

**Variability of Blood Parameters Across ABO and Rh Blood Groups: Insights from a  
Master Health Check-Up data of adult population**

**Running title:** Blood parameters in different ABO and Rh blood groups

**Joseph KI**

Assistant Professor, Department of General Medicine,  
KMCH Institute of Health Sciences and Research, Coimbatore, Tamil Nadu, India  
ORCID: 0009-0009-9673-954X

**Mohan Kumar**

Assistant Professor, Department of Community Medicine,  
KMCH Institute of Health Sciences and Research, Coimbatore, Tamil Nadu, India  
ORCID: 0000-0003-4599-4160

**KanagasabapathySivagami**

Assistant Professor, Department of Community Medicine,  
KMCH Institute of Health Sciences and Research, Coimbatore, Tamil Nadu, India  
ORCID: 0000-0001-6825-5737

**Jeevithan Shanmugam**

Assistant Professor, Department of Community Medicine,  
KMCH Institute of Health Sciences and Research, Coimbatore, Tamil Nadu, India  
ORCID: 0000-0002-6289-587X

**Periasamy Aparnavi \***

Assistant Professor, Department of Community Medicine,  
KMCH Institute of Health Sciences and Research, Coimbatore, Tamil Nadu, India  
ORCID-0000-0001-8671-7261

**Corresponding author:** Aparnavi P

**Email:** abi4shanthi@gmail.com

**Tel:** +91 9080582756

**Address:** Room no 408, 4<sup>th</sup> floor, C Block, KMCH IHSR, Coibatore-641014

## Abstract

**Background:** The ABO and Rh blood group systems have been associated with variations in disease susceptibility. this study aimed to assess the variability in blood parameters including red cell parameters and metabolic parameters (including renal function, hepatic function, blood glucose, lipid profile and thyroid function) by ABO and Rh blood grouping systems.

**Methods:** A secondary data analysis was conducted among patients who came for a preventive health check-up at a private tertiary care hospital in Coimbatore, India. The laboratory database contained records of 62808 adult participants who reported for master health check-ups between January 2017 and February 2024. Among these patients those who reported for the first time were included.

**Results:** Blood grouping and typing data were available for 50,368 and 56,155 participants respectively, with a mean age range of 52.6 to 53.0 years across all blood groups. The most prevalent blood group was O, followed by B, A, and AB, with similar distribution across genders. The mean hemoglobin was highest in the B group ( $13.7 \pm 13.9$  g/dl). MCH and MCV values were elevated in the A and O groups, while MCHC and ESR were higher in the B and AB groups. Renal and liver parameters mostly did not vary by blood group or Rh type, except for elevated urea levels in the A group and higher ALP levels in the O and Rh-positive groups. LDL and total cholesterol were highest in the A group, while HDL was highest in the AB group.

**Conclusion:** The results underscore the importance of considering blood group variations when interpreting blood parameters in clinical practice.

**Keywords:** Blood grouping, ABO system, Rhesus blood grouping, Haematological parameters, Master health checkup, India

## Introduction

Blood groups are a fundamental classification system based on the presence or absence of specific antigens on the surface of red blood cells. The ABO and Rh systems are the main determinants of blood type (A, B, AB, or O), each being either Rh-positive or Rh-negative. The ABO system is based on the presence of A and/or B antigens, while the Rh system is defined by the presence (Rh-positive) or absence (Rh-negative) of the Rh factor (1). However, genetic changes including single-nucleotide polymorphisms (SNPs) can result in changes to the antigenic profile of ABO system resulting in emergence of new antigens(2). Human ABO blood group antigens, which are glycoconjugates found on red blood cells, also appear on leukocytes, specific organs, plasma proteins, platelets, and various enzymes(3). Additionally, these antigens can be present in body fluids like saliva, sweat, breast milk, urine, and gastric secretions(4). They play a significant role in cellular functions and certain disease pathologies(5,6).

