

Research Article

Correlation of blood groups, Bleeding time and Clotting time in male and female students; an observational study

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ABSTRACT

Stoppage of bleeding from damaged blood vessels is hemostasis, an essential factor to be checked before any surgical process. Hence blood test is a routine test for hospital prior to surgeries. The study was aimed to assess the distribution of blood groups and to check gender-based relationship among the blood groups, bleeding and clotting time. The study was conducted in the department of pharmacy, BZU. The available data of 122 students of 1st year of Pharm. D was analysed in every required aspects i.e. sex, age, ABO and Rh blood groups, bleeding and clotting times. The standard antisera (A, B, & D) were used to determine the blood group, Duke Method and Capillary tube method were used to find out bleeding and clotting time respectively. Finally, all the parameters were compared and analysed statistically. In the present study, it is found that B blood group is more prevalent in both the sexes than O, A and AB. Clotting time is found to be significantly higher in O and B blood groups in females and males respectively, whereas Bleeding time was found to be greater than others in AB blood group in females and 'O' blood group in males but the values are not significantly different from each other. In females, the values of bleeding and clotting time are slightly greater than in males, may be because of hormonal differences. Various cardiovascular and gastrointestinal diseases are associated with the blood groups, so people can take preventive measures according to their blood groups.

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INTRODUCTION:

Haematological parameters are a very important diagnostic tool and used as a routine clinical evaluation of health as well as an essential factor for surgeons and anesthesiologist before initiating any surgical procedure. Hence, in hospitals blood tests are performed in routine (Saliu *et al.*, 2012). Evaluation of blood groups, bleeding and clotting time are the most important and initial haematological parameters. The correlation among the blood groups, bleeding and clotting time is important in some clinical conditions like thrombosis, surgery and epistaxis etc. Blood group diagnosis has an important role prior to blood transfusion. The antigenic property of red blood cells is the basis of ABO blood group. Individuals having different blood groups i.e. A, B, and AB

have the antigen A, B, and both while type O is not having A nor B antigens (Wiener, 1943). The surface membrane of Red blood cells contains antigens as complex oligosaccharides with different terminal sugar and their genes are located on the chromosome (Ganong, 2005). Recent researches indicate that diabetes mellitus, various gastrointestinal diseases like ulcers, cancers, UTIs, as well as thrombosis are associated with ABO blood group system (Zhang *et al.*, 2012; Schleef *et al.*, 2005). An important blood glycoprotein vWf (von Willebrand factor) is not only intricate in maintaining hemostasis but also carry the antigens of ABO blood group system. It has also been found that people with O blood group are having a low vWf expression in comparison to other groups of ABO system (Wiggins *et al.*, 2009; Reddy *et al.*, 2008) Hence, bleeding and clotting times have a strong influence of the ABO blood group system. Lack of vWf can lead to hemorrhagic complaints whereas high levels can be a risk factor for venous thrombosis (Ruggeri and Zimmerman, 1981; Gill *et al.*, 1987). Thus, the relationship among

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Bleeding Time (BT), Clotting Time (CT), and blood groups are significant to study clinically. The time interval between the skin puncture and spontaneous (unassisted) stoppage of bleeding is termed as bleeding time. It is mainly a test to assess the platelet adhesion and aggregating. It is found to be prolonged significantly in platelet defects, either congenital or acquired (Issitt, 1985). While the time interval between the blood vessels puncture and fibrin threads formation is called clotting time (Franchini *et al.*, 2007) clotting time is prolonged in conditions in which clotting factors are defective or deficient.

The aim of this study was to evaluate blood group distribution among males and females and to compare Bleeding and clotting Times among various blood groups, and also to identify the gender difference among the same, if any.

METHODS

Data Collection and Project Design

This study was designed and conducted in the Faculty of Pharmacy, Bahauddin Zakariya University, Multan. All the experiments were done among the undergraduate students of the same faculty.

