labeling on the chart were registered on the delivery book if she came to give birth. All women who are alloimmunized were interviewed and questioner was filled by trained data collector.

4.7 Study Variables

Dependent variable

- Rh alloimmunization

Independent variables

- Age of women
- Marital status
- Women's Education
- Women's Employment Status and Women
- Parity
- Previous procedure
- Previous taking anti D

4.8 Operational Definitions

Rh factor: Is an inherited protein found on the surface of red blood cells. If the blood has the protein called Rh positive but if it doesn't contain the protein called Rh negative.

Rh Alloimmunization: Occurs when maternal immune system is sensitized to Rh D Erythrocyte surface antigen**.**

Coombs Test: Are test done to find certain antibodies that attack red blood cells.

Indirect combs test: Find certain antibodies that are in the liquid part of blood. These antibodies can attack RBCs.

Direct combs test: Is used to detect antibodies or complement protein attached to the surface of RBCs.

4.9 Data management

Editing of the questionnaires was done to determine completeness manually. Data entry, cleaning and analysis was performed using SPSS version 24.0. The responses in the completed questionnaires were coded and entered into a data entry template. Summary tables were used for describing data. A relationship among the major variables were described by significant level $P<0.05$. Logistic regression (using $P<0.05$) was used to examine the relationship between the proposed dependent and independent variables. For each regression odds ratios (with the accompanying p-values and confidence intervals) of the relationship was reported.

4.10. ETHICAL CLEARANCE

The proposal was approved by the Ethical Review Committee of Department of Obstetrics and Gynecology Research and Publication Committee Addis Ababa University. Permission was requested from each selected hospitals to access the clients included in the study. Informed oral consent was obtained from each participant before the start of data collection. To ensure confidentially of respondents, their names were not indicated on the questionnaire and it was assured that their responses will be kept strictly confidential.

4.11. RESULT DISSEMINATION

Results will be presented to the Department of Obstetrics and Gynecology research and publication Committee Addis Ababa University. Subsequently, attempts will be made to present it on scientific conferences and publish it on scientific journals. A copy of manuscript will be submitted to the Ministry of Health, Addis Ababa University and other relevant bodies.

5. RESULT

5.1. Socio-demographic characteristics of study participants

During the 8 month study period there were a total of 15,894 pregnant women visited the three teaching hospitals (TAH, ZMH, and GMH) in ANC and delivery service. Of these, 328(2.1%) of them were Rh- D negative all of them were consented to participate in the study giving a response rate of 100%, among them 56 (17.1%) of them were Rh D sensitized (Table 1). The common age group for both Rh immunized and non-immunized women was between 25-29 years. All of the immunized and 97.8% of non-immunized are married. Majority of the women in both group were unemployed and attained primary and secondary school. All of immunized and more than 95% of non-immunized women had two and above pregnancy experience.

Table 1- Sociodemographic characteristics of the study participant in TASH, GMH and ZMH, Addis Ababa, Ethiopia.

Variables	Non-immunized	Immunized	X Value
Age			0.013
15-19	3(1.1%)	0	
20-24	85(31.2%)	6(10.7%)	
25-29	117(43%)	23(41.1%)	
30-34	47(17.3%)	22(39.3%)	
>/=35	20(7.4%)	5(8.9%)	
Marital status			0.145
Married	266(97.8%)	56(100%)	
Other	6(2.2%)	0	
Education			0.522
Primary	113(41.5%)	20(35.7%)	
secondary	84(30.9%)	25(44.6%)	
University	59(21.7%)	8(14.3%)	
Unable to read and write	16(5.9%)	3(5.4%)	
Occupational status			0.002
Unemployed	124(45.6%)	38(41.1%)	

Employed	148(54.4%)	18(58.9%)	
Number of pregnancy			0.008
1	45(16.6%)	0	
2	95(34.9%)	27(48.2%)	
3	70(25.7%)	17(30.4%)	
≥4	62(22.8%)	12(21.4%)	

5.2 Blood group pattern of study participant

Concerning the ABO blood group, in the non-immunized group majority of the women had A and B blood group with equal proportion followed by the O blood group. On the contrary, the immunized group the commonest blood group was B followed by O then A.

