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

Roy B[1], Banerjee I[2], Sathian B[3], Mondal M[4], Saha CG[5]

[1] Assistant Professor, Department of Physiology, Manipal College of Medical Sciences, Pokhara, Nepal

[2] Lecturer, Department of Pharmacology, Manipal College of Medical Sciences, Pokhara, Nepal

[3] Assistant Professor, Department of Community Medicine, Manipal College of Medical Sciences, Pokhara, Nepal

[4] Lecturer, Department of Physiology, Manipal College of Medical Sciences, Pokhara, Nepal

[5] Professor & HOD, Department of Physiology, Manipal College of Medical Sciences, Pokhara, Nepal

Original Article

Corresponding Author:
Mr. Bedanta Roy
Assistant Professor
Department of Physiology
Manipal College of Medical Sciences
Pokhara, Nepal
Email: bedanta.roy@gmail.com

Abstract

Background

Blood group, Bleeding Time and Clotting time are clinically useful tests, extensively used during blood transfusion, platelet disorders and a variety of forms of treatment in hospitals. The objective of our study was to assess the country wise distribution of blood groups and to compare the country wise bleeding time and clotting time with respect to blood group.

Material and Methods

This Observational Study has been performed at Manipal College of Medical Sciences, during the period of 1st May 2010 to 31st August–2011. The study was carried out on the undergraduate medical students of Basic sciences and included assessment of Bleeding time, Clotting time and Blood grouping by standard procedures.

Results

261 medical students participated in the study. Among the Nepalese students, 30.5% had blood group A, 28.9% B, 5.5% AB and remaining 35.2% O. among the Indian students, 22.8% were blood group A, 45.7% B, 7.6% AB and 23.9% O. In Sri Lankan students, 22% had blood group A, 19.5% B, 4.9% AB, and 53.7% blood group O. Among Nepalese students, BT was higher in blood group A (163.85 seconds) as compared to Indians (154.29Sec) and Sri Lankans (133.33 sec). Nepalese students with AB blood group had higher BT (171.43 sec). Indian students with blood group A had comparatively higher clotting time (328.57 sec) followed by Nepali (276.15 sec) and then Sri Lankan students (270 sec). Girls are 4.432 times more prone for bleeding time greater than 4 minutes and 2.453 times for clotting time value above 6 minutes as compared to males.

Conclusion

Our study suggests that O blood group is predominant in Nepalese students while Blood group B was the most common blood group in the Indian students. We found higher BT and CT in females, which are more prominent in Indians, so this gender disparity is an additional risk factor for them. Health Ministries of respective countries have to improve the proper health care policies required for prevention and management of blood group diseases, bleeding time and clotting time related disorders like

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Hypoprothrombinemia and Thrombocytopenia.

Keywords: Blood Group, Bleeding Time, Clotting Time

Background

Hemostasis is stoppage of bleeding from damaged blood vessels which involves only a little number of endothelial cells from capillaries, small venules, or arterioles, then platelet plug is formed and ultimately bleeding stops[1-4]. Evaluation of hemostasis is an essential factor for surgeons and anesthetics before undertaking any surgical procedure. Hence it is a routine preoperative test for hospitals.

The bleeding time test is mainly a test for the assessment of platelet function. It is usually significantly prolonged in congenital or acquired platelet defects.

Scientist Karl Landsteiner first described the ABO blood group in 1900 and it served the beginning of blood banking and transfusion medicine[6]. Blood grouping is based on antigenic property of red blood cells (RBC). The gene for ABO group is present on chromosome 9 while for Rh system it is on chromosome 1. The Rh system is one of the most polymorphic of the human blood groups. According to the presence or absence of Rh antigens blood is classified into Rh positive or negative.

The study of blood grouping is very important as it plays a crucial role in genetics, blood transfusion, forensic pathology and may have some association with diseases like duodenal ulcer, diabetes mellitus, urinary tract infection, feto-maternal incompatibility leading to hemolytic diseases of newborn[7-9].

There is a clear association between ABO blood group status and levels of vWF and FVIII: C. Blood group O is related with lower expression of Von Willebrand factor which leads to relative bleeding tendency[10-12]. It has been reported that Caucasian population with Blood group O have higher admission rates in hospital for epistaxis compared with the general population[13].

