



ASSOCIATION OF ABO AND RHESUS BLOOD GROUPS WITH TYPE 2 DIABETES MELLITUS: A CASE–CONTROL STUDY

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Abstract

Background: Type 2 Diabetes Mellitus (T2DM) is a major global public health concern influenced by complex genetic and environmental factors. ABO and Rhesus (Rh) blood group antigens have been implicated in several metabolic and cardiovascular disorders; however, evidence regarding their association with T2DM remains inconsistent across populations. **Aim:** To evaluate the association between ABO and Rhesus blood groups and Type 2 Diabetes Mellitus and to assess their relationship with glycemic control and age at diagnosis.

Materials and Methods: This hospital-based case–control study was conducted in the Department of Physiology in collaboration with the Department of Endocrinology at Government Medical College, Jammu, over a period of one year (2024–2025). A total of 300 participants were enrolled, including 150 patients with confirmed T2DM and 150 age-unmatched but sex-matched non-diabetic controls. ABO and Rh blood grouping was performed using the microcolumn gel method. Anthropometric parameters, blood pressure, fasting blood glucose, and glycated hemoglobin (HbA1c) were assessed. Statistical analysis was carried out using SPSS version 27.0, and associations were tested using the chi-square test and Student's *t*-test. A *p* value <0.05 was considered statistically significant. **Results:** The mean age of diabetic patients was significantly higher than that of controls (49.58 ± 11.04 vs. 26.39 ± 8.88 years; *p*<0.001). Patients with T2DM had significantly higher body mass index, systolic blood pressure, fasting blood glucose, and HbA1c levels compared to controls

($p < 0.001$). Blood group O was the most common among cases (33.3%), while blood group B predominated among controls (34.7%). However, no statistically significant association was observed between ABO blood groups ($p = 0.259$) or Rh factor ($p = 0.652$) and T2DM. Additionally, no association was found between blood groups and glycemic control or age at diagnosis. In contrast, higher BMI showed a strong and significant association with both T2DM and poorer glycemic control ($p < 0.001$). **Conclusion:** The present study found no significant association between ABO or Rhesus blood groups and Type 2 Diabetes Mellitus. Body mass index emerged as a key modifiable risk factor strongly linked to diabetes and poor glycemic control. These findings suggest that blood group phenotyping has limited utility as a predictive marker for T2DM, and preventive strategies should primarily focus on lifestyle-related risk factors.

Keywords: Type 2 Diabetes Mellitus; ABO blood group; Rhesus factor; Body mass index; HbA1c; Case–control study

INTRODUCTION

The discovery of the circulation of blood by William Harvey in 1616 marked a major milestone in medical science; however, early attempts at transfusing human or animal blood were frequently fatal due to unrecognized incompatibilities between donor and recipient blood. It was not until Karl Landsteiner's seminal experiments at the Pathological Anatomy Institute of the University of Vienna that fundamental differences among human blood were identified. By demonstrating agglutination reactions between the sera and red cells of healthy individuals, Landsteiner established the concept of blood groups, thereby laying the foundation for modern transfusion medicine. His discovery of the ABO blood group system was later recognized with the Nobel Prize in Physiology or Medicine in 1930 [1].

Blood group antigens are specific molecular structures located on proteins, glycoproteins, or glycolipids of the red blood cell membrane that interact with the immune system. Blood group systems are defined as antigens governed by a single gene or a cluster of closely linked genes. At present, the International Society of Blood Transfusion recognizes 47 blood group systems comprising 366 red cell antigens [2]. Among these, the ABO and Rhesus (Rh) systems are of greatest clinical importance due to their strong immunogenicity and relevance in transfusion practice, whereas other systems are primarily of anthropological and genetic significance [3].

The ABO blood group system is encoded by the *ABO* gene located on chromosome 9q34.2, which exists in three allelic forms—A, B, and O. Inheritance of these alleles results in four phenotypic blood groups: A, B, AB, and O [4]. ABO antigens are defined by immunodominant terminal carbohydrate residues—N-acetyl-D-galactosamine for antigen A and D-galactose for antigen B—attached to oligosaccharide chains present on glycoproteins and glycosphingolipids of the erythrocyte membrane. These antigens are secondary gene products, whereas the primary gene products are glycosyltransferase enzymes responsible for their synthesis [5,6]. Individuals with blood group O lack functional glycosyltransferase activity and express only the unmodified precursor H antigen [7]. Landsteiner further demonstrated a reciprocal relationship between red cell antigens and naturally occurring serum antibodies, whereby individuals produce antibodies against the ABO antigens absent on their own erythrocytes [8]. In the Indian population, the distribution of ABO blood groups is reported as O (38%), B (34.5%), A (20.6%), and AB (6.8%), with approximately 93.4% of individuals being Rh positive [9].

