

## Original Article

## Association of Blood Clotting Time with Gender and Blood Group among Young Healthy Individuals

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### Abstract

**Introduction:** Blood clotting is a crucial homeostatic process that prevents excessive bleeding after injury. This study investigates the influence of physiological parameters like blood type and gender on Clotting Time. While previous studies suggest potential associations between pathologic conditions and Blood Clotting Time, the evidence remains inconclusive for such an association between physiological conditions like blood group and gender, and Blood Clotting Time. Additionally, regional variations in Clotting Time are understudied. This study aimed to investigate the relationship between clotting time, blood type, and gender in a Pakistani population.

**Objectives:** The objectives of our study were to investigate the association between blood group (ABO and Rh) and Clotting Time in a healthy young adult population from Pakistan, and to determine if there are significant differences in clotting time between males and females.

**Material and Methods:** A Cross-sectional, observational study was conducted on 208 healthy subjects, 108 males and 100 females. Blood groups were self-reported by participants, while Blood Clotting Time was measured by the Capillary Tube Method. The Association of Clotting Time with gender and blood group was checked.

**Results:** Clotting Time was highest in the O blood group and lowest in AB, and this relation was significant (p value=0.001). Among Rh blood groups, Rh negative had higher Clotting Time than Rh positive, but this relation was not significant (p value=0.564). Females had higher Clotting Time than males with high significance (p value=0.00).

**Conclusion:** Females and O blood group individuals are more susceptible to abnormal bleeding in cases of trauma or surgery; therefore, necessary measures should be taken before undergoing invasive procedures. Studies with a larger number of participants need to be done to determine risk groups more accurately.

**Keywords:** Blood Group Antigens, Young Adults, Gender, Blood Coagulation Factors, Blood Coagulation Disorders, Cross-Sectional Study.

## Introduction

Hemostasis, the physiological response to vascular injury, involves a coordinated sequence of events: vascular constriction, platelet activation, and the coagulation cascade, culminating in the formation of a platelet-fibrin clot, which prevents excessive bleeding and maintains vascular integrity.<sup>1</sup> This is a crucial homeostatic mechanism in which a platelet and fibrin-containing clot is formed on the damaged blood vessel, thus preventing excessive bleeding.<sup>2</sup>

Blood Clotting Time indicates the efficiency of blood coagulation pathways and helps in diagnosing different bleeding disorders. Clotting Time is mainly affected by pathological and dietary disorders like hemophilia, Von Willebrand disease, liver disease, and Vitamin K deficiency. In hemophilia, some Clotting Factors are deficient, which leads to disruption of the coagulation cascade, resulting in excessive or prolonged bleeding.<sup>3</sup> Vitamin K is essential for the synthesis of several key Clotting Factors. A deficiency in Vitamin K impairs the production of these Clotting Factors, leading to an increased Clotting Time.<sup>4</sup> The liver is responsible for producing most of the body's Clotting Factors. Liver diseases, such as cirrhosis or hepatitis, can impair the liver's ability to synthesize these factors, leading to an increased Clotting Time.<sup>5</sup> Von Willebrand disease (VWD) is a genetic disorder caused by a deficiency or dysfunction of the Von Willebrand factor, a protein that is crucial for platelet adhesion and stabilization of Factor VIII.<sup>6</sup> This deficiency leads to impaired platelet plug formation and an increased bleeding tendency. Consequently, individuals

with VWD experience prolonged bleeding, especially after trauma or surgery.<sup>7</sup>

Besides its association with pathological conditions, there is also evidence that variations in physiological conditions like blood group and gender affect the Clotting Time. According to previous studies, females have a longer clotting time than males.<sup>8</sup> Furthermore, blood group differences have also been demonstrated to affect clotting time; people with blood group O frequently have longer clotting times than people in non-O blood groups. The<sup>9</sup> Rh blood type has been shown to have an insignificant correlation with blood clotting time.<sup>10</sup>

There is a significant difference in the mean Clotting Time of people of different ethnic groups because they have different prevalence of type O blood, which is a major factor affecting blood clotting time. Reddy et al. showed that Caucasians were more admitted to hospitals with epistaxis, and found out that they have a greater prevalence of type O blood group.<sup>11</sup> While most of the studies found a significant association between the ABO blood group and Clotting Time, others have not found any significant correlation between these two variables.<sup>12</sup> Understanding the regional differences in Clotting Time is very important to fully understand the risk factors of thrombotic activity of blood, like epistaxis, thrombosis, and cardiovascular disease. Given the limited research on this topic in Pakistan, we conducted this study to explore the relationship between clotting time and physiological factors such as blood type and gender.

