

## Influence of Blood Groups on Bleeding and Clotting Time

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### Abstract

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*Background:* Blood grouping, Bleeding and Clotting time are routinely performed useful hematological tests, mainly done before blood transfusion and any surgery in hospitals. *Aims and Objectives:* To determine the blood group and to evaluate the association of ABO blood groups with BT and CT among medical students. *Materials and Methods:* This study was conducted on medical students, in the Department of Physiology at Karwar Institute of Medical Sciences (KRIMS), Karwar. Total of 299 medical students participated. Out of which 161 were males and 138 females. Blood was collected by finger prick method. Blood grouping was done with the standard anti sera technique. BT and CT were determined by the Duke's filter paper method and Wright's capillary glass tube method respectively. Blood group distribution was expressed in percentage and their correlation with BT, CT was analyzed by Chi-square analysis and Z-test. *Results:* In our study it was found that the percentage distribution of 'ABO' blood group showed predominance of blood group 'O' followed by 'B', 'A' & 'AB'. Our study also shows that bleeding and clotting times are influenced by blood group and gender. Bleeding time was more in females than males and it was statistically significant ( $p < 0.0180$ ). Bleeding time was more in females of blood group O and B, but it was statistically significant only in O blood group ( $p < 0.0016$ ). Clotting time was more in females than males and it was statistically significant ( $p < 0.0118$ ). Clotting time was more in 'O', 'B' & 'AB' blood group in females as compared to males & it was statistically significant ( $p < 0.05$ ). *Conclusion:* In our study, O was the most common while blood group AB was the least. Bleeding time was more in females of blood group O and B, but it was statistically significant only in O blood group. Clotting time was more in 'O', 'B' & 'AB' blood group in females as compared to males and the difference was statistically significant.

**Keywords:** ABO Blood Group; Bleeding Time; Clotting Time; Medical Students.

### Introduction

Haemostasis is the process of forming clots in the walls of damaged blood vessels and preventing blood loss while maintaining blood in a fluid state within the vascular system. A collection of complex interrelated systemic mechanisms operates to maintain a balance between coagulation and anticoagulation [1].

Blood group plays a vital role in the field of transfusion medicine. The relationship between bleeding time, clotting time, and blood group is important in certain clinical conditions such as epistaxis, surgery, and thrombosis. Arrest of bleeding from damaged blood vessels is hemostasis,

an essential factor to be checked before any surgical procedures. Hence blood test is a routine test for hospital prior to surgeries. The discovery of the ABO blood groups by Karl Landsteiner was an important achievement in the history of blood transfusion that was followed by discovery of Rh (D) antigen [2,3].

Some studies have shown clear association between ABO blood group status and von Willebrand factor [4,5]. Von Willebrand factor is a large glycoprotein produced by vascular endothelial cells and platelets. Its main role is in haemostasis. Deficiency of vWF leads to hemorrhagic disorders, while elevated levels are a risk factor for thrombosis [6-8].

Some studies have also shown that people with O blood group have a low vWf expression compared to other groups of ABO system. Hence, ABO blood group system strongly influence bleeding and clotting times [9].

In this regard, present cross sectional study was conducted to observe the percentile distribution of blood groups and to study the influence of blood groups, gender with bleeding time and clotting time in medical students of KRIMS, Karwar.

## Methodology

This study was conducted on undergraduate first and second year medical students, in the Department of Physiology at Karwar Institute of Medical Sciences (KRIMS), Karwar. The Institutional ethical committee clearance was obtained and informed consent was taken from volunteers. All subjects of the study were 18 to 22 years of age group, healthy and were apparently free from diseases. The information of the students regarding their age, gender, blood group, bleeding time and clotting time were collected by pre-designed questionnaire. Total of 299 medical students, volunteered to participate in the study. Out of 299 students, 161 were males and 138 females. Those with history of bleeding disorders and subjects taking NSAIDS were excluded from the study.

The ABO blood grouping and Rh typing was determined by glass slide method. Three slides were marked as A, B, and C and one drop of anti A serum was placed on slide A, one drop of anti B serum on slide B and one drop of anti D serum on slide C. One of the middle three fingers was pricked with aseptic precautions and transferred one drop of blood on the slide C with anti D serum and another drop of blood is transferred to one of the depressions on the tile with normal saline making 5% suspension. A drop of this cell suspension was placed on anti A serum on slide A, similarly on anti B serum with separate droppers for this purpose. Separate applicator pins were used for mixing the blood suspension with anti sera. After waiting for one to two minutes slides were inspected with naked eye to see whether clumping and agglutination has taken place or not. Then clumping and agglutination is confirmed under the microscope especially in the case of micro agglutination for confirmation and carefully compared with the control.