The Rhesus blood group system is the second most clinically significant system after ABO and is particularly notable for its role in hemolytic disease of the newborn. The Rh system consists of 49 defined antigens encoded by two closely linked genes, *RHD* and *RHCE*, located on chromosome 1p34.1–1p36 [10,11]. Among these antigens, the D antigen is the most immunogenic and clinically relevant. Individuals expressing the D antigen are classified as Rh positive, while those lacking it are considered Rh negative [12].

Beyond transfusion medicine, ABO and Rh blood groups have been implicated in the development of various diseases, including cardiovascular disorders, malignancies, infections, and metabolic

conditions [13]. The first reliable evidence linking blood groups to disease was reported by Aird and colleagues, who demonstrated an association between blood group A and gastric cancer, followed by an increased prevalence of duodenal ulcer in individuals with blood group O [14]. Since blood groups are genetically determined, they may be associated with other disorders having a strong genetic component, including diabetes mellitus [15].

Diabetes mellitus is a heterogeneous group of metabolic disorders characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both [16]. Persistent hyperglycemia leads to long-term microvascular and macrovascular complications affecting the eyes, kidneys, nerves, heart, and blood vessels. Diagnostic criteria for diabetes mellitus are based on fasting plasma glucose, oral glucose tolerance testing, glycated hemoglobin (HbA1c), or random plasma glucose levels in symptomatic individuals [17].

Diabetes has emerged as a major global public health challenge, with 537 million affected individuals worldwide in 2021, a number projected to rise to 643 million by 2030. The majority of cases occur in low- and middle-income countries, and diabetes currently ranks as the eighth leading cause of death globally [18,19]. India bears a particularly heavy burden of the disease, with over 100 million adults affected and an additional 136 million classified as prediabetic [20].

Type 2 Diabetes Mellitus (T2DM) accounts for approximately 90–95% of all diabetes cases and is a multifactorial disorder influenced by genetic predisposition, age, obesity, sedentary lifestyle, dietary habits, and environmental factors [12,21]. T2DM is frequently associated with insulin resistance and metabolic syndrome and is accompanied by long-term microvascular and macrovascular complications, with nearly one-third of affected individuals remaining undiagnosed [22]. Long-term prospective studies, notably the Diabetes Control and Complications Trial, have established HbA1c as a reliable marker of mean glycemia and a key predictor of diabetic complications [23].

Despite extensive investigation, the association between ABO and Rh blood groups and Type 2 Diabetes Mellitus remains inconclusive, with conflicting results reported across different populations. Variations in genetic background, ethnicity, lifestyle factors, and study design may account for these inconsistencies. Furthermore, data from certain regions of India, including Jammu, remain limited. In view of these gaps, the present study was undertaken to evaluate the association between ABO and Rhesus blood groups and Type 2 Diabetes Mellitus and to explore their potential role as early diagnostic markers in this population.

Materials and Methods

Study Design and Setting

This hospital-based **case–control study** was conducted in the **Postgraduate Department of Physiology**, Government Medical College (GMC), Jammu, in collaboration with the **Department of Endocrinology**, Super Specialty Hospital, GMC Jammu. The study was carried out over a period of **one year (2024–2025)**.

Ethical Approval

The study protocol was reviewed and approved by the **Institutional Ethics Committee of Government Medical College, Jammu** (IEC/GMCJ/2024/1855 dated 07/06/2024). Written informed consent was obtained from all participants prior to enrollment, in accordance with the principles of the **Declaration of Helsinki**.

Study Population and Sample Size

A minimum sample size of **300 participants** was calculated using **Fleiss’ formula for case–control studies**, assuming a 95% confidence level, 80% power, and a case-to-control ratio of 1:1. The calculation was performed using **OpenEpi software (version 3)**.