Table 2- Blood group profile of the study population in TASH, GMH and ZMH, Addis Ababa, Ethiopia.

Variables	**Non-immunized**	**Immunized**
A	99(36.4%)	14(25%)
B	58(21.3%)	20(35.7%)
AB	17(6.3%)	7(12.5%)
O	98(36%)	15(26.8%)

5.3 Obstetrical characteristics of study participant

In the obstetric history, 21.7% of the non-immunized women were delivered by cesarean section in the contrary about 26.8% of the immunized women delivered by cesarean section. On the other hand 9.2% of the non-immunized women had APH and 8.9% of immunized women reported that they had APH. None of the women in both group had prior history of blood transfusion. About use of anti-D prophylaxis, 60.3% and 25% of non-immunized and immunized utilized anti-D as a prophylaxis's respectively.

Table 3 Percent distribution of maternal obstetric history of study participant in TASH, GMH and ZMH, Addis Ababa, Ethiopia.

Variables		**Non- Immunized (%)**		**Immunized (%)**		**P value**
		Yes	No	Yes	No	
Sensitizing event	APH	25(9.2%)	247(90.8%)	5(8.9%)	51(91.1%)	0.01
	C/S delivery	59(21.7%)	213(78.3%)	15(26.8%)	41(73.2%)	
	Breech delivery	6(18.9%)	266(81.1%)	3(5.4%)	53(94.6)	
Anti-D use on previous pregnancy		164(60.3%)	108(39.7%)	14(25%)	42(75%)	0.022

The commonest reason for not using anti-D as a prophylaxis in the immunized group was due to unaffordability of the drug followed by the fact the health professionals did not inform them about the anti-D. Similarly, the commonest reason for not using anti- D in the immunized group were due to unaffordability of the drug and the second common reason was because the health professionals did not inform them to use the anti-D.

Table 4 Percent distribution of most frequent reason for not using anti-D in TASH, GMH and ZMH, Addis Ababa, Ethiopia.

Reason	Non-Immunized (%)	Immunized (%)
Didn't know my blood type	17(15.7%)	10(23.8%)
Health professional didn't told me	30(27.8%)	11(26.2%)
I can't afford	35(32.4%)	12(28.6%)
Health professional told me not necessary	18(16.7%)	7(16.7%)
Other	8(7.4%)	2(4.8%)

5.4 Fetal and neonatal complication of study participant

Table 5 show that both non-immunized and immunized women had comparable perinatal loss in the previous delivery, the perinatal loss was mainly due to meconium aspiration syndrome, sepsis and preterm delivery. About 8 of the current pregnancy in the immunized women had a fetal complication which was fetal anemia and all of them had inutero blood transfusion but there is none in the non-immunized women.

Table 5- Frequency distribution of fetal/neonatal complication in TASH, GMH and ZMH, Addis Ababa, Ethiopia.

Variable	Non-immunized women	Immunized women
Fetal/neonatal complication in the previous delivery		
Yes	8(3.9%)	6(10.7%)
No	199(96.1%)	50(89.3%)
Fetal complication during the current pregnancy		
Yes	----	8(14.3%)
No	227(100%)	48(85.7%)

5.5 Determinants of Rh alloimmunization in study participant

After adjusting the results of bivariate analysis in order to decrease or remove confounding variables, multivariable logistic regression was performed. In multivariate analyses when adjusted for the other independent variables, occupation of the women was significant factor for respondent's Rh sensitization. Those women who were unemployed found to be 2.281times more Rh sensitized than employed with AOR= [2.281, 95% CI (1.214, 4.282)]. In addition, anti-D use in the previous pregnancy of participant's was found as a significant factor for Rh alloimmunization. Those who didn't utilized anti-D on the previous pregnancy found to be 2.076 times more likely to be Rh sensitized with [AOR=2.076; (1.101, 3.915)](Table 6).

Table 6- Factor associated with Rh D alloimmunization in TASH, GMH and ZMH, Addis Ababa, Ethiopia.