The glycoconjugate structures on red blood cells serve a variety of purposes, such as transporters, channels, adhesion molecules, transporters for foreign ligands, viruses, bacteria, and parasites, and enzymes. ABO antigens and the associated natural iso-agglutinins are crucial in blood transfusion and organ transplantation, though their physiological relevance is still poorly understood(7). It has been demonstrated that there are some correlations between certain infectious and non-infectious disorders and ABO blood types(8). Previous studies have shown the relationship between ABO blood type antigens and various diseases like malaria(9), cognitive disorders(10), circulatory diseases(11), hyperlipidemia(12), diabetes mellitus(13), and thyroid disorders(14).

A preventive health check-up (PHC), or master health check-up (MHC), is a self-initiated, comprehensive medical examination chosen from customizable packages aimed at early diagnosis and assessment of overall health. In the current era of big data analytics, the healthcare system which has always been an evidence-based management system has taken up big data applications in varied fields including clinical decisions on safety and effectiveness, medical records management, laboratory records, epidemiology, and pharmacoeconomic benefits(15). The MHC data serves as one such source of big healthcare data. The automated analyzers in current use accumulate an enormous volume of patient data. Hence, the current study explored the large MHC data for the variability of blood parameters by blood groups. Specifically, the study assessed the variability in blood parameters including red cell parameters and metabolic parameters (including renal function, hepatic function, blood glucose, lipid profile and thyroid function) by ABO and Rh blood grouping systems.

## Method

A secondary data analysis was conducted among patients who came for a preventive health check-up at a private tertiary care hospital in Coimbatore, India. The laboratory database contained records of 62808 adult participants who reported for master health check-up between January 2017 and February 2024. From this database, we included those patients who reported to master health check-up for the first time. The number of patients who reported for the first time were 52,400. Among them, data for blood grouping and typing was available for 50,368 and 56155 patients respectively (Figure 1). Complete data on red cell parameters, renal function, hepatic function, blood glucose, lipid profile and thyroid function were available for all these participants. Cobas 6000 analyzer (Roche diagnostics, Switzerland) was employed for conducting Liver Function Tests (LFT), Renal function tests (RFT), lipid profile assessments, and thyroid profile including thyroid stimulating hormone (TSH); while the Integra 400 Plus system (Roche diagnostics, Switzerland) was utilized for measuring HbA1c. The Beckman Coulter analyzer (Beckman Coulter, Inc., United States) was used to measure red blood cell parameters in this study. Laboratory data was available in Microsoft Excel and was analyzed

using SPSS v26. Categorical variables were expressed as percentages and continuous variables as mean and standard deviation. An Independent 't' test was used to find the association between blood groups and laboratory blood parameters. The present study was approved by the Institutional Human Ethics Committee (EC/AP/1100/12/2023).

## Results

The data for blood grouping was available for 50368 participants and blood typing for 56155 participants. The mean age ranged between 52.6 and 53.0 years for all the blood groups. Among the data available for blood grouping and Rh typing 37.8% (19088/50368) and 36.1% (20309/56155) were females respectively. Blood group distribution was similar for both genders, with the most prevalent being O (41.2% among females, and 41.5% among males), followed by B (31.1% among females, 30.8% among males), A (21.3% among females, 21.5% among males), and AB (6.2% among females, 6.1% among males). The baseline demographic characteristics including age and gender were statistically comparable across the various blood groups and both the Rh types (Table 1).