## Materials and Methods

A cross-sectional observational study was conducted on a sample of 208 healthy, first-year MBBS students enrolled at Rawalpindi Medical University in the year 2024. The sample size was determined using G\*Power software.<sup>13</sup> Assuming a small to moderate effect size (0.2), alpha level of 0.05, and desired power of 0.8, calculations indicated a required sample size of 212 students. This sample size ensures adequate power to detect statistically significant differences in clotting times, while being mindful of resource constraints and the total population size of 366 first-year MBBS students. Our final sample of 208 participants closely approximated this target. The study duration was four months, from February to June 2024. Non-random convenience sampling was used to recruit participants from the study setting. The ages of the participants ranged from 18 to 22 years. Only MBBS students in the first year were recruited. Those from other years of MBBS or from Allied Health Sciences were excluded. Students having a history of bleeding disorders, liver or kidney diseases confirmed from medical history, or using medications that affect clotting time, like NSAIDs, were also excluded. Informed written consent was taken from all participants. The blood group was self-reported by participants based on prior laboratory tests. Those who didn't know their blood group were excluded from the

study. Questionnaires with missing data were discarded. Blood Clotting Time was measured using the Capillary Tube Method. After pricking the ring finger with a sterile lancet, a capillary tube was filled with blood and held at body temperature. Starting two minutes after blood exposure to air, the tube was broken into fragments every 30 seconds until a fibrin strand formed, marking the Blood Clotting Time. Data was analyzed using version 26 of IBM SPSS. Demographics were summarized in the form of frequencies and percentages using descriptive statistics. Appropriate statistical tests were performed to compare clotting times between different blood groups and genders (one-way ANOVA for ABO blood group and independent t-test for gender and Rh blood group). The significance level was set at  $p < 0.05$ .

## Results

Out of the 208 participants, 108 were male and 100 were female. Data analysis revealed that blood group B was the most predominant, followed by blood groups A, O, and AB, respectively. As evident by the table, the relative percentages of individuals of each blood group are as follows: B (35.57%), A (34.61%), O (18.27%), and AB (11.54%) (Table I). Moreover, 184 subjects had a positive Rhesus group and 24 had a negative Rhesus group.

**Table-I** *Frequency distribution of students by gender, ABO type, and Rh type. (N=208)*

Parameters	Subgroups	Frequency	Percentage (%)
Gender	Male	108	51.92
	Female	100	48.08
ABO type	A	72	34.61
	B	74	35.57
	AB	24	11.54
	O	38	18.27
Rhesus type	Positive	184	88.46
	Negative	24	11.54

**Table-II** *Comparison of CT in different blood types and gender groups.*

Groups	Subgroups	Number of Students	Clotting Time (secs) (Mean $\pm$ SD)
Gender	Male	108	218.06 $\pm$ 51.654
	Female	100	311.40 $\pm$ 77.785
	Independent t-test ( <i>p</i> -value)		15.603 ( <i>p</i> < 0.001)
ABO type	A	72	263.33 $\pm$ 78.686
	B	74	260.68 $\pm$ 72.967
	AB	24	213.75 $\pm$ 66.386
	O	38	297.63 $\pm$ 90.507
	One-Way ANOVA F ( <i>p</i> -value)		5.748 ( <i>p</i> = 0.001)
Rhesus type	Positive	184	258.75 $\pm$ 79.892
	Negative	24	295.00 $\pm$ 87.427
	Independent t-test ( <i>p</i> -value)		334 ( <i>p</i> = 0.564)

As interpreted in Table II, the mean clotting time was found to be highest for subjects having blood group O. On the other hand, the shortest mean clotting time was obtained from subjects having blood group AB.

The correlation between Clotting Time and ABO blood groups was assessed using the One-Way ANOVA test and the results were found to be statistically significant with a p-

value of 0.001 ( $p < 0.05$  was considered to be statistically significant). Further analysis was done using Post Hoc test which revealed that there's a significant difference in Clotting Time between blood group O and AB ( $p$  value=0.00), other blood groups also show a significant difference in their Clotting Time, except that the difference in Clotting Time for blood group A and B was statistically insignificant ( $p$  value=0.837). (Table III).