Variables	Non-Immunized	Immunized	COR(95%CI)	P value	AOR(95%CI)	P value
Occupation						
Employed	148(54.4%)	18(58.9%)	1		1	
Unemployed	124(45.6%)	38(41.1%)	2.520(1.370,4.635)	0.003	2.281(1.214,4.282)	0.01
Number of pregnancy						
1	45(16.6%)	0				
2	95(34.9%)	27(48.2%)	0.681(0.321,1.444)	0.316	0.656(0.302,1.425)	0.287
3	70(25.7%)	17(30.4%)	0.797 (0.353,0.799)	0.585	0.736(0.317,1.706)	0.474
≥4	62(22.8%)	12(21.4%)	1		1	
Anti-D use on previous pregnancy						
Yes	139(51.1%)	38(67.9%)	1		1	
No	133(48.9%)	18(32.1%)	2.020 (1.099,3.714)	0.025	2.076(1.101,3.915)	0.024
Sensitizing event						
Yes	102(37.5%)	14(35.7%)	1		1	
No	170(62.5%)	42(64.3%)	0.556(0.289,1.067)	0.078	0.528(0.268,0.1.041)	0.065

6. Discussion

The main purpose of this study was to assess the prevalence of Rh D negative phenotype and determinant factor for being Rh sensitized among pregnant women in the tree teaching hospital.

The study revealed that from 15,894 of total population, 328 (2.1%) were had Rh D negative blood type. Among them 56(17.1%) of them are Rh alloimmunized. From the 328 study population, 113 (34.4%) of them had blood group O and A each with equal proportion. The next highest group was B with 78 (23.7%). In addition the study also shows that 4.5%, 4.2%, 6.1% and 2.13% of the study population with blood groups O, A, B, and AB were Rh D alloimmunized, respectively. In the contrary study done among reproductive age women in Arba-Minch, Ethiopia, show that the proportion of women who are RhD negative were 6.2% (37), Other study done in Ethiopia, Jimma Hospital, over one year period shows that the prevalence of Rh D negative pregnant women were 6.3% and 14.3% of them are Rh alloimmunized(29).

The frequency of RhD negative phenotype is different between populations. In Africa and Asia the Rh negative phenotype is less common. There is a report of 1% in Madagascar, 3.9% in Kenya and 2.4% in Cameron (28). In various region of India Rh negativity was found to be 0.6-8.4% (27). The highest recorded prevalence of Rh D phenotype was 29% in Saudi Arabia and Basques (27).

The possible explanation for the lower prevalence of Rh D in this study can be due to a short study period and difference in study population.

Worldwide after the Introduction of use of prophylaxis anti-D after possible sensitizing event result in reduction of being Rh Alloimmunized (27,30), but in sub- Sahara Africa still there is higher prevalence of Rh Alloimmunization (31). The main reason is poor antenatal practices which fail to identify rhesus negative women, cost of procuring anti-D immunoglobulin, absence of a universal access program for all Rh-negative women, failure to recognize potential sensitizing events in pregnancy as such and to treat them appropriately and failure and absence of facilities to assess the extent of fetomaternal hemorrhage. Other factors include failure to offer

Rh-negative pregnant women anti-D immunoglobulin following potentially sensitizing event during pregnancy and failure to comply with postpartum prophylaxis guidelines to offer further anti-D immunoglobulin to all Rh negative women delivered of Rh-positive babies (33, 35). Similarly our study show that among fifty-six immunized women 12(28.6%) didn't use anti- D due to the cost of the drug and 11(26.2%)of them due to the health professional didn't told them to use the drug. In the multivariate analysis the study also shows that women who are employed and who use anti-D after possible sensitizing event are less likely to be sensitized.

7 Conclusions, Recommendation and Limitation

7.1 Conclusion

The study was conducted among women of Rh D negative women in the three teaching hospitals. Accordingly the findings to conclude are:

1. The prevalence of Rh D negative pregnant women in the three teaching hospital is 2.1% and 17.1% being Rh Alloimmunization
2. Majority of women who are Rh D alloimmunized didn't use anti- D as a prophylaxis
3. The common reason for not using anti- D is due to unaffordability and the health professional didn't prescribe the drug.
4. Rh Alloimmunization is associated with government employment and use of anti-D in the crude analysis.

7.2 Strength and Limitation

This strength of this study, it examines the commonest but neglected obstetric complication in our setting. This study has some limitation that should be noted. First the study done in tertiary hospital that's located in the capital city of Ethiopia so the result cannot be generalized to the other institution. Second due to limitation in data collection time it was difficult to include a large number of participants in the study. Lastly the tests done in our case are combs test only so it's difficult to know the sensitization is due to the other antibody type.

