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ABO and Rh Blood Group Distribution and Its Association with Hemoglobin Levels in Pregnant Women: A Study from Peshawar District

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ABSTRACT

Anemia was a significant global health issue, leading to preterm birth, low weight at birth, and fetal malformations in pregnancy. Determination of blood group distribution and their relationship with anemia was essential for improving anemia management in pregnancy. **Objective:** To assess the distribution of the ABO blood group and Rh type of pregnant women and its relationship with the concentration of hemoglobin. **Methods:** A descriptive cross-sectional design study was conducted at a primary healthcare center in Peshawar from 1st January 2023 to 31st December 2023, enrolling 1049 pregnant women attending antenatal care. ABO and Rh typing were determined, and Hb levels were assayed to assess the presence of anemia in the participants. The data were analyzed using SPSS version 26.0. To examine the relationship between various blood group types and anemia, ANOVA and correlation analysis were employed. **Results:** B-type was the most prevalent blood group among the participants, about 358 (34.1%), followed by O 295 (28.1%), A 279 (26.6%), and AB about 117 (11.2%). The majority of participants, 972 (92.7%), were Rh-positive. Anemia was prevalent in 878 (83.7%) of participants, with 639 (60.9%) having mild anemia. Linear regression analysis indicated no significant impact of ABO blood group or Rh factor on hemoglobin levels ($p > 0.05$). **Conclusions:** In the current study, B-type was the most prevalent blood group among pregnant women, differing from previous reports of blood group O. However, no significant association was observed between blood group types and anemia, suggesting that other factors might play a more significant role in the prevalence of anemia.

INTRODUCTION

Anemia during pregnancy is a major public health concern that can lead to severe complications and negative outcomes for both the mother and the baby [1]. In pregnancy, anemia can result in serious maternal and fetal complications, potentially elevating the risk of maternal mortality [1, 2]. The main causes of anemia during pregnancy include nutritional deficiencies, parasitic infections, and acute blood loss [2]. Among the blood group systems, ABO and rhesus (Rh) antigen systems are fundamental in hematology and transfusion therapeutics [3]. Among the blood grouping systems, ABO and Rhesus are the most prominent and well-known in humans [4]. Approximately 1.93 billion people worldwide are currently affected by anemia [5]. Blood Group Distribution: Research in different populations indicated a predominance of blood group O, followed by groups A, B, and AB. In one study, group O was observed in about 59.1% of pregnant women, with group A at 19.1%, group B at 17.1%, and group AB at 4.8 [6]. The highest prevalence of anemia among pregnant women is observed in sub-Saharan Africa, with rates ranging from 38.9% to 48.7% [7]. The majority of pregnant women are Rh-positive (around 97.1%), while Rh-negative individuals

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constitute a smaller percentage (2.9%) [8]. Another study reported that type O was the most prevalent at 47.7% [4]. In Jordan, another study found that O-type was the commonest, about 38.9%, with 90.2% of the population being Rh positive and 9.8% Rh negative [9]. The ABO system plays a crucial role in blood therapeutics and transplantation, and studies have highlighted its involvement in various disorders, including DM, malignancy, and GI ulcers [3, 10]. The incidence of HDN varies widely, affecting 3–80/100,000 newborns annually, with rates differing across populations and ethnic groups, ranging from 7.2–14.3 per 10,000 births [11, 12]. A comprehensive understanding of blood type patterns is crucial for preventing and managing hemolytic disease of the newborn (HDN), ensuring adequate blood supplies in banks, and reducing neonatal and maternal morbidity and mortality associated with transfusions [11, 12]. Monitoring hemoglobin levels is equally essential, as anemia during pregnancy can lead to adverse maternal and fetal outcomes [5]. Despite the importance, there is a lack of data on blood type prevalence and hemoglobin concentrations in women attending antenatal care centers in the region. This knowledge gap can hinder effective maternal health interventions and blood management strategies.

Therefore, this present study was conducted to assess the prevalence of ABO and RhD typing and hemoglobin concentrations among women seeking antenatal care at a primary healthcare center in Peshawar.

