

EVALUATION OF ERYTHROPOIETIN GENE EXPRESSION IN ABO BLOOD GROUP AMONG DONORS, IN ILORIN, KWARA STATE, NIGERIA

ABSTRACT

Introduction Erythropoietin (EPO) is a critical hormone involved in the regulation of red blood cell production, primarily through its action on erythropoiesis in the bone marrow. In Nigeria, haemoglobin estimation has been the only routine hematology parameter used to screen donors before transfusion. Haemoglobin estimation does not reveal the quality of long term blood supply nor reduce the risk of anemia-related complications in donors as assessing the chances of constant EPO secretion via EPO mRNA expression. This study explores the pattern of EPO mRNA expression in ABO blood groups among prospective blood donors in Ilorin, Kwara State, Nigeria.

Method: One hundred and twenty (120) prospective blood Donors were recruited from hospitals within the three LGAs in Ilorin that render blood transfusion services. Questionnaires were administered to collect demographic information, as well as data on lifestyle and drug use. Venous blood was collected from all the donors for serological screenin; ABO blood group of prospective donors was determined using a commercially prepared monoclonal Anti sera A, B, and D. Serological estimation of erythropoietin was performed using ELISA. Real-time PCR was used to measure EPO mRNA levels, and Red Blood Cell profile was assayed using Haematology autoanalyzer Sysmex XN-350. Data obtained were analyzed using Statistical Package for the Social Sciences (SPSS) Version 27.0, expressed as Mean±SD and frequencies, with statistical significance set at $P<0.05$.

Results: Blood group O Rh+ (63 participants) and B Rh+ (34 participants) were the most prevalent among the participants. Higher EPO gene expression and serum EPO concentration were observed more in individuals with O Rh+ ($p=0.0001, 0.017$) and B Rh+ ($p=0.048, 0.051$).

Conclusion: Individuals with blood groups O Rh+ and B Rh+ had higher EPO gene expression, and are likely to be excellent candidates for frequent blood donation. The study highlights the need for further research into genetic, factors influencing erythropoiesis gene expression in other blood groups to enhance blood donation safety.

Keywords: *Blood donation, Blood donor, ABO Blood group, Erythropoiesis, Erythropoietic Gene expression*

1. INTRODUCTION

Blood donation plays a pivotal role in modern healthcare, providing essential support for a wide range of medical procedures, from surgeries and trauma care to cancer treatments and the management of chronic illnesses (Robert et al., 2019; McGann and Weyand, 2022). Globally, blood demand is immense, with the World Health Organization (WHO) estimating that an average of 118.5 million units of blood are collected annually worldwide (Jhalani, 2021). However, developed countries, despite accounting for just 16% of the global population, collect 40% of this total, while many developing nations, like Nigeria, experience significant blood shortages (Blaise, 2022). Blood donation is associated with the removal of vital components such as red blood cells, plasma, and

other elements (Umar et al., 2023). In healthy donors, the loss of red blood cells triggers a compensatory response, whereby the hormone erythropoietin (EPO) stimulates the production of new red blood cells to replace those lost during donation (Umar et al., 2023). The efficiency of this response is crucial to ensuring that donors do not develop anemia or experience other adverse effects from repeated donations (Vijayram et al., 2019; Karpova et al., 2022). Frequent blood donations without sufficient recovery time can lead to iron depletion and anemia, which in turn impairs erythropoiesis and can compromise the donor's health (Hod et al., 2022).

Erythropoietin is a critical hormone produced primarily in the kidneys, which regulates the proliferation of erythroid progenitor cells in the bone marrow (Sun et al., 2021). Its production is typically triggered by low oxygen levels in the body, a process that enhances red blood cell production (Tichil et al., 2024). The regulation of erythropoietin production is largely governed by hypoxia-inducible factors (HIFs), which respond to oxygen levels in the blood (Suresh et al., 2019). In blood donors, erythropoietin plays a crucial role in the recovery process by stimulating the production of new red blood cells to replace the ones lost during donation (Martinez and Johnson, 2023). The expression of EPO mRNA serves as an important marker of the body's ability to mount an adequate erythropoietic response in response to blood loss, such as that caused by donation (Tsiftoglou, 2021). However, various factors, including genetic polymorphisms, underlying health conditions, and environmental influences such as altitude, can affect erythropoietin mRNA expression (Nath et al., 2021). The expression of EPO mRNA serves as an essential marker of erythropoietic activity and can provide valuable insights into the ability of donors to maintain hematological balance. Despite extensive research on erythropoietin, there remains a gap in understanding erythropoietin mRNA expression specifically within the population of blood donors in Ilorin, Kwara State, Nigeria, making this an important area of study.