**Table-III** Post Hoc Analysis of Comparison of Clotting Time with Different Blood Groups.

ABO Blood Group (Main group)	ABO Blood Group (Comparison group)	Difference of Mean Clotting Time (Main vs. Comparison Groups) $\pm$ Standard Error	Significance ( $p$ -value)
A	B	2.658 $\pm$ 12.868	0.837
	AB	49.583 $\pm$ 18.322	0.007
	O	-34.298 $\pm$ 15.587	0.029
B	A	-2.658 $\pm$ 12.868	0.837
	AB	46.926 $\pm$ 18.260	0.011
	O	-36.956 $\pm$ 15.514	0.018
AB	A	-49.583 $\pm$ 18.322	0.007
	B	-46.926 $\pm$ 18.260	0.011
	O	-83.882 $\pm$ 20.268	0.000
O	A	34.298 $\pm$ 15.587	0.029
	B	36.956 $\pm$ 15.514	0.018
	AB	83.882 $\pm$ 20.268	0.000

Clotting time was also found to be raised in subjects having a negative Rhesus blood group as compared to those having a positive Rhesus blood group. Analysis by

independent t-test revealed a non-significant correlation between Rhesus blood group type and blood clotting time ( $p$  value=0.564).

On the other hand, a comparison of Clotting Time in subjects of different genders showed marked variations. Female subjects showed a larger mean Clotting Time as compared to male subjects. Using an independent t-test, this relation between Blood Clotting Time and gender was found to be statistically significant ( $p=0.00$ ).

### Discussion

Our analysis revealed a statistically significant variation in Clotting Time among different ABO blood groups ( $p<0.001$ ). Blood group O exhibited the longest mean Clotting Time, while blood group AB had the shortest. This observation suggests a potential influence of ABO blood group antigens on coagulation pathways. Previous studies have shown that individuals with blood group O have lower levels of Von Willebrand factor and factor VIII, which may contribute to prolonged Clotting Time. This decreased level of Von Willebrand factor in the blood O group is because, about 30% of genetic determinants are mediated by the ABO gene, which is not expressed in blood group O.<sup>7</sup> Individuals with blood group O have about 25% less von Willebrand factor than other blood groups.<sup>14</sup> The reason is that the presence of ABO antigen increases the level of von Willebrand factor by preventing von Willebrand factor clearance, and not by promoting its production,<sup>15</sup> while others have found that increased von Willibrand clearance in type O blood is an independent phenomenon.<sup>7</sup> This finding aligns with previous studies, which have shown that people with blood group O are more likely to develop type 1 von Willebrand disease<sup>16</sup> and

that non-O blood groups are at an increased risk of thromboembolism.<sup>17</sup>

Our study found an increased clotting time in subjects with a negative Rhesus factor compared to those with a positive Rhesus factor. However, the independent t-test indicated this correlation was not statistically significant ( $p=0.564$ ). This finding is consistent with existing literature, which suggests that while there may be hematological differences associated with the Rhesus factor, these do not significantly affect clotting time.<sup>10</sup>

The comparison of clotting times between genders revealed significant variations, with female subjects displaying a longer mean clotting time than males ( $p=0.00$ ). This gender-related difference in clotting time could be influenced by hormonal variations, particularly estrogen, which is known to affect coagulation processes.<sup>8,18</sup> The statistical significance of this difference highlights the importance of considering gender in clinical assessments of coagulation.

These findings have important clinical implications. Understanding the variability in clotting times across different blood groups and genders can help in personalized medical approaches, particularly in managing bleeding disorders and tailoring anticoagulant therapies.

Future research should focus on larger, more diverse populations to validate these findings and further explore the genetic and molecular bases of the observed differences. Additionally, longitudinal studies could provide insights into how these relationships

may evolve with age and in the presence of co-morbid conditions.

This study highlights a significant correlation between blood clotting time with ABO blood groups and gender, with blood group O showing the longest and blood group AB the shortest clotting times. Gender differences were also significant, with females having longer clotting times than males. Females and individuals having blood group O are more susceptible to hemorrhages, but at the same time less susceptible to thrombotic events. Although the Rhesus factor did not significantly correlate with clotting time, these findings contribute to a deeper understanding of hematological variability and its potential clinical implications.

The limitations of this study should also be considered. The blood groups were self-reported, which may introduce bias. Also, the findings may not be generalizable to older populations or different ethnicities. The study's small sample size, particularly for blood groups like AB and Rh negative, should also be considered, which may have affected the statistical power of certain comparisons. Additionally, potential confounding factors like diet, stress, and menstrual cycle phase were not accounted for. Future research should address these limitations by using laboratory-verified blood group data, increasing sample size and diversity, and controlling for additional variables that may influence blood clotting time.

### Conclusion

In conclusion, there is an association of Blood Clotting Time with both the gender

and ABO type blood group but not with Rh type blood group. So, individuals with O blood type and females may experience a higher risk of abnormal bleeding during trauma or surgery. Therefore, additional precautions might be warranted before invasive procedures. Further research with a larger sample size is necessary to precisely identify risk groups.

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