7.3 Recommendation

Despite the limitations, the determinants we found have useful implications for both health care providers and decision-makers in health programs. The result suggests that special attention should be given to Rh negative pregnancy to be included in maternal health programs by Ministry of health. Also Improvement of access and affordability of anti-D medication. As a nation there is no data that evaluate the determining factor of Rh Alloimmunization so research must be done with a large sample size to improve the management.In addition assessment of knowledge and practice of the health professional also important.

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8. ANNEXES

ANNEX I– Information Sheet

ADDIS ABABA UNIVERSITY COLLEGE OF HEALTH SCIENCE SCHOOL OF MEDICINE

Here, I the undersigned, at Addis Ababa University College of Health Science, Department of Obstetrics and gynecology, currently I will be undertaking research on a topic entitled Prevalence and determinants of Rh alloimmunization in Rh negative women in three teaching hospital in Addis Ababa, Ethiopia. For this study, you are selected as a participant and before getting your assent or permission of your participation, you need to know all necessary information related to the study. Thus, this information is detailed as;

Objective: To assess the prevalence and determinants of Rh alloimmunization in Rh negative women who attended the ANC and delivery service of the study center in Addis Ababa, Ethiopia.

Significance of the study: The study used to know about the determinant of Rh sensitization in other areas of Ethiopia and other country with similar health care issues. Further, the findings of the current study will provide policy makers, health practitioners, mothers and the community at large with useful insights regarding the prevalence and factor that determine the occurrence of Rh sensitization.

Participants to be included: All Rh negative pregnant women who are attending in the antenatal and delivery care and who are voluntary to participate in the study included.

Confidentiality: All information you give will be kept confidential and won't be accessible to any third party. Your name won't be registered on the question sheet so that you will not be identified.

Risks and Benefits of the study: The study will be carried out by interviewer administered questionnaire. The procedure doesn't bear any physical or psychological trauma. Furthermore, you will not be forced to respond to the information you do not know. No payment for your participation in the study but participating in the study and giving your information to questions asked will have great input in efforts to improve health of pregnant and delivered mother's

Assent: Your participation in the study will be totally based on your willingness. You have the right not to participate from the beginning, or stop any time after starting participation. You will not be forced to respond to the question you do not know and you can ask any question whenever you like.

Name of principal investigator (PI):

Melat Benti Date:_____ Signature _____

Address of PI: Mobile: +251- 09- _____ E- Mail _____

Data Collector Name _____ Date _____ Signature _____

Supervisor Name _____ Date _____ Signature _____

ANNEXES II: Consent Form

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE SCHOOL OF MEDICINE

DEPARTMENT OF OBSTATRIC AND GYNACOLOGY

Good morning/afternoon. My name is _____ and I am working _____. I am studying on prevalence and determinants of Rh alloimmunization in Rh negative pregnant women who are attending the antenatal and delivery care service on behalf of Dr. Melat Benti, who is post graduate specialist student at Addis Ababa University, college of health science, Department of OGS/GYN. I would like you to respond to only if you wish to do so. I assure you that the information you provide are will be kept confidential. Your name will be not written on the questionnaire to ensure your confidentiality. Make sure that, there should be no harm caused because you are involved in this study. You have full right to decline to the interview partly or totally. In case you consent for the interview, I need you to provide me your honest answer to the questions you want to respond as this would help us to come up with genuine conclusions and recommendations that would potentially help Ministry of Health of Ethiopia and health facilities improve these services.

Consent I have fully understood its contents and I have agreed to participate in this research project. Yes---------- No----------

Thank you for giving us your consent.

Name of data collector ---------- Sign---------- date ----------

ANNEXES III

Questionnaire

Part -1 Questionnaire on socio-demographic characteristics.