In Nigeria, blood donor eligibility is predominantly assessed based on hemoglobin levels and serological screening for infections, with hemoglobin cutoffs set at 13.5 g/dl for men and 12.0 g/dl for women (Akanmu et al., 2019). While these measures are critical for ensuring donor health and blood quality, they do not provide a comprehensive picture of erythropoietic function. In many Nigerian health institutions, donor deferrals due to low hemoglobin levels are a significant challenge. Studies indicate that up to 54.7% of potential donors in some regions are deferred for this reason, highlighting the urgent need for more nuanced screening and donor management strategies (Olusanya et al., 2022). This rising rate of deferrals negatively impacts the available blood supply. While hemoglobin estimation is a routine part of donor screening, it does not provide a complete picture of erythropoietic function or the capacity of donors to recover from blood loss (Okafor et al., 2023), especially in cases where erythropoietin regulation might be insufficient to maintain red blood cell production. By focusing on erythropoietin mRNA expression, there is a need for more advanced screening approaches, including the molecular quantification of EPO mRNA expression, to better assess erythropoietic potential and minimize the risk of anemia among donors. The study aims to evaluate the pattern of erythropoietin (EPO) mRNA expression in prospective ABO blood group donors in Ilorin, Nigeria. It focuses on the relationship between demographic factors and EPO mRNA levels, and the patterns of serum EPO, EPO mRNA expression, and red blood cell profiles among ABO blood group donors.

2. MATERIALS AND METHOD

2.1 Sample Selection, Population and Area

A total of one hundred and twenty (120) prospective blood donors were recruited from ten hospitals within the sixteen LGAs in Ilorin, Kwara state. A pre-donation questionnaire was created and administered through interviews to collect demographic information and lifestyle history as shown in table 1. Informed consent was obtained from all participants using a consent form prior to sample collection. The recruitment criteria used for sample selection is shown in figure 1.

2.2 Ethical Approval

Ethical approval was sought and gotten from the Kwara State ministry of health, Ilorin with approval number; **ERC/MOH/2024/08/335**.

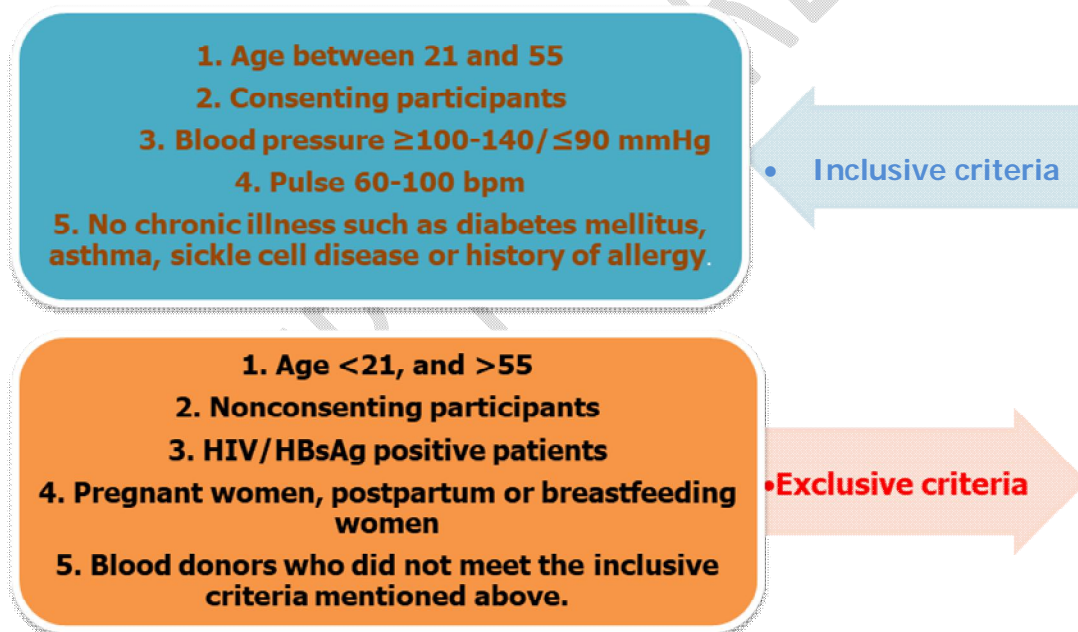


Figure 1: The inclusive and exclusive used for sample selection.