The objective of our study was to assess the country wise distribution of blood groups and to compare the country wise bleeding time and clotting time with respect to blood group.

Material and Methods:

Study design and the participants

This retrospective study was carried out in the Hematology Laboratory of department of Physiology in Manipal College of Medical Sciences, Pokhara Nepal. All the experiments were done on the first year and second year medical students of Basic sciences. Determination of blood group was done by mixing the sample of blood with antisera A and B and appearance for clumping of RBCs under the low power objective of the microscope. Bleeding time estimation was by Duke Method and clotting time was estimated by capillary tube method[14].

Data collection

The present study was undertaken during the period of 1st May 2010 to 31st August–2011. Two investigators of our study performed the experiment and collected data from the students using pre designed questionnaire with the information regarding age, gender, country, blood group, bleeding time and clotting time.

Sample size calculation

From the pilot study of 20 students each for different countries, we found that the percentage of students bleeding time ≤4 minutes were more than 95% for each. So, 99% confidence interval and, significance level α = 1%, P (percentage of students bleeding time ≤4min) = 95%, Q = 5%, allowable error = 10%, required sample size was 35 for each country[15].

Outcome Variable

Outcome variables were Bleeding Time, Clotting Time and Blood group.

Explanatory variables

The demographic factors were defined at individual level. Factors at individual level were age, gender and nationality.

Ethical committee approval

Prior to the study, ethical committee approval was taken from college authorities.

Data management and statistical analysis

Descriptive statistics and testing of hypothesis were used for the analysis. The data collected was analyzed using Excel 2003, Statistical Package for the Social Sciences (SPSS) for Windows Version 16.0 (SPSS Inc; Chicago, IL, USA) and EPI Info 3.5.1 Windows Version. The Chi-square test was used to examine the association between different variables. The associations between the different variables were tested using the Chi-square test and strength of the relationship with logistic regression. We calculated odds ratios (OR) and their 95% confidence intervals (95% CI). $P < 0.05$ is considered as statistically significant.

Inclusion criteria

All the students of Basic Sciences are included in the study.

Exclusion criteria

Students having bleeding time and clotting time related disorders were not allowed to participate in the study to avoid bias.

Results

Response rate and Demographic Characteristics

261 students data out of 338 retrieved from the records maintained in the Hematology Laboratory, giving an overall response rate of 77.22%. Students that had any one of the study variables missing were excluded from the study. The mean age of Indian (92, 35.3%), Nepalese (128, 49.0%) and Sri Lankan (41, 15.7%) were 18.3 ± SD 0.7, 18.6 ± SD 0.8 and 18.0 ± SD 1.5 years respectively. Distribution of students according to religion varied across the countries. Majority of the students were Hindus in India (61.4%) and Nepal (89.6%), while in Sri Lanka, the majority were Buddhist (53.8%).

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Table 1: Country wise distribution of blood group

Country	A	B	AB	O	Total
Nepal	39 (30.5) [22.6, 39.2]	37 (28.9) [21.2,37.6]	7 (5.5) [2.2, 10.9]	45 (35.2) [26.9, 44.1]	128 (100)
India	21 (22.8) [10.6, 37.6]	42 (45.7) [8.8, 34.9]	7 (7.6) [0.6, 16.5]	22 (23.9) [37.4, 69.3]	92 (100)
Sri Lanka	9 (22) [10.6, 37.6]	8 (19.5) [8.8, 34.9]	2 (4.9) [0.6, 16.5]	22 (53.7) [37.4, 69.3]	41 (100)
Total	69 (26.4)	87 (33.3)	16 (6.1)	89 (34.1)	261(100)

Table 1 reveals that the Nepalese students had 30.5% [CI 22.6, 39.2] blood group A, 28.9% [CI 21.2, 37.6] blood group B, 5.5% [CI 2.2, 10.9] blood group AB, 35.2% [CI 26.9, 44.1] blood group O. Indian students had 22.8% [CI 10.6, 37.6] blood group A, 45.7% [CI 8.8, 34.9] blood group B, 7.6% [CI 0.6, 16.5] blood group AB, 23.9% [CI 37.4, 69.3] blood group O. Among the Sri Lankan students, 22% [CI 10.6, 37.6] had blood group A, 19.5% [CI 8.8, 34.9] blood group B, 4.9% [CI 0.6, 16.5] blood group AB, and 53.7% [CI 37.4, 69.3] blood group O.

