

## Blood group and its relationship with bleeding time and clotting time

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### Abstract:

**Background:** Blood group plays a vital role in transfusion medicine. Blood group is genetically determined. The grouping of ABO system is based on the antigenic property of the red blood cells. Recent research data shows that ABO blood groups are associated with various diseases for example, gastric cancer, periodontal disease. Some authors have found that epistaxis is commonly seen in people with O blood group when compared with other ABO blood groups and also observed a lower expression of von Willebrand factor gene (vWf) in them.

**Materials and Methods:** This is a cross sectional study conducted among 543 1st year MBBS students in the age group of 17 - 21 years. Blood grouping was carried out using the standard anti-sera, bleeding time and clotting time was estimated by Duke's filter paper method and Capillary tube method respectively. The bleeding time and clotting time of different blood groups were compared and statistical analysis were done.

**Results:** Blood group O (34.80 %) was the most common blood group in both genders followed by group A(29.83%), group B(23%), group AB (12.38%). Bleeding time of > 4 minutes was observed highest in blood group AB (4.5%) followed by group O (3.3%), group A (1.9%) and group B (1.6%). Bleeding time between blood group B and O was statistically significant (p= 0.007). Clotting time was within normal range. No gender difference in bleeding time and clotting time.

**Conclusion:** In our study, blood group O was the most common blood group in both gender followed by blood group A, B and AB. Bleeding time of > 4 minutes was observed highest in blood group AB followed by O, A and B whereas clotting time was normal. No gender-wise difference in bleeding time and clotting time was observed.

**Key Word:** Blood groups, Bleeding time, Clotting time, vWf, Gender.

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### I. Introduction

In transfusion medicine, blood group plays a vital role and is genetically determined. There are many blood groups and ABO group is the one that has great clinical importance. This blood group was discovered by Scientist Karl Landsteiner in the year 1900<sup>[1]</sup>. The grouping of ABO system is based on the antigenic property of red blood cells and the antigens are inherited as Mendelian determinants. Based on this, the individuals are group in to four major blood groups. Type A individuals have antigen A, type B have antigen B and type AB have both the antigens and type O have neither of these antigens<sup>[2,3]</sup>. These antigens are carbohydrates moieties located at the end of carbohydrate chain of glycoprotein on red blood cell surfaces. The genes coding for these antigens are located on chromosome 9 and 19<sup>[4]</sup>. The Rh system is one of the most polymorphic of human blood groups. There are six common types of Rh antigens C, D, E, c, d and e, each of which is called an Rh factor. Type D antigen is widely prevalent in the population and considerably more antigenic than the other antigens. A person who has this antigen are Rh positive whereas a person who does not have type D antigen is Rh negative<sup>[5]</sup>.

The importance of studying blood grouping is that it plays a crucial role in genetics, blood transfusion, forensic pathology<sup>[6]</sup>. Recent research data shows that ABO blood groups are found to be associated with various diseases for example gastric cancer, periodontal diseases and venous thrombosis. Several authors also reported that epistaxis is commonly encountered in people with O blood group when compare with other ABO blood groups and a lower expression of Von Willebrand factor (vWF) in them.

vWf is a large multimeric blood glycoprotein, synthesized by Weibel-Palade bodies in endothelial cells and alpha granules of megakaryocytes. It has a role in platelet adhesion and aggregation. It is also a special carrier protein for clotting factor VIII. So, vWf plays important role in formation of temporary haemostatic plug

and the conversion of it in to definite clot by activation of clotting mechanism. The gene codes for vWf is located on chromosome 12p12. Some research papers have proved that other gene like gene locus of ABO blood group on the chromosome 9q34 exert a major influence on the vWf gene <sup>[4]</sup>. So, the bleeding time and clotting time can be influence by ABO blood group system.

