ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 15, Issue 7, 2022

Online - 2455-3891
Print - 0974-2441

Research Article

AN OBSERVATIONAL STUDY: ASSOCIATION OF BLOOD GROUPS WITH BLEEDING TIME AND CLOTTING TIME

SWATI GAVIT, SEEMA BHORANIA*

Department of Physiology, Topiwala National Medical College, Mumbai, India. Email: seemabhorania@gmail.com

Received: 28 January 2022, Revised and Accepted: 28 April 2022

ABSTRACT

Objectives: The objective of the present study was to assess the relationship between bleeding time (BT) and clotting time (CT) among various blood groups and also to identify gender differences if any.

Methods: The study was conducted in a tertiary care center. The study included 108 MBBS students, 68 were males and 40 were females. BT was obtained by the Duke method and CT was estimated by Wright's capillary glass tube method. Standard antisera were used to know the blood groups. Blood group and its relation to BT, CT, and gender were analyzed by Chi-square analysis.

Results: Blood Group B (33.33%) was predominant followed by O (32.4%), A (21.29%), and AB (12.96%). CT was found to be more than 6 min in Group O followed by B, A, and AB, and BT was found to be more than 4 min in Group O followed by A, B, and AB. Both were statistically not significant ($p>0.05$). CT more than 6 min was greater in males (52.17%) as compared to females (47.82%), but the variation was insignificant ($p=0.227$). BT was more than 4 min in 68.96% of females as compared to 31.03% of males. The variation was statistically significant ($p=0.0085$*).

Conclusion: In our study, blood Group B was more common followed by O, A, and AB. CT and BT were prolonged in the O group. BT was more in females than males, whereas CT was more in males than males.

Keywords: Blood group, Bleeding time, Clotting time, von Willebrand factor.

 DOI: http://dx.doi.org/10.22159/ajpcr.2022v15i7.44266. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Before the 1900s, it was thought that all blood whether human or animal was the same, a misunderstanding that led to frequently fatal and hazardous transfusions of blood. Even humans' blood is not the same as people belonging to different blood groups, depending on the surface markers found on the red blood cell. The cells that make up the body's tissues and organs are covered with surface markers or antigens. Red blood cells are no different. Blood group antigens are sugars or proteins, and they tend to get attached to various components in the red blood cell membrane. The antigens of the ABO blood group are sugars, while the antigens of the Rh blood group are proteins [1].

The membrane of each red blood cell contains millions of antigens that are considered self-antigens by the immune system. However, during blood transfusions, recipients' immune systems will be activated and attack antigens other than self-antigens. Therefore, matching these surface antigens of blood cells is very essential for safe blood transfusion. Hence the determination of blood groups forms a very important and essential hematological evaluation for transfusions.

The study of blood grouping is very important not only for blood transfusion and organ transplants but also because it plays an important role in genetics, research, forensics, and associations of various diseases. It may have some association with diseases such as duodenal ulcer [2], diabetes Mellitus [3], urinary tract infection [4], and carcinoma cervix [5]. An association has been found between the distribution of various types of fingerprints and blood groups. The correlation is consistent for blood Group "A" with loops, while arches are more common in blood Group "AB" [6].

Von Willebrand factor (VWF) is an adhesive glycoprotein that was first discovered in 1924by Erik von Willebrand who reported a family who had a history of serious hereditary bleeding and consanguineous marriage [7]. It is synthesized by endothelial cells, platelets, and bone marrow. The blood group antigens are covalently bound to asparagine-linked sugar chains of von Willebrand factor (vWF) [8]. The factor is stored in intracellular granules of megakaryocytes and usually co-exists with the coagulation factor VIII. Functionally, vWF helps in the adhesion and aggregation of platelets, thus playing a role in hemostasis [9]. Deficiency of vWF leads to hemorrhagic disorders, while elevated levels are a risk factor for thrombosis [10-12]. According to Mourner *et al*. and Qureshi *et al*., there is a clear association between ABO blood group status and the Von Willebrand factor [13,14]. The absence of ABO antigens hastens the clearance of Vfw from plasma forming another reason for lower levels of plasma vWF in O blood group individuals [15]. Hence, the ABO blood group system influences the bleeding time (BT) and clotting time (CT). BT is the time elapsed between the prick and stoppage of blood flow. CT is the time interval between the puncture of blood vessels and the formation of fibrin threads [16]. Thus, the relationship between BT, CT, and blood groups is very important in clinical practice and hence the current study was carried out. The objective of this study was to assess the relationship between BT and CT among various blood groups and also to identify any gender differences among the same.