Participants were divided into two groups:

- **Cases:** 150 patients with confirmed Type 2 Diabetes Mellitus
- **Controls:** 150 non-diabetic healthy individuals

Cases were recruited from patients attending the **Endocrinology Outpatient Department** of the Super Specialty Hospital, GMC Jammu. Controls included healthy volunteers and attendants of patients visiting the same outpatient department.

Inclusion and Exclusion Criteria

Inclusion criteria included male and female participants aged **18–85 years**. Cases comprised individuals with confirmed Type 2 Diabetes Mellitus diagnosed according to the **American Diabetes Association (ADA) 2024 criteria**.

Exclusion criteria were patients with Type 1 diabetes mellitus, gestational diabetes, secondary causes of diabetes (such as pancreatitis, endocrinopathies, malignancies, or genetic disorders), pregnant women, individuals on medications affecting glucose metabolism (e.g., corticosteroids, thiazide diuretics, phenytoin) within the preceding six months, and those with chronic systemic illnesses or unwillingness to provide consent.

Data Collection and Clinical Assessment

A detailed clinical history was obtained, followed by general physical and systemic examination. Anthropometric measurements included **body weight, height, and body mass index (BMI)**. Body weight was measured using a calibrated electronic weighing scale, and height was recorded using a stadiometer. BMI was calculated using the Quetelet index (kg/m^2) and categorized according to **World Health Organization (WHO) criteria**.

Blood pressure was measured in the seated position after a five-minute rest using a calibrated mercury sphygmomanometer. Three readings were taken, and the mean value was recorded. Pulse rate was measured manually for one minute.

Laboratory Investigations

After an overnight fast of 8–12 hours, **5–6 mL of venous blood** was collected from each participant under aseptic conditions and distributed into appropriate vacutainers.

- **ABO and Rhesus blood grouping** was performed using the **microcolumn gel agglutination method** with commercially prepared gel cards on the ORTHO™ Workstation.
- **Fasting blood glucose** was estimated using the **hexokinase method** on an automated Abbott ARCHITECT C-4000 analyzer.
- **Glycated hemoglobin (HbA1c)** was measured using **chemiluminescent microparticle immunoassay (CMIA)** on the Abbott ARCHITECT i1000SR analyzer and reported as a percentage of total hemoglobin, standardized according to the **National Glycohemoglobin Standardization Program (NGSP)**.

Statistical Analysis

All data were entered into Microsoft Excel and analyzed using **Statistical Package for the Social Sciences (SPSS) version 27.0**. Quantitative variables were expressed as **mean $\pm$ standard deviation**, while qualitative variables were presented as **frequencies and percentages**. Comparisons between groups were performed using **Student's *t*-test** for continuous variables and the **chi-square test (χ^2)** for categorical variables. A two-tailed *p* value <0.05 was considered statistically significant.

RESULTS

A total of **300 participants** were included in the study, comprising **150 patients with Type 2 Diabetes Mellitus (cases)** and **150 non-diabetic individuals (controls)**. The two groups were matched for sex, with **75 males and 75 females** in each group.

The age distribution differed significantly between cases and controls. Most diabetic patients belonged to the **41–50 year age group (36%)**, whereas controls were predominantly younger, with **54.7% in the 21–30 year age group**. The mean age of cases was **49.58 ± 11.04 years**, which was significantly higher than that of controls (**26.39 ± 8.88 years, $p < 0.001$**) [Table 1].

Table 1. Comparison of demographic characteristics between cases and controls

Variable	Cases (n = 150)	Controls (n = 150)	Statistical test	p-value
Age (years), mean $\pm$ SD	49.58 $\pm$ 11.04	26.39 $\pm$ 8.88	t = 20.05	<0.001
Male : Female	75 : 75	73 : 77	$\chi^2 = 0.05$	0.82

Diabetic patients had significantly higher body weight and BMI compared to controls. Mean BMI among cases was **26.43 $\pm$ 2.89 kg/m²**, indicating overweight status, while controls had a mean BMI of **23.99 $\pm$ 3.41 kg/m²** ($p < 0.001$). No statistically significant difference was observed in height [Table 2].