2.3 Sample Size Calculation

The formula used is shown below as described by Lambe et al., (2024):

$$n = \frac{Z^2 P(1-P)}{d^2}$$

This is a sample size calculation formula for a study based on a given confidence level, prevalence, and precision.

- **n** = Sample size (the number of participants needed).
- **Z** = Statistic corresponding to the confidence level (1.96 for 95% confidence).
- **P** = Expected prevalence (0.08 or 8% in this case).
- **(1 - P)** = The complement of the prevalence (0.92).
- **d** = Precision or the margin of error (set at 0.05, or 5%).

Substituting the values:

- $(1 - P) = 1.0 - 0.08 = 0.92$
- Using the equation:
$$n = (1.96)^2 \times 0.08 \times (0.92) / (0.05)^2,$$

the required sample size (n) was calculated to be 120.

2.4 Sample Collection

About 5mls of venous whole blood sample each was collected from eligible prospective donor into EDTA bottle and plain bottle. The blood in the plain bottle was centrifuged to obtain serum for serological assessment of erythropoietin. A portion of blood in EDTA bottle was used for hematological tests (red blood cell profile) while the other portion was centrifuged to obtain plasma which was then stored at -20°C for molecular assessment of erythropoietin.

2.5 Biochemical and Hematological Analyses

A monoclonal blood grouping reagent kit [LORNE LAB. LTD. BERKS, U.K. REF: 610010E] comprising of anti-A, anti-B anti-AB and anti-D were used to determine the blood group of the donors. Red Blood Cells profile was assayed using Haematology autoanalyzer Sysmex XN-350. Sandwich Enzyme Linked Immunosorbent Assay (ELISA) kit was used for serological estimation of erythropoietin (E-EL-H3640, LOT: GY02260H7587). The level of EPO mRNA expression was measured using real-time polymerase chain reaction (RT-PCR).

2.6 Statistical Analysis

Demographics and Laboratory data were analyzed using Statistical package for social sciences (SPSS) version 27.0. Categorical data were represented as frequencies and percentages, while continuous variables were expressed as means and standard deviations (SD). The association between categorical variables was evaluated using the chi-square test, Student t-test was applied to compare the means of continuous variables. Pearson's correlation coefficient was used to examine the relationship between ABO blood groups and EPO mRNA expression. $P < 0.05$ was considered to be statistically significant.

3. RESULTS

Table 1: Family replacement donors are the most prevalent group ($p=0.045$), with 73.3% of whole participants. The majority of participants were aged 21 to 35 years (64.2%) and

predominantly male (91.7%). Approximately 70% were married, and educationally, the least level observed was primary school, while more than half were university graduates and about half were skilled worker. Less than 3% of participants reported a history of alcohol use or smoking. The most common blood donation frequency was first-time donation (51.7%). Also, the majority of the participants (72%) reported engaging in moderate physical exercise ($p=0.048$).

Figure 2: Figure 2 displayed the frequency of ABO Rhesus factors of the participants. As revealed on the bar chart, the frequency of each blood group among the total number of participant is as follow, A Rh+ (10), B Rh+ (34), AB Rh+ (4), O Rh+ (63), A Rh- (3), B Rh- (2), and O Rh- (4). Hence, O Rh+ has the highest prevalence among the prospective blood donors.

Table 2: No association was observed in the expression of the erythropoietin gene and across the demographic factors (age, gender, frequency of donation, type of donor, and physical exercise).

Table 3: Participants with blood group O+ and B+ only showed high expression of erythropoietin mRNA ($p=0.0001$ and 0.048 respectively). However, no significant association was observed among other blood groups.

Table 4: Participants with blood group O+ significantly displayed high serum EPO concentration ($p=0.017$) compared to other blood groups, followed by participants with blood group B+.

Table 5: The outcome of the comparative analysis revealed no significant differences comparing the plasma levels of different RBC profile parameters (PCV, HBG, MCH, MCHC, MCV, RDW-SD and RDW-CF) and serum erythropoietin concentration to ABO blood group and Rhesus factor among the potential donors in Ilorin.

