

## RESEARCH ARTICLE

## No association of bleeding time and clotting time with four ABO blood groups in healthy young adults: An observational study

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## ABSTRACT


**Background:** The bleeding time, clotting time, and ABO blood groups are important in many clinical conditions such as thrombosis, epistaxis, or cardiac surgery. Although there are many reports on the association of bleeding time and clotting time among the ABO blood groups, the results found conflicting. **Aims and Objectives:** The aim of the study was to study the association of bleeding time and clotting time among the four ABO blood groups in healthy adults. **Materials and Methods:** This study was conducted among the undergraduate medical students belonging to age group of 18–22 years. The bleeding time was examined by Duke's method using Whatman filter paper and clotting time was determined by capillary tube method. ABO blood grouping was done in all the students. The possible association of bleeding time and clotting time with the ABO blood groups was analyzed by Chi-square test. **Results:** Two hundred twenty-seven healthy students were recruited in this study with 59.9% being males. Blood groups revealed, 47.58% (108/227), 27.75% (63/227), 18.06% (41/227), and 6.6% (15/227) students with blood group "O," "B," "A," and "AB," respectively. Both bleeding time ( $\chi^2 = 1.401$ ;  $P = 0.7769$ ) and clotting time ( $\chi^2 = 2.21$ ;  $P = 0.537$ ) were comparable in all the four blood groups. Although bleeding time was found to be high in female students compared to males, it did not reach statistically significant ( $\chi^2 = 1.725$ ;  $P = 0.1891$ ). For clotting time, both genders have equal number of students ( $\chi^2 = 7.08$ ;  $P = 0.993$ ). **Conclusion:** Neither bleeding time nor clotting time in the study, participants were associated with ABO blood groups. Further there was no difference in both bleeding time and clotting time among males and females students.

**KEY WORDS:** ABO Blood Group; Bleeding Time; Clotting Time; Gender

## INTRODUCTION

ABO blood group antigens are inherited as Mendelian determinants, on the basis individuals are categorized into four blood groups as "A," "B," "AB," and "O." ABO blood

group has clinical importance for human life and plays a key role in the field of transfusion medicine. The incidences of these four ABO blood groups are different in different geographical regions.<sup>[1]</sup> The ABO blood groups have found to have a profound effect on hemostasis,<sup>[2]</sup> and thought to exert major quantitative effects on plasma levels of von Willebrand factor and factor VIII.<sup>[3,4]</sup> It has been studied that deficiency of von Willebrand factor leads to hemorrhagic disorders, while elevated levels are a risk factor for thrombosis.<sup>[5]</sup> Again many research data evidenced for the association of ABO blood groups with various diseases such as gastric carcinoma, diabetes mellitus, urinary tract infections, duodenal ulcer, venous thrombosis, and epistaxis.<sup>[2,3,6-9]</sup>

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Bleeding time and clotting time are examined for blood transfusion for various purposes and to diagnose various diseases in relation to the functions of platelets and clotting factors.<sup>[10,11]</sup>

Bleeding time can be defined as the time interval between the movement when bleeding starts and the movement when the bleeding stops due to the formation of temporary platelet plug. Bleeding time usually lasts for 3–4 min. Clotting time is the time interval from onset of bleeding to formation of first fibrin thread and normal clotting time usually lasts for 5–8 min.

The studies on the association of bleeding time and clotting time with four ABO blood groups found conflicting results.<sup>[11-25]</sup> Majorities of these studies have concluded with high bleeding and clotting time for blood group “O” compared to non “O” blood groups. This has been found that individuals with blood group “O” have the lowest level of plasma von Willebrand factor for which there is a decreased thrombotic risk among the blood group “O” individuals.<sup>[26]</sup> Only one study has been carried out in the state of Odisha with a significant association of bleeding time and clotting time among the four ABO blood groups.<sup>[17]</sup> With the paucity of literature in the state of Odisha and conflicting results of the previous study undertaken in India, this present study was carried out with an aim to find out the association of bleeding time and clotting time with four ABO blood groups in healthy young adults (students).