The mean hemoglobin value was statistically higher among those in B group ( $13.7 \pm 13.9$  g/dl). The MCH and MCV values were statistically higher in A blood group ( $28.6 \pm 2.9$  pg/cell and  $85 \pm 6.9$  mm<sup>3</sup> respectively) and O groups ( $28.6 \pm 2.9$  pg/cell and  $84.9 \pm 7.1$  mm<sup>3</sup> respectively). Conversely, the MCHC and ESR were statistically higher in B blood group ( $33.7 \pm 1.2$  g/dl and  $16.1 \pm 13.7$  mm/hr respectively) and AB ( $33.7 \pm 1.2$  g/dl and  $15.9 \pm 13.6$  mm/hr respectively) group. The PCV was highest in B blood group ( $40.6 \pm 5$  cells/ $\mu$ l) and was lower in O ( $40.5 \pm 4.9$  cells/ $\mu$ l), followed by AB ( $40.4 \pm 4.9$  cells/ $\mu$ l), and A ( $40.3 \pm 4.9$  cells/ $\mu$ l) blood groups. PCV and ESR were higher among patients with Rh-positive blood group ( $40.8 \pm 5$  cells/ $\mu$ l and  $15.5 \pm 13.5$  mm/hr respectively), in comparison with the Rh-negative blood group ( $40.5 \pm 5.2$  cells/ $\mu$ l and  $12.6 \pm 12$  mm/hr respectively) (Table 2).

Most of the renal and liver parameters did not differ based on the blood group or Rh type, except for the urea and ALP levels. Urea was higher in the A group (21.2 g/dl) followed by O, AB and B. The ALP was higher in O ( $80.3 \pm 25.9$  U/L) group, followed by B, AB, and A. ALP was higher in Rh positive ( $78 \pm 26.3$  U/L) group than the Rh-negative group (Table 3). The LDL and total cholesterol were highest in A group at  $130.1 \pm 37.4$  mg/dl and  $189.4 \pm 40.3$  mg/dl respectively. The HDL was highest in AB group at  $41.9 \pm 10.5$  mg/dl (Table 4).

## Discussion

The distribution of ABO system showed that O was the most frequent type with more than two-fifth of the people, followed by B and A. AB was present only in about 6.0%. The same pattern of distribution with nearly similar prevalence was observed in both national and regional (Tamil Nadu) data(16). The order of prevalence with  $O > B > A > AB$  was also found in literature from other countries including Australia(17), Britain(18), and USA (19). Saudi Arabia also had high prevalence of O and lowest prevalence of AB(20). However, Europe and Africa had higher prevalence of A and B groups. Majority (94.3%) overall and 94.1% of the females were Rh positive. This high Rh positivity rate was in accordance with the findings of a systematic review from India (94.1% both in India and Tamil Nadu)(16).

The interference of ABO blood grouping in disease expression has been in research since 1900s. The ABO blood type has also shown to significantly impact haemostasis, primarily by influencing von Willebrand factor (VWF) and factor VIII (FVIII) levels to be higher in non-O blood groups due to ABH structures in VWF N-linked oligosaccharides(21–23). Regional analysis within India showed difference in distribution of ABO system and this in addition to geographical and environmental influences were related to occurrence of diseases like cholera and malaria(24–26). The current report is novel in its kind and explored the relation of ABO and Rh system with blood parameters. The mean haemoglobin was normal (above 13 g/dl)

across all groups, likely because the data is from preventive check-ups, mostly involving apparently healthy individuals, and nearly 75% were males, among whom anemia prevalence in India is low(27). Because the AB antigens are majorly located on RBCs, conditions such as aplastic anemia have shown undetectable levels of A or B antigen(28). But beyond that the biological role of AB antigens in disease pathology is questionable. The current study reported that the mean of all red blood cell indices including RBC count, haemoglobin, MCH, MCHC and MCV were within normal range but differed significantly across the various ABO types. Despite higher mean haemoglobin levels in blood group B compared to others, it had the lowest MCH, while groups A and O, with lower haemoglobin levels, had higher MCH. This suggests that haemoglobin alone isn't sufficient to diagnose anemia since MCH varies by blood type, disclosing a potential need to redefine anemia cutoffs for each ABO group. However, the need for further research to determine the role of ABO system in anemia pathogenesis is also to be considered.