METHODS

This descriptive cross-sectional study was conducted at a Primary Healthcare Center in District Peshawar, from 1st January to 31st December 2023. About 1,049 sample size was determined with a 99.9% confidence interval and a margin of error at 1%. This substantial sample size was determined based on expected outcome factor frequency of 41.1% and a population of 6,000,000 [12]. A non-probability convenient sampling technique was employed for the selection of study participants. The inclusion criteria for this present study consisted of pregnant women aged 18 years or older who provided written informed consent to participate. Participants were required to be attending the primary healthcare center in Peshawar during the study period and to have no history of blood transfusion within the past six months. Women were excluded from the study if they refused to give consent, had known blood disorders such as thalassemia, sickle cell disease, or other hematological conditions, or had received a blood transfusion within the last six months. All the participants meeting this current study inclusion criteria were enrolled in the study after approval from the Hospital Ethics Committee (Ref. No: 1042/HEC/B&PSC/2022). The confidentiality of the collected data was thoroughly maintained during the study. Blood samples were collected from all patients by antecubital venipuncture and transferred into EDTA anticoagulant tube. The blood samples were thoroughly mixed by placing on the rolling mixer. ABO and Rh blood typing were performed by forward agglutination methods with anti-A, anti-B, and anti-Rh antisera. Agglutination was observed when erythrocyte antigens reacted with the corresponding antibodies in the serum or plasma sample, then Reverse blood typing was done to confirm the blood type. A full blood count was conducted on the blood samples using the Ruby Cell-dyn Hematology Analyzer, and hemoglobin (Hb) levels were recorded for all participants. Anemia was identified in females with Hb levels below 12.5g/dL. Anemia was classified according to the National Cancer Institute's grading system as follows: hemoglobin levels between 10.0g/dL and the lower limit of normal were classified as having mild anemia. Those with hemoglobin levels between 8.0-10.0g/dL were categorized as having moderate anemia, and individuals with hemoglobin levels between 6.5-7.9g/dL were classified as having severe anemia [13]. The statistical analysis of the collected data, including the demographic and blood groups distribution, was performed using SPSS version 26.0. The distribution of blood group types was expressed as frequencies and percentages. Quantitative variables such as Hemoglobin concentration was summarized using Mean and SD. Tables and figures were used to illustrate the findings. ANOVA, Linear regression and Chi-square tests were employed to assess any relation between blood group types and prevalence of anemia.

RESULTS

The analysis of the ABO and Rh blood typing of the 1049 participants revealed that B-type was most common, observed in 358 (34.1%) of the participants, followed by blood group O, which was present in 295 (28.1%) of the women. Blood group A accounted for 279 (26.6%) of the sample, while the least common was blood group AB, found in 117 (11.2%) of the participants. Regarding the Rh factor, a significant majority of 972 (92.7%) were Rh-positive, with only 77 (7.3%) being Rh-negative (Figure 1).

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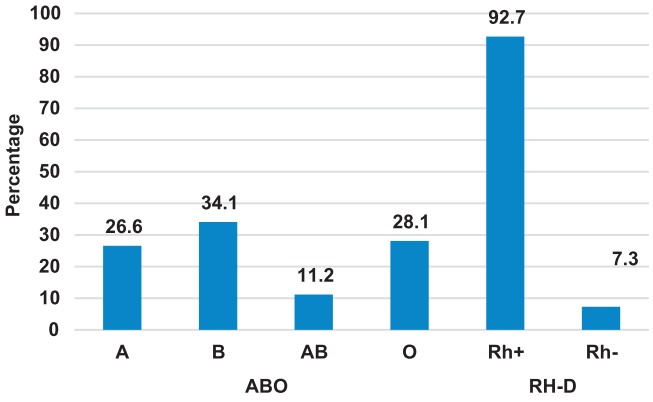

Figure 1: Prevalence of Different Blood Group Systems

The statistical analysis of the presence of RhD-Ag in each ABO blood groups distribution among the pregnant women reveals that the most prevalent was B+ about 322 (30.7%) of the participants. Blood groups O+ and A+ were equally prevalent, each accounting for 269 (25.6%). AB+ constituted 112 (10.7%) of the sample, and AB- was the least common, observed in only 5 (0.5%), as illustrated in figure 2.