Table 1: Demographic characteristics of the participants

Demographic Characteristics		Frequency (%)	P- value
Type of donation	Voluntary	15 (12.5)	0.045*
	Family replacement	88 (73.3)	
	Commercial	17 (14.2)	
Age (years)	21-35	77 (64.2)	0.115
	36-45	34 (28.3)	
	46-55	9 (7.5)	
Gender	Male	110 (91.7)	0.987
	Female	10 (8.3)	
Marital status	Single	37 (30.8)	0.762
	Married	82 (68.3)	
Frequency of donation	1st timer	62 (51.7)	0.452
	Occasionally	11 (9.2)	
	Yearly/regularly	47 (39.2)	
Educational level	Primary	10 (8.3)	0.452
	Secondary	45 (37.5)	
	Tertiary	65 (54.2)	

Occupation	Students Civil servant Artisans Business man Farmer	8 (6.7) 38 (31.7) 44 (36.7) 25 (20.8) 5 (4.2)	0.623
History of alcoholism	Yes No	1 (0.8) 119 (99.2)	0.837
History of cigarette smoking	Yes No	3 (2.5) 117 (97.5)	0.823
Physical exercise	Mild Moderate High	7 (5.8) 73 (60.8) 40 (33.3)	0.048*

Data were presented in frequencies and percentages. Chi-square Test was used to determine the significant of association between the variables at $P < 0.05$ (95% confidence level) with degree of freedom 1.

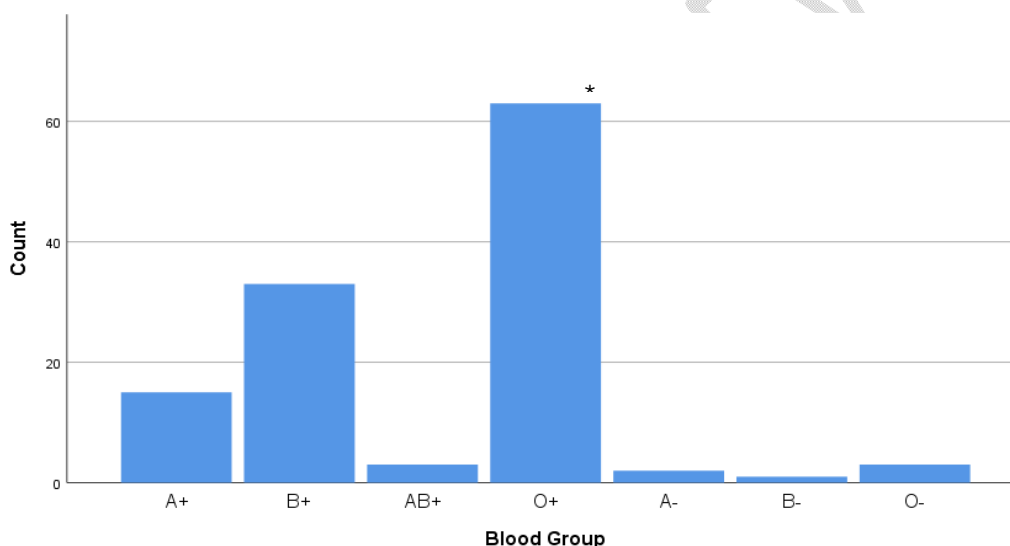


Figure 2: Bar chart showing the ABO and Rhesus group pattern of the participants. Data were presented in frequencies and percentages *= Highest frequency

Table 2: Erythropoietin mRNA expression in relation to demographic characteristics

Characteristics		Low	Normal	High	P- value
Age (years)	21 – 35	21 (17.5)	12 (10)	42 (50.4)	0.502
	36 – 45	19 (15.8)	6 (5)	14 (11.7)	
	46 – 55	3 (2.5)	2 (1.6)	1 (0.8)	
Gender	Male	46 (38.3)	19 (15.8)	45 (37.5)	0.580
	Female	4 (3.3)	3 (2.5)	3 (2.5)	

Type of donation	VD	5 (4.2)	2 (1.7)	8 (6.7)	0.592
	FRD	44 (36.7)	14 (11.7)	29 (24.2)	
	CD	7 (5.8)	3 (2.5)	8 (6.7)	
Freq. of donation	1 st time	31 (25.8)	7 (5.8)	19 (15.8)	0.510
	Occasionally	19 (15.8)	9 (7.5)	13 (10.8)	
	Yearly	11 (9.2)	6 (5)	5 (4.2)	
Physical exercise	Mild	3 (2.5)	1 (0.8)	2 (1.7)	0.576
	Moderate	40 (33.3)	11 (9.2)	26 (21.7)	
	High	13 (10.8)	7 (5.8)	17 (14.2)	

The values are finding (%). Chisquare Test was used to determine the significant of association between the variables at $P < 0.05$ (95% confidence level) with degree of freedom 1.