METHODS

This retrospective study was carried out in a tertiary care center in a Metropolitan city. As a part of the curriculum, all 1st-year MBBS students have to perform bleeding time, clotting time, and blood group practical's in hematology. All these practical's are performed in the hematology laboratory of the Physiology department. A total of 108, 1st-year MBBS students were included in this study. Ethical clearance was obtained from the Institutional Ethical committee. The available reports of 108 students were analyzed concerning age, gender, blood group, bleeding time, and clotting time. All students were in the age group of 17–20 years with 68 males and 40 females respectively. The blood group, bleeding time, and clotting time were determined by the following procedures.

Blood group determination

Blood samples were collected by finger prick under aseptic conditions and the red cell suspension was prepared by mixing blood with citrate and normal saline. The red cell suspension was mixed with antisera anti-A, anti-B, and anti-D in Reins slide and covered with a Petri dish for 8–10 min. Blood groups were determined based on the presence or the absence of agglutination. Confirmation of agglutination was done by observing under the low-power objective of a compound microscope [16].

Bleeding time and clotting time

BT was determined by Duke's filter paper method. Under aseptic conditions, a Finger prick was taken using a lancet. A stopwatch was started and the time required for bleeding to stop was recorded. The drop of blood coming out of the incision was blotted every 30 s using blotting paper. This was continued till no further drop of blood was seen on the paper. BT was calculated by multiplying the number of spots on the filter paper by 30 and was recorded in seconds. The normal BT by Duke's method is usually in the range of 1–5 min [16].

Wright's capillary glass tube method was used to estimate CT. Under the aseptic conditions, a finger prick was made and the blood was taken into a capillary glass tube. The stopwatch was started and the tube was first broken after 1 min of the prick. Thereafter the capillary tube was broken approximately 1 cm in length from one end every 30 s till the appearance of fibrin thread. Normal clotting time was 3–6 min [16].

Statistical analysis

Statistical analysis was done with SPSS software, version 16. The Chi-square analysis was applied to examine the relationship between blood groups and BT, CT; $p<0.05$ was considered to be significant.

RESULTS

In the present study, data collected from 108 students were analyzed. All the participants were in the age group of 17–20 years. Out of 108 students, 68 were males and 40 were females (Table 1). Our results stated that blood Group B was predominant, followed by blood Groups O, A, and AB. The percentage distribution (Table 1) of blood groups was in order of B (33.33%) > O (32.40 %) > A (21.29) > AB (12.96%).

The distribution of bleeding time and clotting time according to gender and blood groups are given in Tables 2 and 3, respectively. Bleeding time of more than 4 min. was predominant in blood Group O followed by A, B, and AB. The distribution of bleeding time more than 4 min. was found more in the O blood group for both males and females. There was no statistically significant difference between blood group groups and bleeding time (p=0.133). The clotting time of more than 6 min was predominant in blood group O and <6 min was predominant in blood Group B. However, there is no statistically significant association between blood group and clotting time (p=0.714).

Table 4 shows the gender-wise distribution of bleeding time and clotting time. Bleeding time of more than 4 min was found greater in females than the male with a percentage of 61.9%. We found that the association between bleeding time and gender was statistically significant with a p=0.0085. The clotting time of more than 6 min was found greater in males than females with a percentage of 52.17% but which was statistically not significant.

DISCUSSION

In the present study, 108 1st year MBBS students in the age group of 17–20 years were selected. Out of 108, 68 were males and 40 were females, respectively. It was found that the B blood group was predominant in our study followed by O, A, and AB. Our study is by many studies done in the past [17-19]. Our study showed contradictory results to the Asiatic trend as different researchers reported that the prevalence of ABO blood groups was O>B>A>AB in them [20-23].