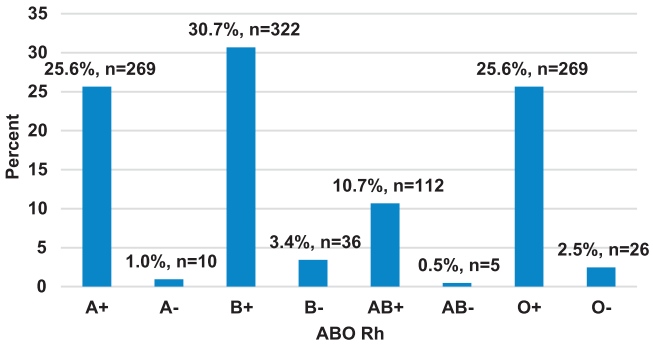

Figure 2: Prevalence of Rh-D Antigen in ABO Blood System

The mean Hgb level was 11.19 ± 1.61g/dL ranging from 6.0-16.6 g/dL. About 878 (83.7%) of the participants were found to be anemic, while 171 (16.3%) were non-anemic, as illustrated in figure 3. Within the anemic population, 639 (60.9%) had mild anemia. Moderate anemia was observed in 219 (20.9%) of the participants. While severe anemia was present in 20 (1.9%) of the women.

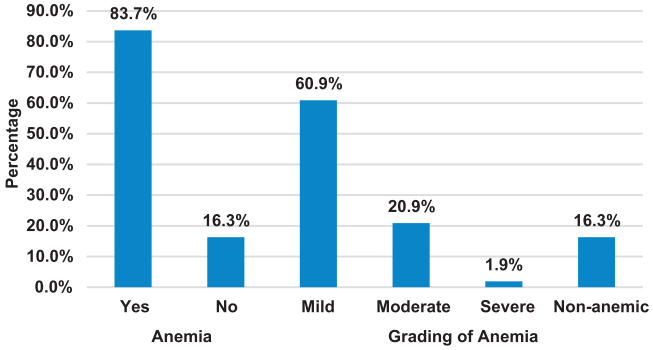

Figure 3: Prevalence and Grading of Anemia among Study Participants

To assess the impact of ABO blood group and Rh factor on hemoglobin (Hb) levels among participants, the linear regression analysis was performed. The model yielded an R-value of 0.052 and an R-squared value of 0.003, which indicated a very weak correlation and minimal explanatory power. The results of ANOVA showed an F-value of 1.396 with a p-value of 0.248, suggesting that the model was not statistically significant. Among the predictors, ABO had a coefficient of 0.071 (p=0.098), reflecting a marginal and non-significant effect, while Rh had a coefficient of -0.027 (p=0.889), indicating no significant impact on Hb levels. The overall analysis showed that ABO and Rh-D antigen did not significantly affect the hemoglobin levels in the study participants, as illustrated in figure 4.

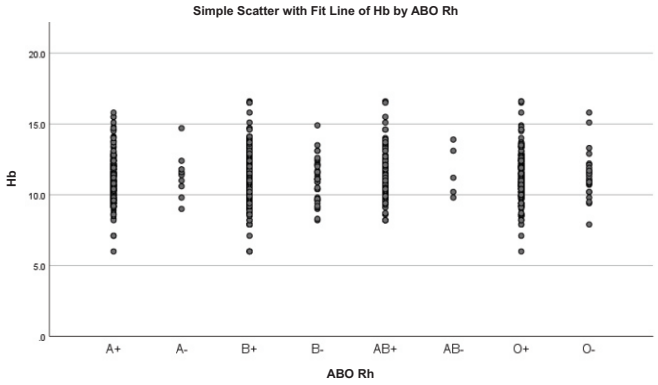

Figure 4: Scatterplot of Hb Levels by ABO and Rh Blood Groups

The crosstab analysis examined the link between ABO blood groups and Rh type with the prevalence of anemia, as shown in Table 1. The Chi-square test for ABO blood groups yielded a Pearson Chi-Square value of 9.743 with a p-value of 0.372, indicating no significant association between ABO blood group and anemia severity. The Chi-square test for Rh factor resulted in a Pearson Chi-Square value of 0.245 with a p-value of 0.970, suggesting no significant association between Rh status and anemia severity.