## MATERIALS AND METHODS

This prospective observational study was carried out in the Department of Physiology, Maharaja Krishna Chandra Gajapati Medical College, Berhampur, Odisha, India, from August 2018 to February 2019.

### Study Subjects

The study subjects were selected as follows.

### Inclusion Criteria

The following criteria were included in the study:

- Undergraduate medical students belonging to the age group of 18–22 years
- Both male and female students.

### Exclusion Criteria

The following criteria were excluded from the study:

- Students with a history of any bleeding and/or clotting disorders
- Students with a history of any medication causing abnormal bleeding and clotting time like nonsteroidal anti-inflammatory drugs
- Students refused to be a part of the study.

### Laboratory Analysis

Blood samples were collected by finger prick under aseptic conditions, and red blood cells suspension was prepared with mixing blood and normal saline. The red blood cells suspension was mixed with antisera anti-A, and anti-B and looking for clumping of red blood cells under the microscope. ABO blood grouping was done by agglutination reaction observing under low-power objective of a compound microscope.<sup>[27]</sup>

Bleeding time was determined by Duke’s filter paper method.<sup>[28]</sup> In detail, a deep finger prick was made, and the length of time required for bleeding to stop was recorded by blotting the drop of blood coming out of the incision every 30 s using blotting paper. Bleeding time was calculated by multiplying the number of spots on the filter paper and 30 s. The normal bleeding time by Duke’s filter paper method is usually in the range of 1–5 min.

Clotting time was determined by Wright’s capillary glass tube method.<sup>[28]</sup> In details, a standard incision was made in the skin under an aseptic condition, and the blood was taken into a clean capillary glass tube. The length of time taken for the blood to clot was calculated by breaking the capillary tube after 2 min, 1–2 cm from one end every 30 s till appearance of fibrin thread. The normal clotting time estimated by this capillary glass tube method falls in the range of 5–11 min.

### Statistical Analysis

The generated data were entered in a predesigned Excel Microsoft window sheet for further analysis. All the students categorized into two groups on the basis of their bleeding time, i.e., students bleeding time with <4 min and students bleeding time with >4 min. Similarly, all the students categorized into two groups on the basis of their clotting time, i.e., students clotting time with <6 min and students clotting

**Table 1: Gender-wise distribution of study subjects among the four ABO blood groups (n=227)**

ABO blood group	Total number (%)	Male number (%)	Female number (%)	Chi-square test; P value
A	41 (18.06)	30 (73.17)	11 (26.83)	$\chi^2=5.582$ ; $P=0.1338$
B	63 (27.75)	36 (57.14)	27 (42.86)	
AB	15 (6.60)	11 (73.33)	4 (26.27)	
O	108 (47.58)	59 (54.63)	49 (45.37)	
Total	227 (100.0)	136 (59.91)	91 (40.09)	

**Table 2:** Distribution of bleeding time and clotting time among the ABO blood groups of healthy students ( $n=227$ )

Blood groups	Bleeding time			Clotting time		
	<4 min number (%)	>4 min number (%)	Chi-square test; <i>P</i> value	<6 min number (%)	>6 min number (%)	Chi-square test; <i>P</i> value
A	39 (95.12)	2 (4.88)	$\chi^2=1.401$ ; $P=0.7769$	36 (87.8)	5 (12.2)	$\chi^2=2.210$ ; $P=0.537$
B	61 (96.83)	2 (3.17)		60 (96.24)	3 (4.76)	
AB	15 (100.0)	0		14 (93.33)	1 (6.67)	
O	103 (95.37)	5 (4.63)		97 (89.81)	11 (10.19)	
Total	218 (96.04)	9 (3.96)		207 (91.19)	20 (8.81)	