1 Present maternal age _____Years

2 What is the highest level of schooling you have ever attended?

1. Never attended
2. Only read & write
3. Primary school
4. Secondary school
5. Tertiary (Certificate-Degree)
6. Other Specify_____

3 What ethnic group do you belong?

1. Oromo
2. Amhara
3. Gurage
4. Tigre
5. Other specify_____

4 What is your occupation?

1. House wife
2. Farmers
3. Maid
4. Civil servant
5. Merchant
6. Student
7. Other specify_____

5 What is your religion?

1. Muslim
1. Orthodox
2. Catholic

3. Protestant
4. Other specify

6 What is your marital status?

1. Married
2. Divorced
3. Widowed
5. Never married

7 What is the average family income per months? _____

Part- 2 Questionnaire on Blood type

1. What is your blood group
    1. A
    2. B
    3. AB
    4. O
2. What is your Rh status
    1. +ve
    2. –ve
3. Did combs test done during the current pregnancy?
    1. Yes
    2. No
4. What is the result?
    1. Negative
    2. Positive

Part-3 Questionnaire on previous delivery experience.

1. Number of previous delivery?

1. 0
2. 1
3. 2
4. 3 or more

2. Number of previous abortion/Ectopic or Molar pregnancy
    1. 0
    2. 1
    3. 2
    4. 3 or more
3. Place of previous delivery
    1. Home
    2. HC
    3. Hospital
4. Did you take Anti- D during the previous pregnancy?
    1. Yes
    2. No
5. If no, why?
    1. I didn't know my blood group at that time
    2. I can't afford
    3. The health professional didn't told me
    4. The health professional told me not necessary
    5. The drug not available
    6. My husband blood group is similar with me
    7. other, specify ……
6. When did you take anti-D during the previous pregnancy
    1. Before 7 month
    2. At 7 month
    3. After 7 month
7. Is there any procedure/complication during the previous pregnancy?
    1. Yes

2. No

8. If yes what was it?
    1. Abdominal trauma
    2. ECV
    3. Amniocentesis
    4. Ante partum bleeding
    5. Other (specify)……..
9. Did you take anti-D during the previous delivery?
    1. Yes
    2. No
10. If no, why?
    1. Because I deliver at home
    2. I didn't know my blood group at that time
    3. I can't afford
    4. The health professional didn't told me
    5. The health professional told me not necessary
    6. My child blood type was similar with me
    7. The drug not available
    8. other, specify ……
11. Did you take anti-D during the previous abortion/ectopic/molar pregnancy?
    1. Yes
    2. No
12. If no, why?
    1. Because I deliver at home
    2. I didn't know my blood group at that time
    3. I can't afford
    4. The health professional didn't told me
    5. The health professional told me not necessary
    6. My child blood type was similar with me
    7. The drug not available
    8. other, specify ……

13. When did you take anti-D after delivery/abortion/ectopic pregnancy?
    1. Within 24 hr
    2. Within 72hr
    3. >72hr
14. Is there complication during the previous delivered neonate?
    1 Yes
    2 No
15. If yes
    1. Intra uterine transfusion
    2. Require phototherapy
    3. Exchange transfusion
    4. IUFD
    5. Neonatal death
    6. Other(specify)_____

Part 4. Questioner on current pregnancy experience

1. Did you take Anti- D during the current pregnancy?
    1. Yes
    2. No
2. If no, why?
    1. The health professional didn't told me
    2. The health professional told me not necessary
    3. I didn't take also in the previous delivery
    4. other, specify ……
3. Is there complication during the current pregnancy?
    1. Yes
    2. No
4. If yes
    1. Decrease in neonatal hemoglobin
    2. Hydrops fetalis

3. IUFD
4. Other (specify)……

5. Is there complication during the current delivery?
    1. IUFD
    2. Neonatal death
    3. Admitted to NICU
    4. Other(specify)_____

DECLARATION

By my signature below, I declare and affirm that this thesis is my own work. I have followed all ethical principles of scholarship in the preparation, data collection, data analysis and completion of this thesis. All scholarly matter that is included in the thesis has been given recognition through citation. I affirm that I have cited and referenced all sources in this document. Every serious efforts been made to avoid any plagiarism in the preparation of this thesis.

This thesis is submitted in partial fulfilment of the requirement for the degree of specialization in Obstetrics and Gynaecology in Addis Ababa University School of Medicine. I would like to declare that this thesis has not been submitted to any other institution anywhere for the award of any academic specialization, degree, diploma or certificate.

Name: _____ Signature _____ Date _____