In hemostasis, VWF is very essential. It is needed for platelet adhesion to the subendothelial layer as well as platelet aggregation in the blood vessels. It also acts as a specific carrier of factor VIII (FVIII) in plasma and protects it from proteolytic degradation and prolongs its half-life. The nature of the interaction between ABO blood group antigens and plasma VWF levels along with its clinical implication has been extensively studied. Studies have revealed that the ABO gene locus accounts for approximately 30% of the genetic determinant of vWF levels, which implies that the ABO blood group does influence plasma vWF levels. Hence, the absence of A and B antigens can indirectly lower the plasma levels of vWF forming one reason why bleeding tendencies are predominantly seen in the O blood group [24]. In a large twin study, Orstavik and colleagues [25] found that 66% of the total variation in plasma VWF levels was genetically determined, some of which was explained by the ABO blood group. Franchini *et al.* stated that there is an increased risk of thrombosis in non-O blood groups as compared to the O group due to the higher levels of vWF [26]. They also stated that the ABO group can affect the vWF catabolism, meaning that the plasma vWF levels may depend upon the blood group of the individual. This concept was accepted by other studies done by Jenkin's *et al.* [27], who stated that vWF is 25% more in non-O group individuals compared to group O individuals. Another study conducted by Gill *et al*. [28] showed that Group O individuals had the lowest plasma vWF levels and non-O groups (A, B, and AB) had elevated levels of plasma vWF, which would reflect as increased clotting time and bleeding time among the individuals with blood group O.

Table 1: Gender-wise distribution of Blood group

Blood group	Female	Percentage	Male	Percentage	Total	Percentage
A	12	30	11	16.17	23	21.29
B	11	27.50	25	36.76	36	33.33
AB	5	12.50	9	13.23	14	12.96
O	12	30	23	33.82	35	32.40
Total	40	100	68	100	108	100

Table 2: Distribution of Bleeding time according to blood group and gender

Blood group	Male		Female		Total	
	BT < 4 Min.	BT>4 Min.	BT < 4 Min.	BT>4 Min.	BT < 4 Min.	BT>4 Min.
A	9	2	8	4	17	6
B	24	1	9	2	33	3
AB	8	1	4	1	12	2
O	19	4	6	6	25	10
Total	60	8	27	13	87	21
p-value	0.458		0.853		0.133	

Table 3: Distribution of Clotting Time according to blood group and gender

Blood group	Male		Female		Total	
	CT < 6 Min.	CT > 6 Min.	CT < 6 Min.	CT > 6 Min.	CT < 6 Min.	CT > 6 Min.
A	10	1	10	2	20	3
B	21	4	7	4	28	8
AB	7	2	4	1	11	3
O	18	5	8	4	26	9
Total	56	12	29	11	85	23
p-value	0.802		0.685		0.714	

Table 4: Gender-wise distribution of clotting time and bleeding time

Variables	Clotting time		Bleeding time	
Time (minutes)	< 6	>6	< 4	>4
Male	56	12	60	8
Female	29	11	27	13
p-value	0.227		0.0085*	

In our study prolonged clotting time and bleeding time were associated with blood Group O as compared to non-O blood groups but were found to be statistically not significant. Results similar to ours were observed by Benjamin and Geetha [29] Gavali *et al*. [30] and Patil *et al.* [14] who did not find any significant difference in bleeding time between O and non-O blood groups.

Contrary to our findings studies by Mahapatra and Mishra [31] showed that bleeding time was increased in the AB group and clotting time was increased in the B group and was statistically significant. Another study done by Sasekala and Saikumar [23] showed CT was prolonged in blood group AB and BT was prolonged in group B which were statistically significant.

A study done by Reeta *et al*. [32] and Mirdha and Jena [20] showed that both bleeding times of more than 4 min and clotting times of more than 6 min were found predominantly in O blood group individuals with a statistically significant p-value.