**Table 3:** Gender-wise comparison of bleeding time and clotting time among the study subjects ( $n=227$ )

Gender	Bleeding time			Clotting time		
	<4 min number (%)	>4 min number (%)	Chi square test; <i>P</i> value	<6 min number (%)	>6 min number (%)	Chi square test; <i>P</i> value
Male ( $n=136$ )	133 (97.8)	3 (2.2)	$\chi^2=1.725$ ; $P=0.1891$	124 (91.2)	12 (8.8)	$\chi^2=7.08$ ; $P=0.993$
Female ( $n=91$ )	85 (93.4)	6 (6.6)		83 (91.2)	8 (8.8)	

**Table 4:** Summaries of studies based on the association of bleeding time and clotting time with four ABO blood groups

Studies	Association of blood groups with CT	Association of blood groups with BT	Association of gender with CT	Association of gender with BT
Mahapatra and Mishra 2009 <sup>[12]</sup>	significant	significant	Not significant	Not significant
Patil et al., 2013 <sup>[15]</sup>	Not significant	Not significant	-	-
Kohli et al., 2014 <sup>[13]</sup>	Not significant	significant	-	-
Maleki et al., 2014 <sup>[14]</sup>	-	-	significant	significant
Kaur 2015 <sup>[16]</sup>	Not significant	Not significant	Not significant	Not significant
Mirdha and Jena, 2016 <sup>[17]</sup>	significant	significant	significant	significant
Monika et al., 2016 <sup>[18]</sup>	Not significant	Not significant	-	-
Baishya et al., 2017 <sup>[19]</sup>	significant	significant	significant	significant
Gantela and Thotakura 2017 <sup>[20]</sup>	significant	significant	significant	significant
Jha et al., 2017 <sup>[21]</sup>	Not significant	Not significant	Not significant	Not significant
Adhana et al., 2018 <sup>[11]</sup>	-	-	significant	significant
Farzana et al., 2018 <sup>[22]</sup>	Not significant	Not significant	significant	significant
Nazeer et al., 2018 <sup>[23]</sup>	Not significant	significant	significant	Not significant
Waghmare and Muniyappanavar, 2018 <sup>[24]</sup>	significant	significant	significant	significant
Verma et al., 2019 <sup>[25]</sup>	Not significant	Not significant	-	-

time with >6 min. The categorical data were compared using the Chi-square test.  $P < 0.05$  was considered for statistical significance. GraphPad Instat version 3 for window was used for all statistical data analysis.

This present study was approved by the Institutional Ethical Committee of Maharaja Krishna Chandra Gajapati Medical College, Berhampur, Odisha, India.

## RESULTS

A total of 227 healthy students have participated in this study. The mean age of the students was  $19.91 \pm 1.34$  years (ranges 18–22 years). Out of 227 students, 136 (59.9%) students were male and 91 (40.1%) students were female. The mean age of

the male students was  $20.3 \pm 1.3$  years compared to female students of  $20.0 \pm 1.12$  years. There was no statistically significant difference observed in the age of male and female students ( $P > 0.05$ ).

The results of ABO blood group typing in 227 health students showed 108 (47.58%) healthy students with blood group O, 63 (27.75%) healthy students with blood group B, 41 (18.06%) healthy students with blood group A, and 15 (6.60%) healthy students with blood group AB. The distribution of male and female students among the four ABO blood groups was shown in Table 1. In blood groups A and B, approximately 75.0% of the students were males, whereas in blood groups AB and O, approximately 50% of students were males. However, these differences did not

reach statistical significance when compared among the four ABO blood groups ( $\chi^2 = 5.582$ ;  $P = 0.1338$ ).

We have compared bleeding time and clotting time across the four ABO blood groups. For a bleeding time, none of the students with blood group AB has bleeding time more than 4 min. As per the Chi-square test analysis, there was no statistical difference in the bleeding time among the four blood groups ( $\chi^2 = 1.401$ ;  $P = 0.7769$ ). For clotting time with more than 6 min, students with blood group A have maximum number (12.2%), followed by blood group O (10.19%), blood group AB (6.67%), and blood group B (4.76%). As revealed by Chi-square test, there was no association of clotting time >6 min to any blood groups ( $\chi^2 = 2.21$ ;  $P = 0.537$ ). The distribution of bleeding time and clotting time in relation to four ABO blood groups is depicted in Table 2.