Bleeding time is the time interval between the skin puncture and unassisted stoppage of bleeding, test of bleeding time mainly assess platelet function. Clotting time is the time interval between the entry of blood in to the glass capillary tube up to formation of fibrin threads <sup>[2]</sup>. The blood groups, bleeding time and clotting time relationship has influence in certain clinical conditions like thrombosis, cardiac surgery and epistaxis etc. Bleeding time was found to be longer in blood group O individuals when compared with non O groups individuals. Several studies <sup>[3]</sup> also noticed an association between non O blood groups and thromboembolic disease including ischemic heart disease and peripheral vascular disease.

Although several studies have succeeded in correlating the association between bleeding disorders with ABO blood groups, other workers could not find out any association between bleeding disorder with different ABO blood groups <sup>[4]</sup>. Hence the study was undertaken with the objective to assess the relation between blood groups with Bleeding time (BT) and Clotting time (CT) and to see gender difference if any.

## II. Material And Methods

The cross sectional study was conducted in the Department of Physiology, RIMS, Imphal after getting approval from the Research Ethics Board, RIMS, Imphal. Our study was conducted over a period of five years from 2013 - 2018 among 543 1st year MBBS students in the age group of 17 - 21 years. Students who had any history of bleeding disorders and drug intake (non-steroidal anti-inflammatory drugs) were excluded from the study.

Blood group was determined during practical time in Physiology laboratory by using standard anti-sera. The student did their own blood grouping and calculated their BT and CT with the help of technicians and tutors as a part of their study curriculum.

For blood group determination, blood samples were collected by finger prick with sterile lancet after taking aseptic precautions. The first drop of blood was wiped away with clean piece of cloth and a drop of blood is added to the test tube containing 3ml of isotonic saline (0.9% NaCl). This constituted the red cell suspension. Then, a drop of red cell suspension was mixed with anti-A serum, anti-B serum and anti-D serum. Agglutination was confirmed by observing under low power objective of the compound microscope.

BT was determined by Duke's Filter paper method. A deep skin puncture 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 seconds. BT was calculated by multiplying the number of drops on the filter paper by 1/2 to get BT in minutes. The normal BT by Duke's Filter paper method is usually in the range of 2-6 minutes.

CT was determined by capillary glass tube method. A standard incision was made in the skin of the subjects and blood was taken in the capillary glass tube. The length of time taken for the blood to clot can be calculated by breaking the capillary tube of 1 cm from one end after 1 minute, followed by breaking the capillary tube every 30 seconds. It was then looked for the appearance of fibrin thread. The normal clotting time estimated by this method was in the range of 3-8 minutes at 37°C <sup>[7]</sup>.

The data obtained were analyzed using SPSS version 21, P value of < 0.05 was considered statistically significant.

## III. Result

Among the 543 students in the age group of 17 -21 years, 55.4% were males and 44.6% were females. 33.9% belong to O<sup>+ve</sup> blood group (34.3% were females and 33.6% were males). A<sup>+ve</sup> blood group was 29.7% (30.6 % females & 28.9% males). B<sup>+ve</sup> blood group 22.7% (22.9% males & 22.3% females). AB<sup>+ve</sup> blood group 12.3% (13.3% males & 11.2% females). Blood group O<sup>-ve</sup> 0.9% (1.2% females & 0.7% males). Blood group B<sup>-ve</sup> 0.4% (0.7% males only). Blood group A<sup>-ve</sup> 0.2% (0.4% females only) as shown in table 1.