In the case of gender-wise distribution of bleeding time, we observed higher bleeding time in females compared to males. We found the association between gender and bleeding time as statistically significant (Table 4). Results similar to ours were found in various studies [19,23,33], while no such difference was reported in a study by Mahapatra and Mishra [31]. Contrary to our finding study done by Benjamin and Geetha [29] found bleeding time to be prolonged in males as compared to females. Female individuals having comparatively more bleeding time and clotting time may be due to the presence of the hormone estrogen, which lowers the plasma level of fibrinogen and increases the clotting time [34]. On the other hand, the presence of androgens hastens the activation and aggregation of platelets and thus shortens the bleeding time [35]. In our study, the association between gender and clotting time was not significant.

CONCLUSION

In our study, blood Group B was more common followed by O, A, and AB. CT and BT were prolonged in the O group. BT was more in females than males, whereas CT was more in males than females. We will be extending our research further in the next phase with a greater number of participants, levels of plasma vWF, and hormonal assays.

AUTHORS CONTRIBUTION

Both authors have contributed equally to the preparation of the manuscript.

CONFLICT OF INTEREST

Nil.

SOURCE OF FUNDING

The study was not supported by any grants or funds.

REFERENCES

1. Dean L. Blood group antigens are surface markers on the red blood cell membrane. In: Blood Groups and Red Cell Antigens. Ch. 2. Bethesda: National Center for Biotechnology Information. US; 2005. Available from: https://www.ncbi.nlm.nih.gov/books/NBK2264
2. Qureshi Ma BR. Frequency of ABO blood groups among the Diabetes Mellitus type-2 patients. J Coll Phys Surg Pak 2003;13:453-5.
3. Ziegler T, Jacobsohn N, Fünfstück R. Correlation between blood group phenotype and Virulence properties of Escherichia coli in patients with chronic UTI. Int J Antimicrob Agents 2004;24:70-5.
4. Kaur I, Singh IP, Bhasin MK. Blood groups in relation to carcinoma of cervix uteri. Hum Hered 1992;42:324-6. doi: 10.1159/000154091
5. Bharadwaja A, Saraswat PK, Aggarwal SK, Banerji P, Bharadwaja S. Pattern of finger prints in different ABO blood groups. J Indian Acad Forensic Med 2004;26:971-3.
6. von Willebrand EA. Hereditarpseudohemofili. Fin Laekaresaellsk Hand 1926;68:87-112.
7. Yamamoto F, Cid E, Yamamoto M, Blancher A. ABO research in modern era of genomics. Transfus Med Rev 2012;26:103-18. doi: 10.1016/j.tmrv.2011.08.002, PMID 21945157
8. Matsui T, Titani K, Mizuochi T. Structures of the aspargine linked oligosaccharide chains of human von Willebrand factor. Occurrence of blood group A, B and H(O) structures. J Biol Chem 1992;267:8723-31. doi: 10.1016/S0021-9258(19)50338-6, PMID 1577715
9. Ruggeri ZM, Zimmerman TS. The complex multimeric composition of factor VIII/Vwf. Blood 1981;57:1140-3. PMID 6784794
10. Sadler JE. Biochemistry and genetics of Von Willebrand factor. Annu Rev Biochem 1998;67:395-424. doi: 10.1146/annurev.biochem.67.1.395, PMID 9759493
11. Ruggeri ZM. Structure of von Willebrand factor and its function in platelet adhesion and thrombus formation. Best Pract Res Clin Haematol 2001;14:257-79. doi: 10.1053/beha.2001.0133, PMID 11686099
12. Mourant AE. Blood Relations: Blood Groups and Anthropology. Oxford: Oxford University Press; 1983. p. 1-146.
13. Favaloro EJ, Soltani S, McDonald J, Grezchnik E, Easton L, Favaloro JW. Reassessment of ABO blood group, sex, and age on laboratory parameters used to diagnose von Willebrand disorder: Potential influence on the diagnosis vs the potential association with risk of thrombosis. Am J Clin Pathol 2005;124:910-7. doi: 10.1309/W76QF806CE80CL2T, PMID 16416741
14. Ganong WF. Review of Medical Physiology. 22nd ed. New York: McGraw-Hill Education; 2005. p. 537.
15. Ghai CL. A Textbook of Practical Physiology. 5th ed. New Delhi: Jaypee Brothers; 1999. p. 84-101.
16. Talib HV. Handbook of Medical Laboratory Technology. 2nd ed. New Delhi: CSB Publishers; 1991. p. 205-10.
17. Patil SV, Gaikwad PB, Vaidya SR, Patil US, Kittad SD. To study the blood group distribution and its relationship with bleeding and clotting time in dental students. Asian J Pharm Sci 2013;1:1-4.
18. Banerjee RB, Sathian B, Mondal M, Saha CG. Blood group distribution and its relationship with bleeding time and clotting time: A medical school based observational study among Nepali. Indian and Sri Lankan students. Nepal J Epidemiol 2011;1:135-40.
19. Mirdha M, Jena SK. Distribution of blood group and its relation