Bleeding Time (BT) was determined by Duke's filter paper method. It was determined under aseptic precautions by pricking the one of the middle three fingers deep and gently blotting the blood after 15 seconds. Procedure was repeated with fresh site of blotting paper every 15 seconds till no blood appeared on the paper. Number of blots counted and multiplied by 15 (seconds) which gives the bleeding time.

Clotting Time (CT) was determined by capillary tube method. It was determined by pricking one of the middle three fingers with aseptic precautions and the time of appearance of blood noted. The blood was made to flow through capillary tube. At an interval of thirty seconds the capillary tube of 0.5 cm length was broken. The procedure was continued till fibrin threads formed and bridged between the broken ends. Clotting time was obtained by noting the time interval between the appearance of blood and appearance of fibrin thread.

The normal BT by Duke's filter paper method is usually in the range of 1-5 min and the normal Clotting Time valued by the capillary tube method ranges between 5-11 min [10].

The glass slide method of blood group determination is based on antigen antibody agglutination. The antigen present on the membrane surface of RBC agglutinates with the agglutinins present in the anti sera. Hence, blood group was determined based on agglutination with the corresponding anti sera. If agglutination was observed in the blood drop on slide marked A, then it belongs to A blood group, agglutination in blood drop slide B, B group, agglutination in both A and B drops, AB group and if there was no agglutination in both A and B drops, then O group. Similarly, agglutination in blood drop on glass slide marked D was considered as Rh-positive and no agglutination as Rh negative. The data was expressed as percentages

## Statistical Analysis

The blood group data was analyzed and the results were listed according to percentage distribution of ABO and Rh blood groups and were expressed in percentages. The Chi-square analysis was carried out to examine gender wise relation between BT and CT. The 'Z' test was applied to examine gender wise relation between BT, CT and Blood groups. The p-value of <0.05 was considered to be statistically significant.

## Results

In the present study out of 299 medical students, 161 were males and 138 females. Gender wise distribution of 'ABO' blood group is shown in Table 1. The distribution of 'ABO' blood group system among Males & Females was same but the 'O' blood group had highest frequency followed by 'B', 'A' & 'AB'.

Gender wise distribution of bleeding time with Chi-square analysis is shown in Table 2. It shows bleeding time was more than 2.30 minutes in 9.03% females as compared to 5.35% in males. This variation was statistically significant. Distribution

of bleeding time according to 'ABO' blood groups in males & females is shown in Table 3. The bleeding time was more in 'O' & 'B' blood group of females as compared to males but it was statistically significant ( $p < 0.05$ ) in 'O' blood group only. Gender wise distribution of clotting time with Chi-square analysis is shown in Table 4. The clotting time was more than 4 minutes in 14.05% of females as compared to 9.70% in males. This variation was statistically significant ( $p < 0.05$ ). Distribution of clotting time according to 'ABO' blood groups in males & females is shown in Table 5. The clotting time was more in 'O', 'B' & 'AB' blood group in females as compared to males & it was statistically significant ( $p < 0.05$ ).

**Table 1:** Gender wise distribution of 'ABO' blood group system

Blood Group	Males	Females
A	41(13.71%)	36(12.04%)
B	43(14.38%)	38(12.71%)
AB	8(2.68%)	10(3.34%)
O	69(23.07%)	54(18.06%)
Total	161(53.85%)	138(46.15%)

**Table 2:** Distribution of bleeding time according to gender with 'Chi-square' analysis

Bleeding time	Gender	Total	$\chi^2 = 5.5934$
	Male	Female	
≤ 2.30 min	145(48.50%)	111(37.12%)	p=0.0180
>2.30 min	16(5.35%)	27(9.03%)	The result is significant at
Total	161(53.85%)	138(46.15%)	p<0.05

**Table 3:** Distribution of bleeding time according to 'ABO' blood groups in males & females

Blood Group	Males (N=161) Mean + SD	Females (N=138) Mean +SD	SE	Z' value	'p' Value
A	127.32 +28.2864	126.69 +27.8773	6.4111	0.0983	0.4801
B	124.88+38.1334	127.89 +40.4808	8.7716	-0.3432	0.3632
O	126.09 +29.3667	145+38.9848	6.3752	-2.9661 *	0.0016*
AB	135 +22.6779	120 +37.4166	14.2929	1.0495	0.1469