ABO blood group system has been implicated in many solid tumours, including ovarian,(29) gastric,(30) pancreatic,(31) and renal cell carcinomas (RCC). The relationship between ABO and kidneys were studied extensively in terms of RCC, and one such study by Martino et al.,(32) reported that O blood type has lesser lymph node metastasis, but did not translate into better survival rate. This study is the first to explore the relationship between renal function markers and the ABO blood system, revealing that blood urea levels are higher in groups A and O, while creatinine levels remain consistent across all groups.

The LDL, total cholesterol and HbA1c was least in B group and highest in A antigen containing blood groups (A, AB); thus, indicating that B group was less prone for cardiovascular diseases. However, because the outcome variables were not available, the need for redefining lipid profile cut offs with respect to ABO system might also be put forth. Notably, the mean cholesterol values across all ABO groups were within the normal range; the LDL and HDL values show deviations; and this might be attributed to higher mean age of the participants(33,34).

One of the major strength in the study is that the participants had similar age distribution across the various blood groups. The higher mean age of participants over 50 enabled better assessment of metabolic changes, as younger individuals often show normal values, making differences harder to detect. The major limitation is that being laboratory-based data, it lacks information on pre-existing comorbidities thereby limiting disease specific stratified analysis. Pathologies of kidney or lipid metabolism needed clinical correlation (data not available) in addition to RFT, LFT values for patient diagnosis. However, for conditions including anemia (Hb values) and diabetes (HbA1C values) where diagnosis can be made, stratified analysis was done to determine variability in blood parameters across ABO and Rh blood groups stratified among anemic and diabetic patients.

## **Conclusion**

This study provides novel insights into the variability of blood parameters across different ABO and Rh blood groups in a large cohort from a tertiary care setting in Coimbatore. The findings confirm that the prevalence patterns of ABO blood groups are in line with national and regional data, with the O blood group being the most common. Significant variations were observed in red blood cell indices, lipid profiles, and renal markers across different blood groups, suggesting a potential influence of ABO blood types on these parameters. Hence, further large dataset studies are to be carried out in this respect. Notably, the B blood group exhibited the lowest mean LDL, total cholesterol, and HbA1c levels, indicating a potentially lower risk for cardiovascular diseases compared to A antigen-containing groups. Further research on disease outcomes may indicate the potential protective effect of blood groups against cardiovascular diseases.

## **Acknowledgement**

Nil

## **Conflicts of interest**

The authors declare no conflicts of interest regarding this manuscript.

## **Ethical statement**

A Waiver for consent was obtained from the Institutional Ethical committee of KMCHHSR.

## **Consent for publication**

The article does not contain any personal identifiers and hence consent for publication was not applicable.

## **Availability of data and materials**

The data owner is the institution and permission was obtained by the authors to use the data for the research purpose. The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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## **Authors' contributions**

JK, PA and MK conceived the idea for the study. MK, SK, JS and AP were involved in data, cleaning, and analysis, writing the first draft, review and editing. All authors provided technical inputs to the manuscript and approved the final version of the paper.

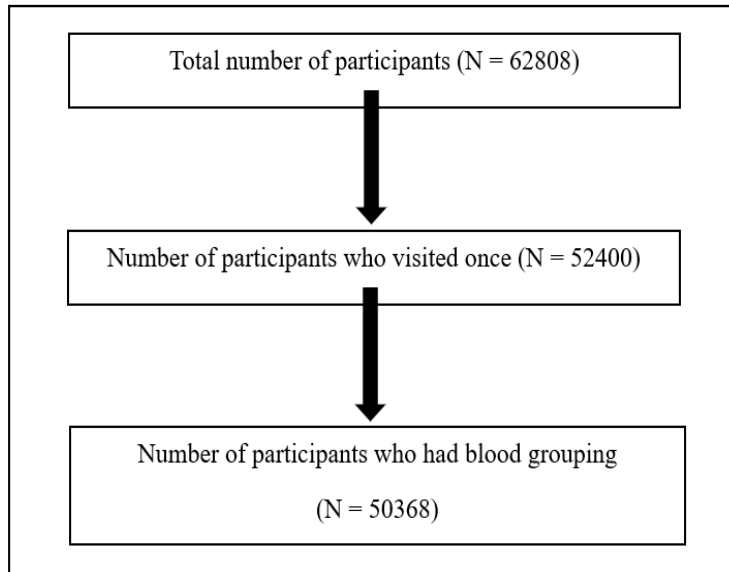
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**Figure 1:** Workflow for enumeration of participants