**Table 1 : Percentage of ABO groups among the subjects**

Blood group	Males	Females	No. of subjects
A <sup>+</sup>	87 (28.9)	74 (30.6)	161 (29.7%)
B <sup>+</sup>	69 (22.9)	54 (22.3)	123 (22.7%)
AB <sup>+</sup>	40 (13.3)	27 (11.2)	67 (12.3%)
O <sup>+</sup>	101 (33.6)	83 (34.3)	184 (33.9%)
A <sup>-</sup>	0	1 (0.4)	1 (0.2%)
B <sup>-</sup>	2 (0.7)	0	2 (0.4%)
AB <sup>-</sup>	0	0	0
O <sup>-</sup>	2 (0.7)	3 (1.2)	5 (0.9%)
<b>Total</b>	301 (55.4)	242 (44.6)	543

**Table 2:** Gender percentage among study group

Gender	No. of subjects
Male	301 (55.4%)
Female	242 (44.6%)
<b>Total</b>	<b>543</b>

**Table 3:** Distribution of bleeding time based on blood groups

	<1 min (%)	1 to 4 min (%)	>4 min (%)	Total
A <sup>+</sup>	0	158 (98.1)	3 (1.9)	161
B <sup>+</sup>	0	121 (98.4)	2 (1.6)	123
AB <sup>+</sup>	0	64 (95.5)	3 (4.5)	67
O <sup>+</sup>	0	178 (96.7)	6 (3.3)	184
A <sup>-</sup>	0	1 (100)	0	1
B <sup>-</sup>	0	2 (100)	0	2
AB <sup>-</sup>	0	0	0	0
O <sup>-</sup>	0	5 (100)	0	5
<b>Total</b>	<b>0</b>	<b>529</b>	<b>14</b>	<b>543</b>

Table 3 shows that majority of the subjects had bleeding time within the normal range, bleeding time of > 4 minutes was observed in 4.5 % of AB<sup>+ve</sup> blood group, 3.3% of O<sup>+ve</sup> group, 1.9% of A<sup>+ve</sup> group and 1.6% of B<sup>+ve</sup> blood groups.

**Table 4:** Distribution of clotting time based on blood groups

	<2 min (%)	2 to 6 min (%)	>6 min (%)	Total
A <sup>+</sup>	0	161 (100)	0	161
B <sup>+</sup>	0	122 (99.2)	1 (0.8)	123
AB <sup>+</sup>	0	67 (100)	0	67
O <sup>+</sup>	0	184 (100)	0	184
A <sup>-</sup>	0	1 (100)	0	1
B <sup>-</sup>	0	2 (100)	0	2
AB <sup>-</sup>	0	0	0	0
O <sup>-</sup>	0	5 (100)	0	5
<b>Total</b>	<b>0</b>	<b>542</b>	<b>1</b>	<b>543</b>

In Table 4, almost all the subjects had normal clotting time except one B<sup>+ve</sup> blood group which show clotting time of > 6 minutes .

**Table 5:** Comparison of BT and CT within different blood groups for significance

	BT (p value)	CT (p value)
A and B	0.285	0.647
A and AB	0.471	0.494
A and O	0.115	0.018
B and AB	0.098	0.796
B and O	0.007*	0.100
AB and O	0.647	0.272

Bleeding time difference of blood group B & O is found to be statistically significant

**Table 6:** Gender wise distribution of bleeding time

	<1 min (%)	1 to 4 min (%)	>4 min (%)	Total
M	0	293(97.3)	8(2.7)	301
F	0	236(97.5)	6(2.5)	242
<b>Total</b>	<b>0</b>	<b>529(97.4)</b>	<b>14(2.6)</b>	<b>543</b>

**Table 7:** Gender wise distribution of clotting time

	<2 min (%)	2 to 6 min (%)	>6 min (%)	Total
M	0	300(99.7)	1(0.3)	301
F	0	242(100)	0	242
<b>Total</b>	<b>0</b>	<b>542(99.8)</b>	<b>1(0.2)</b>	<b>543</b>

#### IV. Discussion

In this study conducted on 543 students, the percentage distribution of blood group showed predominance of blood group O (34.80 %) in both genders followed by group A (29.83%), group B (23%), group AB (12.38%). Bleeding time of > 4 minutes was observed highest in blood group AB (4.5%) followed by

group O (3.3%), group A (1.9%) and group B (1.6%). Bleeding time between blood group B and O was statistically significant ( $p=0.007$ ). Clotting time observed was within normal range. There was no gender difference in bleeding time and clotting time.