Table 1: Correlation of Anemia with Different Blood Group Systems

Blood Group System		N	Prevalence of Anemia					
			Mild	Moderate	Severe	Non-Anemic	Total	p-Values
ABO	A	279	176	61	4	38	279	0.372
	B	358	206	82	10	60	358	
	AB	117	71	22	0	24	117	
	O	295	186	54	6	49	295	
	Total		639	219	20	171	1049	
Rh	Negative	77	47	17	1	12	77	0.970
	Positive	972	592	202	19	159	972	
	Total		639	219	20	171	1049	

The crosstab analysis assessed the association between Rh-D antigen in each ABO blood groups with the severity of anemia among the participants, as presented in table 2. Evaluating the relationship between ABO and Rh type and

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anemia severity yielded a Pearson Chi-Square value of 15.934 with a p-value of 0.773. This result suggests that there was no relationship between the ABO and Rh type and the severity of anemia, as many cells had expected counts of less than 5.

Table 2: Association of Rh-D Antigen in ABO Blood type with Anemia

ABO	N	Rh type	Severity of Anemia					
			Mild	Moderate	Severe	Non-Anemic	Total	p-Values
A	279	Rh+	169	59	4	37	269	0.992
		Rh-	7	2	0	1	10	
B	358	Rh+	186	71	10	55	322	0.500
		Rh-	20	11	0	5	36	
AB	117	Rh+	69	21	0	22	112	0.512
		Rh-	2	1	0	2	5	
O	295	Rh+	168	51	5	45	269	0.712
		Rh-	18	3	1	4	26	