**Table 1:** Demographic patterns of the study subjects

Demography	Blood grouping (N = 50368)					Rh typing (N = 56155)		
	A	B	AB	O	P-value	Rh +	Rh –	P-value
Age (years) Mean (SD)	53.0 (12.2)	52.9 (12.1)	52.6 (12.0)	52.9 (12.2)	0.28 <sup>#</sup>	53.2 (12.2)	53.3 (12.0)	0.69 <sup>#</sup>
Female n. (%)	4068 (37.7)	5955 (38.2)	1198 (38.4)	7867 (37.7)	0.71 <sup>s</sup>	19121 (36.1)	1188 (37.2)	0.21 <sup>s</sup>

<sup>#</sup>Independent test, <sup>s</sup>Chi-square test

**Table 2:** Depicting difference in CBC across various blood groups and Rh types

Blood parameters	A	B	AB	O	P value <sup>#</sup>	Rh +	Rh –	P value <sup>#</sup>
	Mean (SD), N = 50368					Mean (SD), N = 56155		
Haemoglobin (g/dl)	13.6 (1.8)	13.7 (1.9)	13.6 (1.8)	13.6 (1.8)	<0.001*	13.7 (1.8)	13.6 (1.9)	0.06
RBC (million/microlitre)	4.8 (0.5)	4.8 (0.5)	4.8 (0.5)	4.8 (0.5)	<0.001*	4.8 (0.5)	4.8 (0.6)	0.017*
WBC (cells/cumm)	7575.5 (2917.6)	7644.8 (2800.9)	7678.3 (5412.2)	7654.4 (1995.1)	0.08	7672.9 (2762.5)	7579.3 (1935.8)	0.010*
MCH (pg/cell)	28.6 (2.9)	28.5 (2.9)	28.5 (2.8)	28.6 (2.9)	<0.001*	28.6 (2.8)	28.6 (2.9)	0.88
MCHC (g/dl)	33.6 (1.2)	33.7 (1.2)	33.7 (1.2)	33.6 (1.2)	<0.001*	33.6 (1.2)	33.6 (1.2)	0.008*
MCV (µm3)	85.0 (6.9)	84.4 (7.0)	84.7 (6.9)	84.9 (7.1)	<0.001*	85.0 (7.0)	84.8 (7.0)	0.18
Platelets (cells/µl)	2.9 (0.4)	2.2 (0.5)	4.1 (0.0)	2.7 (0.7)	0.11	2.6 (0.7)	2.7 (0.7)	0.09
PCV (cells/µl)	40.3 (4.9)	40.6 (5.0)	40.4 (4.9)	40.5 (4.9)	<0.001*	40.8 (5.0)	40.5 (5.2)	0.006*
ESR (mm/hr)	15.6 (13.4)	16.1 (13.7)	15.9 (13.6)	15.1 (13.2)	<0.001*	15.5 (13.5)	12.6 (12.0)	<0.001*