Further, we have analyzed the association of gender with both bleeding time and clotting time of the healthy students. The number of students with bleeding time >4 min was found to be high in female students (6.6%) compared to male students (2.2%), although this difference has not been reached to statistical significance as revealed by Chi-square test ( $\chi^2 = 1.725$ ;  $P = 0.1891$ ). For clotting time, both male and female students have equal percentage of students (8.8%) with clotting time >6 min ( $\chi^2 = 7.08$ ;  $P = 0.993$ ). Gender-wise distribution of bleeding time and clotting time in healthy students is shown in Table 3.

## DISCUSSION

This observational study was carried out in 227 healthy young students of the age group of 18–22 years and around 60% of students being males. The age of both male and female students was comparable. ABO blood grouping resulted with students with blood group “O” in 108 (47.58%) students, followed by “B” blood groups in 63 (27.75%) students, “A” blood groups in 41 (18.06%) students, and “AB” blood groups in 15 (6.6%) students. In the present study, the comparison of bleeding time among the four ABO blood groups revealed no possible associations. Further, we have compared the gender-wise distribution of students with categorized bleeding time and clotting time and found non-significant association.

In a study undertaken in Nepalese students, the highest number of students had blood group “A,” followed by blood group “O,” “B,” and “AB.”<sup>[29]</sup> The distribution of ABO blood groups was found to be different in different geographical regions belonging to different races.<sup>[29]</sup> There was no difference in the gender-wise distribution of students among the four ABO blood groups. The bleeding time and clotting time were found comparable in the four ABO blood groups in our study subjects. Similar observation has also been reported from various studied undertaken in Sangli,<sup>[15]</sup> Amritsar,

Punjab,<sup>[16]</sup> Pune, Maharashtra,<sup>[18]</sup> and Utter Pradesh<sup>[25]</sup> among the Indian states, and Nepal<sup>[21]</sup> and Pakistan.<sup>[22]</sup> Other researchers found a significant association of bleeding time with ABO blood groups resulted a high bleeding time in individuals with “O” blood groups compared to non “O” blood groups.<sup>[12,17,19,20,23,24]</sup> All of these studies have explained this rise in bleeding time in blood group “O” individuals is due to the presence of low plasma von Willebrand factor in blood group “O” individuals.<sup>[5,30]</sup> Similar observation has also been reported for clotting time in association with the ABO blood groups. For the better comparison, we have provided a summary of the studies based on the association of bleeding time and clotting time with four ABO blood groups, as shown in Table 4.

Our result on the comparison of bleeding time and clotting time among the male and female students was in accordance with some of the earlier studies.<sup>[12,16,21]</sup> However, others have reported contradictory results, i.e., with significant differences in both the bleeding time and clotting time between male and female students.<sup>[11,14,17,19,20,22,24]</sup> Majorities of the studies with statistically significant difference observations in the bleeding time and clotting time have noticed high bleeding time and clotting time in female compared to male individuals. This may be due to the presence of hormone estrogen, which lowers the plasma level of fibrinogen and increases the clotting time.<sup>[31]</sup>

This is the second study to elucidate the association of bleeding time and clotting time in young adults with different ABO blood groups in the state of Odisha. The present study and the earlier study<sup>[17]</sup> from the same state of Odisha have found contradictory results on this association. We have included only 227 students from a single year for analysis in the present study. Inclusion of more number of students from the conjugative years for analysis may help to fill the knowledge gap on this possible association.

## CONCLUSION

First, both bleeding time and clotting time were comparable among the four ABO blood groups in young adults. Second, there is no evidence of association of genders with both bleeding time and clotting time in our study subjects. Further, a large cohort study required to fill the knowledge gap of this association.

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