Table 3: Association between the participants ABO Rhesus positive blood group and Erythropoietin mRNA expression

Blood group	EPO mRNA expression	Freq. (%)	P-value
A +	Low	6 (5)	0.549
	Normal	6 (5)	
	High	3 (2.5)	
B +	Low	7 (5.8)	0.048*
	Normal	9 (7.5)	
	High	18 (15)	
AB +	Low	1 (0.8)	0.173
	Normal	0	
	High	3 (2.5)	
O +	Low	32 (26.7)	0.000*
	Normal	7 (5.8)	
	High	22 (18.3)	
A-	Low	0	0.275
	Normal	3 (2.5)	
	High	0	
B -	Low	0	0.217
	Normal	2 (1.6)	
	High	0	
O-	Low	1 (0.8)	0.263
	Normal	0	
	High	3 (2.5)	

Data were presented in frequencies and percentages. Chi-square Test was used to determine the significant of association between the variables at $P < 0.05$ (95% confidence level).

Table 4: Serum erythropoietin concentration among ABO blood group of the participants.

Blood group	Serum EPO concentration	Frequency	P-value
A +	Low Normal High	1 (0.8) 7 (5.8) 7(5.8)	0.668
B +	Low Normal High	3(2.5) 9(7.5) 21(17.5)	0.051
AB +	Low Normal High	1 (0.8) 0 2 (1.6)	0.987
O +	Low Normal High	11 (9.2) 12 (10) 40 (33.3)	0.017*
A-	Low Normal High	1 (0.8) 1 (0.8) 0	0.721
B -	Low Normal High	0 0 1 (0.8)	0.899
O-	Low Normal High	0 2 (1.6) 1 (0.8)	0.987

Data were presented in frequencies. Chi-square Test was used to determine the significant of association between the variables at $P < 0.05$ (95% confidence level).

Table 5: Comparison of level of serum erythropoietin and RBC profile in relation to different ABO Rhesus positive blood group

RBC profile	A Rh+	B Rh+	AB Rh+	O Rh+	p-value
PCV (%)	41.57 ±4.32	42.21±3.72	45.33±4.51	41.56±3.39	0.061
HBG (g/dL)	14.88±1.54	14.91±1.71	16.03±1.19	14.72±1.32	0.342
RBC(x10¹²/L)	5.31±2.05	4.84±0.58	4.78±0.25	4.76±0.81	0.381
MCH	34.48±2.61	31.63±4.47	37.00±1.00	33.30±3.25	0.926
MCHC	35.52±1.70	34.60±2.65	37.33±1.52	38.72±2.57	0.501
MCV	87.75±15.7	90.99±8.40	98.66±2.08	92.35±9.53	0.472
RDW-CV	9.36±2.38	9.73±1.62	10.43±2.88	10.09±1.67	0.460
RDW-SD (fL)	37.57±7.28	38.68±4.94	40.70±2.65	39.81±4.09	0.340

The values are expressed as mean \pm standard deviation, Student t-test was used to compare the means and $p < 0.05$ is considered statistical significant. PCV = Packed cells volume, HBG = hemoglobin level, RBC = red blood cell. MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, MCV = mean corpuscular volume, RBC = red blood cells count.

DISCUSSION

Erythropoietin plays a critical role in stimulating red blood cell (RBC) production (Ghosh et al., 2024). Its regulation is not a major determinant of blood donation outcomes in healthy individuals. But, the process of RBC production and recovery following blood donation may be influenced by other factors, such as iron levels, and recovery time between donations. For regular blood donors, the ability to produce new RBCs efficiently is critical for both their health and the sustainability of the blood supply (Gammon et al., 2023). Repeated blood donations without adequate recovery time can also strain the body's ability to maintain optimal red blood cell production, potentially leading to decreased oxygen-carrying capacity and overall fatigue, which underscores the importance of proper donor management and recovery protocols (Jahr et al., 2021). This study reveals two major discoveries about blood donors in Nigeria. Over 70% of participants were family replacement donors, reflecting a cultural trend in sub-Saharan Africa where familial ties drive blood donation, often to meet immediate needs (Okocha et al., 2019; Nyakunga, 2023). Additionally, 72% of participants engaged in regular physical exercise, improving donor health. Interesting finding was the high rate of moderate physical exercise among participants, with over 72% engaging in regular physical activity. This is a positive indicator, as physical fitness is associated with better cardiovascular health, increased hematopoiesis, and fewer complications during and after blood donation (Schloss et al., 2020). Engaging in moderate physical exercise may also help stimulate erythropoietin production, promote the formation of red blood cells, and improve the quality of blood donated (Gibson *et al.*, 2024), thereby reducing the risk of adverse effects for both donors and recipients (Avancini et al., 2021). O Rh+ was the most common blood group among participants and its significance lies in its role as the universal donor for plasma and red blood cell transfusions, making it especially crucial for blood transfusion programs.