In our study, majority of the subject belongs to blood group O. Similar observations were also reported by other researchers<sup>[3,4,8,9,10]</sup>. Our findings is in contrast to the findings of the other studies<sup>[2,6,10,11,12]</sup>, where they observed blood group B the most common blood group followed by O, A and AB.

We observed bleeding time of > 4 minutes in 4.5% of AB blood group, 3.3% of blood group O, 1.9% of blood group A & 1.6% group B. This is in line with another study<sup>[9]</sup>. The difference between bleeding time of blood group B and O was found statistically significant ( $p=0.007$ ) similar to the findings of Poonam G Kohli et al<sup>[3]</sup>. In their study, they found statistically significant difference of bleeding time between B and O ( $p=0.002$ ), B and AB ( $p=0.001$ ), A and B ( $p=0.021$ ), the bleeding time was significantly increased in blood group B in contrast to our finding. Bleeding time of > 4 minutes were also observed in the study conducted by other researchers<sup>[1,2,4,10]</sup>. Several studies have been carried out to correlate between blood groups, bleeding time and clotting time. Study conducted by Manjeet Kaur et al and Poonam G. Kohli et al reported that blood group O individual have a longer bleeding time<sup>[2,3]</sup>. In a study, a longer bleeding time observed in blood group O was found associated with lower expression of vWf. Another study denies any relationship between plasma vWf and bleeding time<sup>[3]</sup>. According to review article written by Franchini et al, when compared to the type of O group, non O blood groups can have increased risk of thrombosis due to higher levels of vWf, ABO blood group can affect the vWf metabolism. This means that bleeding time & clotting time will be elevated among the O group individuals compared to other groups<sup>[2,9]</sup>. Another study also found that 66% of the total variation in plasma vWf level was genetically determined and 30% of this genetic component was explained by ABO blood group<sup>[3]</sup>.

Clotting time observed was within normal range in almost all the subject except one blood group B which showed clotting time of > 6 minutes. No significant difference between clotting time of different blood groups were observed similar to the observation made by other studies<sup>[3,4,13]</sup>. This is in contrast to the findings of Nadera Yasmeen et al<sup>14</sup> and Gowri SR et al<sup>15</sup> where they observed more clotting time in blood group O & AB groups, O & A groups respectively.

We did not observed significant difference in clotting time as well as bleeding time among males and females sexes. This is in line with the findings reported by B. Mahapatra and N. Mishra<sup>[8]</sup>. Similar observations were also reported by Aurobinda Chinara et al<sup>[13]</sup>. But an increased in bleeding time and clotting time in females than male sexes have been noticed by other studies<sup>[2,6,9,12,15,16]</sup>. These differences may be due to hormonal differences that occur in males and females. Females have higher levels of oestrogen and lower level of fibrinogen in blood plasma in comparison to males. The oestrogen decreases the plasma level of fibrinogen that increases the clotting time. It also causes blood vessels dilatation and increases the bleeding time<sup>[14,16]</sup>.

Our study was conducted among 543 students which is more in comparison to other studies conducted by Manjeet Kaur et al<sup>[2]</sup>, Poonam G Kohli et al<sup>[3]</sup>, and Thenmozhi S et al<sup>[4]</sup>. As the tests were done by the students themselves as a part of their study curriculum, there is possibility of technical errors during the tests, which can be considered as one of the limitations of the study.

## V. Conclusion

Blood group O was the most common blood group & AB is the least common blood group. Bleeding time of > 4 minutes was observed highest in blood group AB followed by O, A & B. There was statistically significant relation between bleeding time of blood group B & O. Clotting time of different blood groups were found within normal limits. No difference was found among males and females sexes with regards to bleeding time and clotting time. Further research with conduct of larger multicentric study is necessary to confirm the association of different ABO blood groups with different bleeding tendencies, so as enable us to find out the risk groups and take precautions early.

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