to bleeding time and clotting time. Int J Med Sci Public Health 2016;5:2566-9. doi: 10.5455/ijmsph.2016.13052016526
20. Thenmozhi S, Neelambikai N, Aruna P. Comparison of bleeding time and clotting time in different ABO blood groups. Natl J Physiol 2013;1:19-24.
21. Kohli PG, Kaur H, Maini S. Relationship of bleeding time and clotting time with blood groups. Res J Pharm Biol Chem Sci 2014;5:1780-3.
22. Sasekala S, Saikumar P. Relationship between bleeding time and clotting time among gender difference and varying blood groups in UG medical students. IOSR JDMS 2013;10:40-3. doi: 10.9790/0853-1064043
23. Gallinaro L, Cattini MG, Sztukowska M, Padrini R, Sartorello F, Pontara E, *et al*. A shorter von Willebrand factor survival in O blood group subjects explains how ABO determinants influence plasma von Willebrand factor. Blood 2008;111:3540-5. doi: 10.1182/blood-2007-11-122945, PMID 18245665
24. Orstavik KH, Magnus P, Reisner H, Berg K, Graham JB, Nance W. Factor VIII and factor IX in a twin population: Evidence for a major effect of ABO locus on factor VIII level. Am J Hum Genet 1985;37:89-101. PMID 3919575
25. Franchini M, Capra F, Targher G. Martina Montagnana4 and Giuseppe Lippi4Relationship between ABO blood group and von Willebrand factor levels: From biology to clinical implications. Thromb J 2007;5:14.
26. Jenkins PV, O'Donnell JS. ABO blood group determines plasma von Willebrand factor levels: A biologic function after all? Transfusion 2006;46:1836-44. doi: 10.1111/j.1537-2995.2006.00975.x, PMID 17002642
27. Gill JC, Endres-Brooks J, Bauer PJ, Marks WJ Jr., Montgomery RR. The effect of ABO blood group on the diagnosis of von Willebrand disease. Blood 1987;69:1691-5. PMID 3495304
28. Benjamin JJ, Geetha MB. Study of association of bleeding and clotting time with blood group among young adults. Indian J Clin Anat Physiol 2020;7:350-3.
29. Monika G, Sameer S, Yogesh G, Preeya M, Krishnakant P. Comparison of BT (bleeding time)/CT (clotting time) with respect to blood group in medical students. Int J Health Sci Res 2017;7:75-8.
30. Mahapatra B, Mishra N. Comparison of bleeding time and clotting time in different blood groups. Am J Infect Dis 2009;5:113-5. doi: 10.3844/ajidsp.2009.113.115
31. Baishya R, Sarkar R, Barman B. Blood group and its relationship with bleeding time and clotting time- an observational study among the 1st MBBS students of Gauhati medical college, Guwahati. Int J Res Med Sci 2017;5:4147. doi: 10.18203/2320-6012.ijrms20174000
32. Kumar SS, VK. JM, George J, Mukkadan JK. Bleeding time and clotting time in healthy male and female college students of Karukutty village, Kerala. Health Prospect 2013;12:7-9.
33. Ercan M, Yegin E, Akdeniz H, Irmak H, Bayiroglu F, Tuncer I. Effect of estrogen on fibrinogen clotting time in rabbits. Trans J Vet Anim Sci 1998;22:137-40.
34. Ferenchick G, Schwartz D, Ball M, Schwartz K. Androgenic-anabolic steroid abuse and platelet aggregation: A pilot study in weight lifters. Am J Med Sci 1992;303:78-82. doi: 10.1097/00000441-199202000-00002. PMID 1539613