Table 2. Comparison of anthropometric parameters between cases and controls

Parameter	Cases (Mean $\pm$ SD)	Controls (Mean $\pm$ SD)	t-value	p-value
Weight (kg)	70.27 $\pm$ 8.57	66.77 $\pm$ 10.79	3.11	0.002
Height (cm)	163.15 $\pm$ 8.0	166.84 $\pm$ 9.0	1.72	0.086
BMI (kg/m ²)	26.43 $\pm$ 2.89	23.99 $\pm$ 3.41	6.67	<0.001

Mean systolic blood pressure (SBP) was significantly higher among diabetics compared to controls ($p = 0.001$). No statistically significant differences were observed in diastolic blood pressure or pulse rate [Table 3].

Table 3. Comparison of cardiovascular parameters between cases and controls

Parameter	Cases (Mean $\pm$ SD)	Controls (Mean $\pm$ SD)	p-value
Pulse (beats/min)	80.35 $\pm$ 11.56	79.60 $\pm$ 10.64	0.561
SBP (mmHg)	123.36 $\pm$ 18.25	117.73 $\pm$ 10.08	0.001
DBP (mmHg)	78.37 $\pm$ 10.08	77.94 $\pm$ 7.28	0.675

As expected, fasting blood sugar (FBS) and HbA1c levels were significantly higher in diabetic patients compared to controls ($p < 0.001$) [Table 4].

Table 4. Comparison of glycemic parameters between cases and controls

Parameter	Cases (Mean $\pm$ SD)	Controls (Mean $\pm$ SD)	p-value
FBS (mg/dL)	157.78 $\pm$ 54.68	86.19 $\pm$ 12.62	<0.001
HbA1c (%)	7.67 $\pm$ 1.62	4.88 $\pm$ 0.55	<0.001

Blood group **O** was the most common among cases (33.3%), whereas blood group **B** predominated among controls (34.7%). However, no statistically significant association was observed between ABO blood groups and T2DM ($p = 0.259$) [Table 5].

Table 5. Distribution of ABO blood groups among cases and controls

Blood Group	Cases n (%)	Controls n (%)	χ^2	p-value
A	39 (26.0)	43 (28.7)		
AB	20 (13.3)	12 (8.0)		
B	41 (27.3)	52 (34.7)		
O	50 (33.3)	43 (28.7)	4.02	0.259
Total	150 (100)	150 (100)		

Rh-positive blood group predominated in both cases (98.7%) and controls (98.0%). No statistically significant association was found between Rh factor and T2DM ($p = 0.652$) [Table 6].

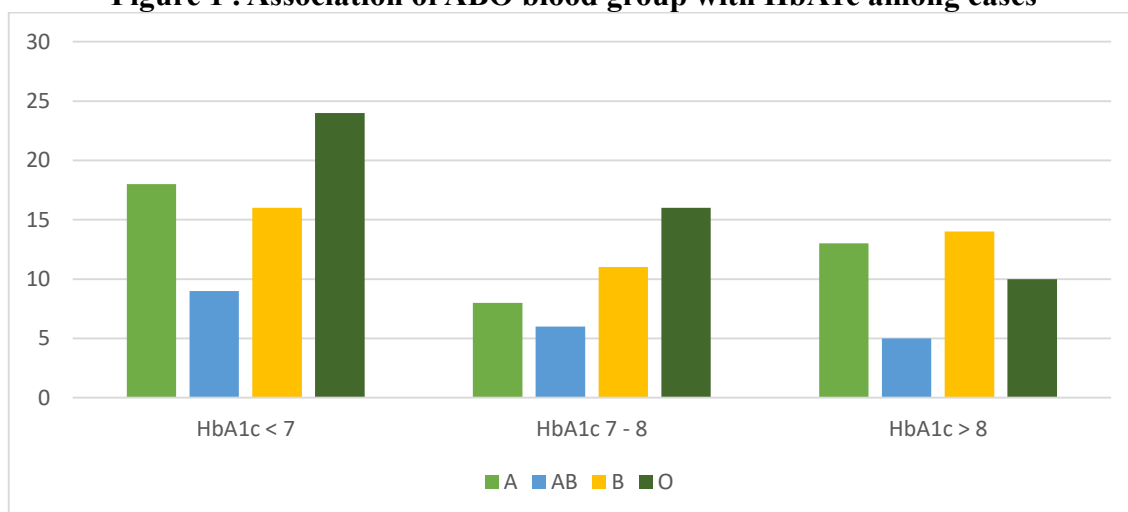
Table 6. Distribution of Rh blood groups among cases and controls