Blood Group

Blood groups were determined by using standard antisera during physiology practical time in a physiology laboratory. Sterile lancet was used to prick the finger after sterilising the puncture site with spirit. A blood sample was taken on glass slides and mixed with anti-sera A, b and D, and presence or absence of agglutination was checked to determine the blood group. The low-power objective of a compound microscope can be used to confirm the agglutination (Pal and Pal, 2001).

Bleeding Time

Duke's filter paper method was used to determine the bleeding time. A deep skin puncture in the ear lobe was made and the time period required to stop the bleeding from incision was recorded every 30

sec using blotting paper. Bleeding time was calculated by multiplying the number of drops on the filter paper with time (30 sec). The normal values of BT by Duke's filter paper method generally lie in the range of 1–5 min (Pal and Pal, 2001).

Clotting time

Capillary tube method was used to find out the clotting time. A skin puncture was made and the first drop was wiped away. A special capillary tube was filled with blood and time was noted when the blood first appeared in the capillary. The tube was held between the thumb and index finger of both hands, and the time taken to clot the blood was counted by gently breaking the tube every 30 sec, 1-2 cm from the end, until the formation of fibrin thread across the gap between the ends of the tube. The normal Clotting Time valued by the method ranges between 5–11 min (Pal and Pal, 2001).

Data Collection

The study was done during Dec, 14 and Jan, 15. A pre-designed questionnaire was used to collect the information from the students regarding their age, gender, blood group, bleeding time and clotting time.

Outcome Variable and Explanatory variables

Bleeding Time, Clotting Time and Blood group were the Outcome variables, while age and gender the explanatory.

Inclusion criteria

All the students of 1st professional of Pharm.D were included in the study.

Exclusion criteria

Students having any bleeding/clotting time disorders and drug intake (nonsteroidal anti-inflammatory drugs) were not included in this study to avoid bias.

RESULTS

Students of same age group participated in our study (17–20 years), all students belonged to the Pharm. D, 1st professional class. Students having any missing study variables were omitted from the study. The mean ages were 18.3 ± 0.7 , 18.6 ± 0.8 and 18.0 ± 1.5 years respectively. The presented data of 122 students was analysed. Out of 122 students, 35 were males while 87 were females as shown in Table 1. Our results indicated that blood group B was the most common blood group among male and female students, followed by the other blood groups i.e. O, A, and AB, while B-, O- and A- were at least. The total ABO blood groups distribution was in the order of A+ (18), AB+(15), B+(43),O+(36), A-(1), B-(5),O-(4).

Table 2 shows that the bleeding time among students, Females had higher bleeding time among

Table 1. Distribution of students under Blood group

	Gender		Total
	Female	Male	
A+	15	3	18
AB+	11	4	15
B+	31	12	43
O+	25	11	36
A-	1	0	1
B-	3	2	5
O-	1	3	4
Total	87	35	122

Table 2. Distribution of students under Bleeding Time

	Gender		Total
	Female	Male	
<1	1	8	9
1 - 2.30	44	21	65
>2.30	42	6	48
Total	87	35	122

as compared to males. 1 female had bleeding time less than 1 minute out of 87, while 8 males had less than 1 minute and 21 were less than 2.30 minutes whereas only 6 male students were had more than 2.30 minutes. In females, the study contrast with males students and 44 females were had bleeding time within range of 1-2.30 minutes while 42 were above 2.30. When Data of males and females bleeding time were compared followed by One-way ANOVA analysis it showed the $p > 0.001$ and showed significant difference among both genders ($p > 0.05$ were considered significant) as shown in Table 3.

Females had no significant difference in clotting time parameters, almost equally distributed in <3, 3-4 and >4 ranges, whereas in males it shown the difference behaviour, in >4 clotting time had only 8 students while others equally distributed in <3 and 3-4(Table 4). When Data of males and females clotting time were compared followed by One-way ANOVA analysis it showed the $p > 0.001$ and showed significant difference among both genders ($p > 0.05$ were considered significant) (Table 5)

When Bleeding time and Clotting time on above-mentioned data correlated by Pearson correlation, a straight line of the linear graph was obtained with $r^2 = 0.1111$ $r = 0.333$, $p > 0.0001$ and 95%CI (1.144 to 1.352) It is highly significant that both are interdependent on each other. It was found that as bleeding time or clotting time increase another factor will also increase. So, bleeding time is directly proportional to clotting time (Fig1)