The key findings of this study are the higher expression of EPO mRNA and serum EPO concentration in blood groups O+ and B+. While the literature on the association between ABO blood group and EPO expression is limited, several studies have suggested that blood group antigens might play a role in various physiological processes (Kronstein-Wiedemann et al., 2023). The findings in this study suggest a potential link between specific ABO blood groups (O+ and B+) and higher EPO gene expression. This association was particularly strong in participants with blood groups O Rh+ and B Rh+. Blood group O+ individuals generally have been found to possess lower levels of von

Willebrand factor (vWF) and Factor VIII, which reduces their risk of thrombosis compared to non-O+ blood groups (Ward et al., 2020). These differences in hemostatic factors can influence vascular responses to hypoxia, potentially affecting erythropoietin (EPO) gene expression (Bermudez et al., 2020). Lower vWF levels in blood group O+ and B+ individuals may impact oxygen delivery and blood flow characteristics, potentially altering hypoxia-driven pathways and EPO genes expression. However, these associations underline the complex interplay between ABO blood groups, coagulation factors, and physiological responses to oxygen deprivation. Another potential explanation could be related to genetic or epigenetic factors associated with the ABO blood group locus (Groot et al., 2020). In agreement with our findings, variations in genes linked to erythropoiesis, including those involved in erythropoietin synthesis or receptor signaling, could interact with O+ and B+ blood group antigens, thereby influencing the expression of the EPO gene and EPO secretion.

Despite the notable association between blood group O+ and B+ and increased EPO gene expression as well as serum EPO concentration, no significant correlations were found between EPO mRNA expression or serum EPO concentration and the demographic factors, such as age, gender, type of donor, donation frequency, or physical exercise. These findings suggest that the variations in EPO gene expression observed across blood groups are independent of commonly studied factors in blood donation research (Kronstein-Wiedemann *et al.*, 2023; Kurhaluka *et al.*, 2024). For instance, while regular physical exercise and moderate physical activity were associated with increased health benefits in many donor populations, these factors did not appear to influence EPO gene expression in the present study.

Another aspect of the study focused on comparing the plasma levels of various red blood cell (RBC) profile parameters such as packed cell volume (PCV), hemoglobin concentration (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), red cell distribution width (RDW). The findings from this analysis indicated that there were no significant differences in any of these RBC profile parameters when stratified by blood group or Rh factor. These results suggest that while erythropoietin is crucial for regulating red blood cell production, serum erythropoietin concentration and the associated RBC profile parameters, such as PCV and hemoglobin levels, may not be directly influenced by blood group type or Rhesus factor in healthy and non-anemic prospective donors (Kalra and Auron, 2024) used in this study. It is important to consider that erythropoietin levels can fluctuate based on a variety of factors, including donor health status, hydration levels, and recent donation history. In this study where healthy prospective blood donors were selected, these variations were minimized, resulting in the lack of significant differences observed between the blood groups. Moreover, other biological factors that influence erythropoiesis, such as kidney function, iron status, and overall health, may have had a more substantial impact on erythropoietin levels and RBC profile parameters than ABO and Rh status.

In conclusion, prospective donors with blood groups O Rh+ and B Rh+ who exhibit higher EPO gene expression and serum EPO concentration are likely to be excellent candidates for frequent blood donation. Their enhanced ability to regenerate RBCs efficiently makes them valuable to the blood donation system, ensuring that their contributions are both safe for them and beneficial for recipients. This study highlights

the complexity of erythropoietin regulation and its potential implications for blood donation practices. This study provides a foundation for further research into the factors that influence the expression of erythropoiesis genes in other blood groups for blood donation safety.

Disclaimer (Artificial intelligence)

Option 1:

We hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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