Rh Factor	Cases n (%)	Controls n (%)	χ^2	p-value
Rh-positive	148 (98.7)	147 (98.0)		
Rh-negative	2 (1.3)	3 (2.0)	0.20	0.652

Association of ABO Blood Groups with Glycemic Control (HbA1c)

No statistically significant association was found between ABO blood groups and glycemic control categories ($p = 0.716$).

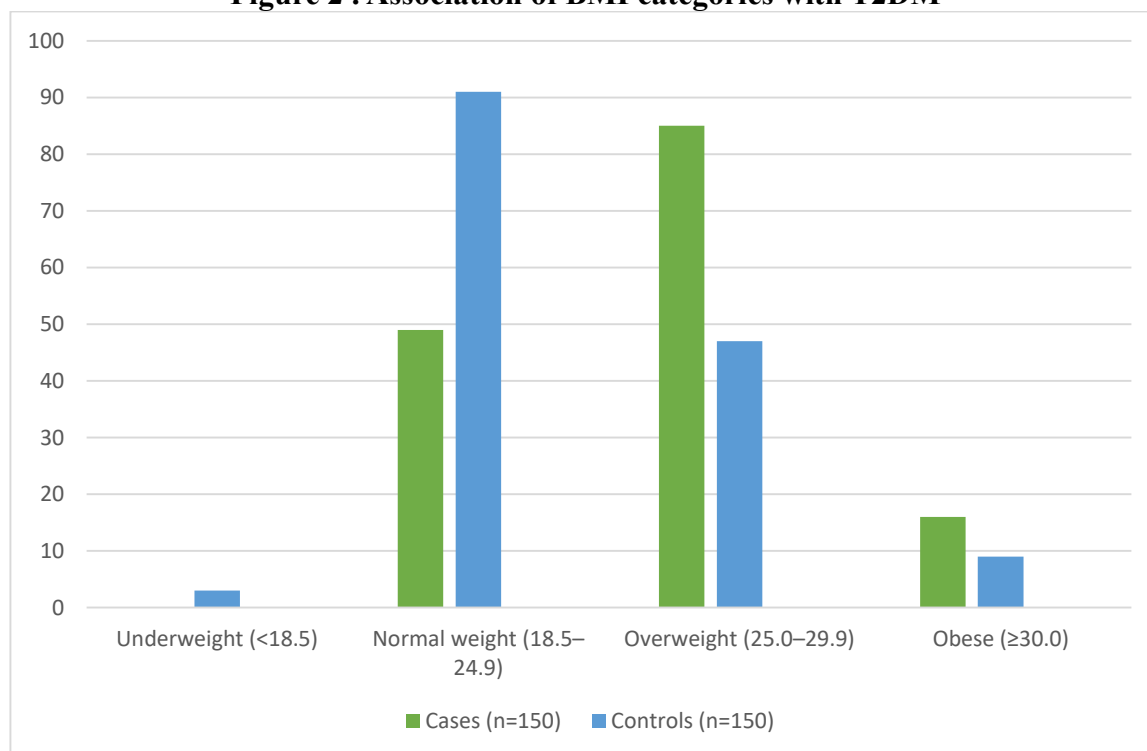
Figure 1 . Association of ABO blood group with HbA1c among cases



Association of BMI with Type 2 Diabetes Mellitus

BMI categories differed significantly between cases and controls ($p < 0.001$), with overweight and obesity being more prevalent among diabetics.