\* $p < 0.05$  is significant

**Table 4:** Distribution of clotting time according to gender with 'Chi-square' analysis

Clotting time	Gender	Total	$\chi^2 = 6.3327$
	Male	Female	
≤4 minute	132(44.15%)	96(32.10%)	p=0.0118
>4 minute	29(9.70%)	42(14.05%)	The result is significant at
Total	161(53.85%)	138(46.15%)	p<0.05

**Table 5:** Distribution of Clotting time according to 'ABO' blood groups in males & females

Blood Group	Males (N=161) Mean + SD	Female (N=138) Mean +SD	SE	Z' value	P value
A	212.44 + 42.8241	199.17 +41.9098	9.6705	1.3722	0.0885
B	211.40 + 55.4025	253.42 +76.9442	15.0726	-2.7878*	0.0031*
O	229.57 +54.4069	272.59+103.1062	15.4845	-2.7783*	0.0031*
AB	203.75 +48.67898	297 +74.0945	29.0724	-3.2075*	0.0006*

\* $p < 0.05$  significant

## Discussion

Evaluation of blood groups, bleeding and clotting time are the most important and initial haematological parameters. They are done as a routine clinical evaluation for anesthesiologist and surgeons before doing surgical procedures. The correlation among the blood groups, bleeding and clotting time is important in some clinical conditions like epistaxis, thrombosis and surgery etc. Blood grouping has important role before blood transfusion.

Many studies are conducted so far to correlate the association between blood groups and the Bleeding and Clotting time. Studies have shown that people with O blood group have less risk of venous thromboembolism when compared with the individuals of A, B and AB. The main reason might be that, the von Willebrand factor and factor VIII levels are on the higher side in non-O group individuals [11,12]. Several studies have shown the influence of ABO blood groups on plasma VWF levels [13-15]. These studies indicate that CT and the BT will be elevated among the O group individuals compared to the other groups. Study conducted by Kaur M et al, shown that BT was more prolonged in blood group O followed by B, AB and A, but the difference was not statistically significant [16].

In our study it was found that the percentage distribution of 'ABO' blood group showed predominance of blood group 'O' followed by 'B', 'A' & 'AB'. The similar prevalence O>B>A>AB has been noted by many research studies [17-20]. Dissimilar to our study prevalence of blood group B>O>A>AB was observed in other studies [21-23]. Our study also shows that bleeding and clotting times are influenced by blood group and sex of an individual. Bleeding time was more in females than males and it was statistically significant ( $p<0.0180$ ). Bleeding time was more in females of blood group O and B, but it was statistically significant only in O blood group ( $p<0.0016$ ). Clotting time was more in females than males and it was statistically significant ( $p<0.0118$ ). Clotting time was more in 'O', 'B' & 'AB' blood group in females as compared to males & it was statistically significant ( $p<0.05$ ). Studies conducted by Baishya R et al. [24], Ambreen Aleem, Muqet Wahid [25], showed similar increase in BT, CT in blood group O and female subjects compared to males. Studies conducted by Mahapatra and Mishra [18], Sasekala and Saikumar [20], noted prolonged CT in blood group B followed by O, AB, A which was statistically significant. Some other

studies also observed similar findings of statistically significant prolonged BT and CT in females as compared to males [26-27].

Females have more estrogen levels and low levels of plasma fibrinogen. This could be the possible physiological basis for prolongation of bleeding and clotting time in female subjects than males [28]. The subjects with blood group "O" have the highest values of CT and BT which could be due to low expression of vWf (von Willebrand factor) in this blood group.

Further research at different centers should be carried out with greater sample size and other than ABO blood group system must be evaluated along with measuring plasma levels of vWF to rule out any reasons for the difference in clotting and bleeding time among ABO blood groups, so that preventive measures could be adopted at the earliest.

## Conclusion

In our study, O was the most commonly occurring blood group while blood group AB was the least. Bleeding time was more in females of blood group O and B, but it was statistically significant only in O blood group. Clotting time was more in 'O', 'B' & 'AB' blood group in females as compared to males and the difference was statistically significant. Therefore, it can be concluded from our study that blood groups and gender have influence over bleeding and clotting times. Females especially belonging to blood group 'O' are more susceptible for bleeding tendencies compared to males. Screening for bleeding disorders and preventive measures must be initiated before the onset of such disorders.

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