DISCUSSION

Current study findings explored the association of ABO and Rh blood types and Hb concentrations among participants. The statistical analysis showed that group B was the most common type (34.1%), followed by group O (28.1%), and AB was the least common (11.2%). A significant majority of the participants were Rh-positive, with 92.7% exhibiting the Rh-D antigen. Karami M *et al.*, reported a 36.8% anemia prevalence among pregnant women, classifying 70.8% of these cases as mild anemia. In contrast, the present study found a substantially higher anemia prevalence of 83.7%. Of these, 60.9% had mild anemia, 20.9% had moderate anemia and 1.9% had severe anemia [1]. In comparison, Nwauche CA *et al.*, reported an anemia prevalence of 93.1% among 1,000 pregnant women in Pakistan. This prevalence was notably higher than the present finding of 83.7% [14]. Liyew AM *et al.*, reported an occurrence of anemia of about 41.85% in pregnant women. Current study findings highlight a moderate level of anemia in their study population, which was much lower than the 83.7% observed in the current study [2]. Group B was reported in 35.96% of the population by Barot T *et al.*, and group AB was the least common at about 8.43%. Previous studies also found that 93.45% of individuals were Rh-positive, which was consistent with the high Rh-positive prevalence observed in the present study (92.7%) [15]. Akogu SP *et al.*, reported a higher prevalence of blood group B at 27.97% compared to other blood types, whereas the current study found an even greater prevalence of 34.1% [16]. Blood group O was reported at about 41.1% and 37.44% by Al-Kuran O *et al.*, and Chanko KP *et al.*, respectively [8, 12]. While present findings underscore a notable regional variation, with blood group B being the most common in the current study sample, contrasting with the predominance of blood group O in other studies. In comparison with Alemu M *et al.*, the current study observed a lower prevalence of blood group O at 28.1% compared to 41.5% reported by Megbaru. Conversely, the present study found a higher prevalence of blood group B at 34.1%, compared to 25% in Alemu M *et al.*, and a higher prevalence of blood group AB at 11.2%, compared to 5.5%. The prevalence of blood group A in the present study was 26.6%, which was similar to the 28% reported by Megbaru. Both studies reported similar frequencies for the Rh-D antigen, with 92.7% of women being RhD positive and 7.3% RhD negative [17]. About 28.0% of the anemic individuals in the current study belonged to blood group O, while Nwabuko O *et al.*, reported that 61.2% of individuals of O blood type were anemic, indicating a significantly higher incidence of anemia in this group compared to other ABO blood groups. Additionally, it was reported that O positive blood group was particularly prone to anemia by Nwabuko O *et al.* In contrast, the present study did not find a significant association between ABO blood types and anemia severity (p-value = 0.372). While a substantial percentage of the anemic population had blood group O, the anemic individuals with blood group O (28.0%) in the present study were notably lower than the 61.2% reported by Nwabuko O *et al.* This discrepancy suggests that while blood group O was more prevalent among the anemic in the study, it does not exhibit the same heightened susceptibility to anemia as observed by Nwabuko O *et al.* [18]. The individuals of group O had lower Hgb thresholds reported by Alemu M *et al.*, compared to other group types, suggesting that blood group O individuals might experience anemia at lower Hb levels. In contrast, the linear regression analysis in the present study showed no significant impact of the ABO blood group on hemoglobin concentrations [17]. Magtooph MG *et al.*, found no difference in Hgb levels between blood groups A, B, and AB, with p-values >0.05 [19]. However, they observed group O in women had much lower Hgb concentrations than other ABO groups (p-values of <0.029). While the linear regression analysis in the present study showed no significant relationship between ABO types and Hgb levels (R-squared = 0.003 and p-value = 0.248). Similarly, the current study found no significant variance in hemoglobin concentrations between ABO blood groups, in contrast to Kumar BA *et al.*, findings, which associated blood group O with lower hemoglobin thresholds [20]. Among thalassemia major patients, the B+ (positive) blood group was found more common in the district of Peshawar [20]. Biologically, blood group O has been associated with lower hemoglobin concentrations in previous studies, potentially due to increased susceptibility to gastrointestinal blood loss and iron deficiency [17]. However, the present findings did not support this association, as no significant relationship between ABO types and hemoglobin levels was observed in the current study (R-squared=0.003, p-

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value=0.248). This discrepancy could be due to differences in genetic background or environmental factors, such as nutritional status or access to healthcare. There were a few limitations to the present study: cross-sectional design and data from a single center, which cannot make causal inferences about the association between the prevalence of anemia and different blood types. Potential confounding factors such as age, dietary habits, and socioeconomic status were not accounted for, which could have influenced the observed associations. Selection bias may also have affected present results, as women attending antenatal care at the healthcare center may not represent the broader population of pregnant women in the region. Furthermore, data collection methods relied on self-reported information, which may introduce recall bias. Future studies should address these limitations by including multiple centers, controlling for confounding factors, and using longitudinal designs to clarify the relationship between blood groups and anemia. This would allow for more robust conclusions about the clinical and biological factors influencing anemia risk in pregnant women.

CONCLUSIONS

Present study provides valuable insights into the distribution of blood groups and their correlation with anemia among pregnant women attending antenatal care at a primary healthcare center in Peshawar. Present study found that blood group B was the most prevalent, differing from previous studies where group O was more common. While present findings were consistent with other studies regarding the high prevalence of Rh positivity, no significant association was observed between ABO/Rh blood groups and anemia prevalence. This suggests that blood group systems may not play a significant role in determining anemia risk. Instead, other factors such as nutritional status, genetic predisposition, and socioeconomic conditions may be more influential.

Authors Contribution

Conceptualization: NJ
Methodology: SJK
Formal analysis: NJ, NS, SA, MK
Writing, review and editing: NJ, NS, SA, MK, SJK, SNM

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

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