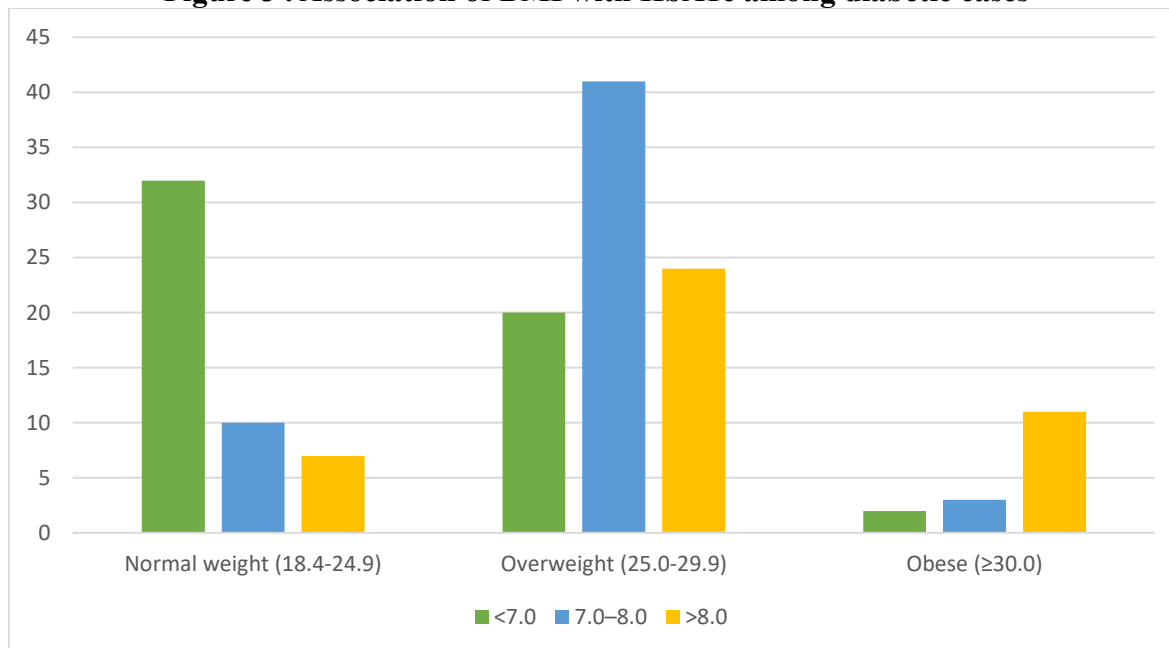
Figure 2 . Association of BMI categories with T2DM



Association of BMI with Glycemic Control among Diabetics

A statistically significant association was observed between BMI and HbA1c levels among cases ($p < 0.001$), indicating poorer glycemic control with increasing BMI.

Figure 3 . Association of BMI with HbA1c among diabetic cases



Patients with Type 2 Diabetes Mellitus exhibited significantly higher age, body mass index, systolic blood pressure, fasting blood glucose, and HbA1c levels compared to non-diabetic controls. However, no statistically significant association was observed between ABO or Rhesus blood groups and the presence of Type 2 Diabetes Mellitus, nor with glycemic control or age at diagnosis. In contrast, increased BMI showed a strong association with both diabetes status and poorer glycemic control, underscoring the importance of adiposity as a key metabolic determinant in this population.

DISCUSSION

The present case–control study was conducted to evaluate the association between ABO and Rhesus blood groups and Type 2 Diabetes Mellitus (T2DM) in a North Indian population. A total of 300 participants were included, comprising 150 patients with T2DM and 150 non-diabetic controls. The primary objective was to assess whether ABO and Rh blood groups are associated with T2DM and whether these genetic markers could be useful in early risk stratification.

The ABO blood group system is one of the most extensively studied genetic polymorphisms in humans, yet its functional significance beyond transfusion medicine remains incompletely understood [24 – Cserti CM & Dzik WH]. Since the discovery of ABO blood groups, numerous studies have examined their association with a wide range of diseases, including peptic ulcer disease, malignancies, cardiovascular disorders, infections, and hematological conditions [25 – Abegaz SB]. Given that ABO and Rh blood groups are genetically determined, it has been postulated that they may be associated with other disorders with a strong genetic component, including diabetes mellitus [26– Bener A & Yousafzai MT].

In the present study, no statistically significant association was observed between ABO blood groups and T2DM ($p = 0.259$), nor between Rh factor and T2DM ($p = 0.652$). Blood group O was the most prevalent among diabetic cases (33.3%), followed by B (27.3%), A (26.0%), and AB (13.3%), whereas blood group B predominated among controls (34.7%). This distribution closely resembles the known blood group distribution in the Indian population, where blood groups O and B are most common [9 – Soumee B.etal], suggesting that the observed frequencies likely reflect background population patterns rather than a disease-specific association.