<sup>#</sup>Independent test, \*Statistically significant at p<0.05

**Table 3:** Showing renal and liver function variables across various blood groups and Rh types

Renal and liver function tests	A	B	AB	O	P-value <sup>#</sup>	Rh +	Rh –	P-value <sup>#</sup>
	Mean (SD), N = 50368					Mean (SD), N = 56155		
Renal Function test								
Urea (mg/dl)	21.2 (8.6)	20.8 (8.3)	20.9 (8.5)	21.0 (8.4)	0.01*	21.0 (8.5)	20.9 (7.8)	0.41
Uric acid (mg/dl)	5.2 (1.4)	5.2 (1.4)	5.1 (1.4)	5.2 (1.4)	0.19	5.2 (1.5)	5.2 (1.4)	0.16
Creatinine (mg/dl)	0.8 (0.4)	0.8 (0.3)	0.8 (0.4)	0.8 (0.4)	0.89	0.8 (0.4)	0.8 (0.3)	0.44
Liver Function Test								
Direct Bilirubin (mg/dl)	0.2 (0.2)	0.2 (0.2)	0.2 (0.1)	0.2 (0.2)	0.13	0.2 (0.2)	0.2 (0.1)	0.65
Indirect Bilirubin (mg/dl)	0.4 (0.3)	0.4 (0.5)	0.4 (0.3)	0.4 (0.3)	0.21	0.4 (0.4)	0.4 (0.3)	0.73
Total Bilirubin (mg/dl)	0.6 (0.4)	0.6 (0.4)	0.6 (0.4)	0.6 (0.4)	0.18	0.6 (0.4)	0.6 (0.4)	0.74
SGOT (U/L)	24.5 (21.2)	24.4 (17.2)	24.2 (16.5)	24.9 (31.2)	0.24	24.7 (24.4)	24.5 (18.2)	0.59
SGPT (U/L)	27.8 (24.4)	27.8 (24.2)	27.6 (20.3)	27.7 (47.5)	0.99	28.0 (35.4)	27.3 (20.9)	0.30
ALP (U/L)	72.8 (24.7)	79.3 (27.9)	73.8 (25.2)	80.3 (25.9)	<0.001*	78.0 (26.3)	76.8 (25.8)	0.011*
Albumin (g/dL)	4.4 (0.3)	4.4 (0.3)	4.4 (0.3)	4.4 (0.3)	0.77	4.4 (0.3)	4.4 (0.3)	0.13
Total Protein (g/dL)	7.3 (0.4)	7.3 (0.4)	7.3 (0.4)	7.3 (0.4)	0.041*	7.3 (0.4)	7.3 (0.4)	0.86

<sup>#</sup>Independent test, \*Statistically significant at p<0.05

**Table 4:** Showing lipid, thyroid, and glucose profiles across different blood groups and types

Metabolic profile	A	B	AB	O	P-value <sup>#</sup>	Rh +	Rh –	P-value <sup>#</sup>
	Mean (SD), N = 50368					Mean (SD), N = 56155		
Lipid profile								
VLDL (mg/dl)	29.9 (20.8)	30.0 (28.8)	30. 2 (22.0)	30.3 (20.8)	0.78	30.3 (24.6)	29.4 (18.2)	0.11
LDL (mg/dl)	130.1 (37.4)	126.8 (36.9)	129.5 (37.7)	127.8 (36.7)	<0.001*	129.6 (37.4)	130.0 (36.4)	0.64
HDL (mg/dl)	41.5 (10.1)	41.3 (9.9)	41.9 (10.5)	41.2 (9.9)	0.02*	41.3 (10.1)	41.3 (10.0)	0.92
Total Cholesterol(mg/dl)	189.4 (40.3)	185.4 (40.4)	188.1 (39.8)	186.0 (39.9)	<0.001*	186.8 (40.3)	186.7 (38.5)	0.82
Thyroid profile								
TSH (μU/mL)	3.6 (7.0)	3.5 (6.6)	3.6 (6.4)	3.5 (7.0)	0.792	3.6 (6.9)	3.4 (5.3)	0.11
Blood Glucose								
FBS (mg/dl)	117.7 (50.7)	117.7 (50.8)	118.2 (51.7)	116.6 (49.3)	0.07	117.0 (49.7)	115.8 (48.3)	0.204
HbA1c (%)	6.8 (2.1)	6.7 (1.9)	6.8 (1.9)	6.7 (1.9)	0.021*	6.7 (2.0)	6.7 (1.9)	0.633

<sup>#</sup>Independent test, \*Statistically significant at p<0.05