The comparison of clotting time in different blood groups in male and female shows that females have higher clotting time than males except blood group B⁻, in which female have lower clotting time than male. While females with O⁻ blood group have highest clotting time among all blood groups(Fig 2A).One way ANOVA analysis showed significant difference among clotting time in blood groups ($p > 0.05$ were considered significant)

Comparison between bleeding time shows that females have higher bleeding time than male,

Table 3. Comparison of Bleeding time of Males and Females

		Gender		P
		Female	Male	
Bleeding Time	<1	1	8	<i>p</i> <0.001
	1 - 2.30	44	21	
	>2.30	42	6	
Total		87	35	

Females when compared to males, it was found bleeding time was significantly increasing *p*<0.001

Table 4. Distribution of students under Clotting Time

		Gender		Total
		Female	Male	
Clotting Time	<3	26	15	41
	3-4	27	12	39
	>4	34	8	42
Total		87	35	122

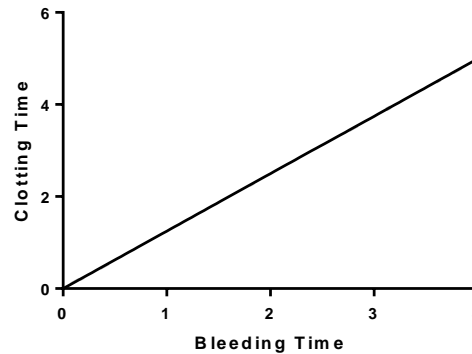
Table 5. Comparison of clotting time of Males and Females

		Gender		P
		Female	Male	
Clotting Time	<3	26	15	<i>P</i> <0.001
	3-4	27	12	
	>4	34	8	
Total		87	35	

Females when compared to males, it was found bleeding time was significantly increasing *p*<0.001

except the female with blood group who have lower bleeding time as compared to male. On the other hand, females with blood group AB+ have highest bleeding time among all blood groups (Fig 2B). One way ANOVA analysis showed significant difference among clotting time in blood groups (*p*>0.05 were considered significant)

Figure 1. Pearson Correlation between Bleeding and Clotting Time



DISCUSSION

Hemostasis consist of two phases, one is a vascular (platelet) phase and the other is coagulation phase. The activity of first phase can be checked by using the parameters like bleeding time, platelet count and platelet function assay (Harker and Slichter, 1972; Day and Rao, 1986) while the available test to check the second phase(Coagulation phase) include clotting time(whole blood), prothrombin time, plasma fibrinogen and activated plasma thromboplastin time (Kinra *et al.*, 2009). Some diseases are also having relation with blood groups. Like in Hemophilia bleeding time remains normal because the platelet adhesion and aggression are the main cause (Zucker, 1980). A study showed that people with blood group O were more susceptible to gastrointestinal infection in an endemic, which was due to Escherichia coli (O157) in 1996 in Scotland. Another study showed that people with A, B and AB blood groups are more vulnerable to thrombotic diseases (arterial/venous) as compare to blood group O people. Persons with O blood group have less risk of venous thromboembolism (VTE) when compared with the individuals of other blood groups (A, B and AB). One main reason might be that the levels of von Willebrand factor (vWF) and factor VIII (FVIII) are on the higher side in non-O group individuals (Kamphuisen *et al.*, 2001; Jenkins and O'Donnell, 2006).

The aim of this study was to check the blood groups distribution, correlate the BT with CT as well as to study the effect of gender and blood groups on bleeding time and clotting time.

The study was done in 122 students of which 87 were female and 35 were male. The most prevalent blood group among the students of Pharm .D was B⁺, followed by O⁺ and A⁺. The rarest blood group was A⁻ which was found in only one female student and no male student has A⁻ blood group. About 65 students were having bleeding time in the range of 1-2.30 minutes, 48 were having more than 2.30 minutes while only 9 were having bleeding time less than 1 minute. The comparison of clotting time shows that 42 students were having >4 minutes clotting time, 41 are having <3 minutes and 39 were having 3-4 minutes Clotting time.