Several biological mechanisms have been proposed to explain a possible association between ABO blood groups and metabolic disorders. It has been suggested that ABO gene variants may influence inflammatory and endothelial pathways, thereby affecting insulin resistance and glucose metabolism [28 – He M *et al.*, 2012]. However, despite these theoretical mechanisms, the findings of the present study do not support a clinically significant association between ABO or Rh blood groups and T2DM. The absence of association in the present study is consistent with several earlier investigations. Craig and Wang [29 – Craig J & Wang I] reported similar ABO blood group distributions among diabetics and non-diabetic controls. Similarly, Dali-Sahi *et al.* [30 – Dali-Sahi M *et al.*] and Dodiya *et al.* [31 – Dodiya D *et al.*] found no statistically significant relationship between ABO blood groups and T2DM. These findings support the view that ABO blood group distribution among diabetics often mirrors that of the general population.

Conversely, some studies have reported positive associations between ABO blood groups and T2DM. Blood group A has been reported to be more prevalent among diabetics in certain populations, while other studies have suggested blood group B as a risk factor [28 – El-Sayed MIK & Amin HK; 32– Bener A & Yousafzai MT]. These discrepancies across studies may be attributed to ethnic diversity, regional variation in blood group distribution, environmental influences, lifestyle factors, and differences in study design and sample size.

With respect to the Rhesus blood group system, the present study found no significant association between Rh factor and T2DM. This finding is in agreement with several studies that failed to demonstrate an independent role of Rh status in diabetes pathogenesis [33– Alanazi M *et al.*].

Sex-based analysis in the present study showed no statistically significant association between ABO blood group distribution and sex among diabetic cases ($p = 0.760$). Blood group O was most common among male diabetics, while blood groups B and O were equally distributed among female diabetics. These findings suggest that sex does not modify the relationship between blood group and T2DM in this population.

The secondary objective of the study was to assess whether blood group influences the age at diagnosis of T2DM. Most patients were diagnosed after 35 years of age, and no significant association was found between blood group and age at diagnosis ($p = 0.5175$). This observation indicates that blood group does not influence the timing of disease onset. In contrast, age itself emerged as a significant determinant, with diabetic patients being significantly older than controls ($p < 0.001$). Aging is known to be associated with increased insulin resistance and impaired β -cell function [34– Chang AM & Halter JB].

Body mass index (BMI) was a major determinant of T2DM in the present study. Diabetic patients had significantly higher body weight and BMI compared to non-diabetic controls, with a greater proportion of diabetics being overweight or obese. These findings are consistent with epidemiological data demonstrating a strong association between obesity and T2DM [35– CDC]. Obesity, particularly visceral adiposity, plays a central role in the development of insulin resistance and metabolic dysfunction.

Furthermore, higher BMI was significantly associated with poorer glycemic control among diabetics, as reflected by elevated HbA1c levels ($p < 0.001$). In contrast, no significant association was observed between ABO blood groups and glycemic control ($p = 0.716$), indicating that glycemic control is influenced primarily by modifiable metabolic factors rather than inherited blood group antigens.

Hypertension was also more prevalent among diabetic patients in the present study, with significantly higher systolic blood pressure observed among cases. The coexistence of obesity, hypertension, and diabetes observed in this study aligns with the concept of metabolic syndrome, which substantially increases cardiovascular risk [36– Michaelidou M *et al.*, 2023].

CONCLUSION

Overall, the findings of this study indicate that **ABO and Rhesus blood groups do not play a significant role in the development, age of onset, or glycemic control of Type 2 Diabetes Mellitus**, whereas **modifiable metabolic risk factors such as increased body mass index and associated**

cardiovascular parameters exert a far greater influence. These results emphasize that preventive and management strategies for T2DM should focus on lifestyle modification, weight control, and blood pressure management rather than on blood group–based risk assessment.

Based on the present findings, **blood group phenotyping cannot be recommended as a screening or predictive tool for Type 2 Diabetes Mellitus.** Further large-scale, multicentric studies across diverse populations may be required to explore subtle genetic associations; however, within the context of this study, metabolic and lifestyle factors remain the primary determinants of disease risk and control.

Conflict of interest: Nil

Funding: Nil

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