The comparison of gender with bleeding and clotting times shows that females have greater values of BT and CT as compared to males, which might be because of hormonal difference in male and female. Females are having higher levels of oestrogen and lower levels of fibrinogen in blood plasma as compared to males. This may cause the difference of bleeding time and clotting time in male and female (Ercan *et al.*, 1998)

Pearson correlation between BT and CT shows that these two values are interdependent on each other, as both the tests are used to check the activity of two different phases of hemostasis.

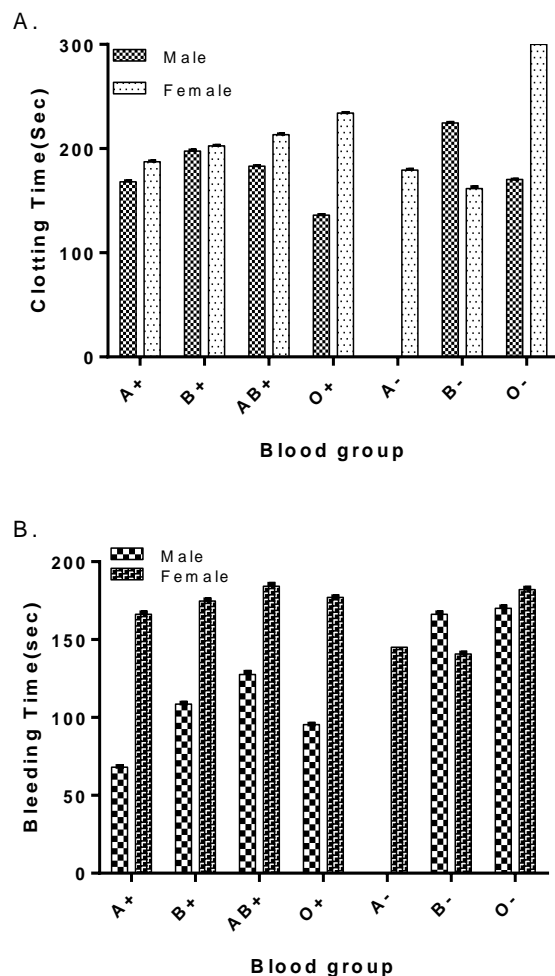
The comparison of CT among different blood groups shows that Females with blood group O⁻ have the highest values of Clotting time, followed by O⁺ group. While the females with B⁻ blood group have the lowest CT values. In male students, CT was higher in individuals having blood group B⁻, followed by B⁺. While the males with O⁺ have lowest CT values.

A similar comparison of BT among different blood groups shows interesting results. Females with blood group AB⁺ have slightly higher BT values, followed by females with blood groups B⁺, O⁺ and

O⁻ have almost same BT values. While the blood group B⁻ females have lowest BT values.

The blood group B⁻ and O⁻ have the almost same highest values of BT in males, while the males with A⁺ blood group have lowest values of BT. Mostly the students with blood group “O” have the highest values of CT and BT which may be because of low expression of vWf (von Willebrand factor) in this blood group.

Figure 2. Comparison of (A) Clotting time and (B) Bleeding time in different blood groups in male and female.



Conclusion:

This study suggested that blood group “B” is the most prevalent in the observed students. A

previous study revealed that people with blood group A, B and AB have more chances to develop arterial and venous thrombotic diseases. As these diseases are the major problem and cause of majority deaths in Pakistan. So, people with all blood groups except O should take preventive measures and regular screening tests. While the O blood group is the second most common one, which is an influencing factor in the development of GIT infections and epistaxis (Blackwell *et al.*, 2002). So, the people with blood group O should take preventive measures to reduce the risk of infection, morbidity and mortality by these diseases.

Furthermore, the study also shows that blood groups also affect the Clotting and bleeding times which may be because of difference in expression of vonWillebrand factor (vWf) in different blood groups. The increased values of CT and BT in females as compared to males are may be because of hormonal differences

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