Table 2: Distribution of blood group, Bleeding Time (<4min and >4min) and clotting Time (<6min and > 6mins) in Nepal

Variable	Blood group	Nepal			
		<4min (%)	>4min (%)	Total	P value
Bleeding Time	A	36 (92.3)	3(7.7)	39	0.0001†
	B	35 (94.6)	2(5.4)	37	0.0001†
	AB	7(100)	0(0)	7	-
	O	42(93.3)	3 (6.7)	45	0.0001†
	Total	120 (93.8)	8 (6.3)	128	0.0001†
Clotting Time		<6min (%)	>6min (%)		P value
	A	33 (84.6)	6(15.4)	39	0.0001†
	B	32 (86.5)	5(13.5)	37	0.0001†
	AB	6 (85.7)	1(14.3)	7	0.0001†
	O	41 (91.1)	4(8.9)	45	0.0001†
	Total	112 (87.5)	16(12.5)	128	0.0001†

† p<0.01, statistically significant
- p value cannot calculate

Table 3: Distribution of blood group and Bleeding Time (<4min and >4min) and clotting Time (<6min and > 6mins) in India

Variable	Blood group	India			
		<4min (%)	>4min (%)	Total	P value
Bleeding Time	A	21 (100)	0	21	-
	B	40 (95.2)	2 (4.8)	42	0.0001†
	AB	7 (100)	0 (0)	7	-
	O	21 (95.5)	1 (4.5)	22	0.0001†
	Total	89 (96.7)	3 (3.3)	92	0.0001†
		<6min (%)	>6min (%)		P value
Clotting Time	A	16 (76.2)	5 (23.8)	21	0.0001†
	B	30 (71.4)	12 (28.6)	42	0.0001†
	AB	4 (57.1)	3 (42.9)	7	0.589×
	O	18 (81.8)	4 (18.2)	22	0.0001†
	Total	68 (73.9)	24 (26.1)	92	0.0001†

† p<0.01, statistically significant
- p value cannot calculate

Table 2, 3 and 4 depicts the variations in Bleeding time and clotting time among different students from different countries. Among the Nepalese students having A blood group, 92.3% had bleeding time less than 4 minutes, but only 7.7% showed greater than 4 minutes. Similarly in B blood group 94.6% showed BT less than 4 minutes, remaining 5.4% had more than 4 minutes. Among the AB group, all had BT below 4 minutes, but among the O blood group 93.3% was below 4 minutes, and only 6.7% showed more than 4 minutes in BT. Similarly for Indian students having blood group A, all had BT value less than 4 minutes. In blood group B, majority (95.2%) showed BT less than 4 minutes, and remaining 4.8% was greater than 4 minutes. All students with blood group AB had a BT of less than 4 minutes, and for O blood group, 95.5% had BT less than 4 minutes and only 4.5% greater than 4 minutes. Sri Lankan students having blood groups A, B, AB had BT less than 4 minutes, Only in O blood group, 95.5% showed below 4 minutes and the remaining 4.5% was greater than 4 minutes.

84.6% of the Nepalese students had CT below 6 minutes, only 15.4% had more than 6 minutes. B blood group showed almost same, where 86.5% was below 6 minutes, and 13.5% was above 6 minutes. For AB Blood group, 85.7% was less than 6 minutes and remaining 14.3% was above 6 minutes. In case of blood group O, majority (91.1%) was below 6 minutes, only 8.9% was above 6 minutes. In Indian students 76.2% of blood group A people was less than 6 minutes and remaining 23.8% was greater than 6 minutes. It was quite similar with blood group B, where 71.4% was less than 6 minutes CT and 28.6% and showed more than 6 minutes. In case of CT in blood group AB, the percentages below 6 minutes and above 6 minutes were 57.1 and 42.9 respectively. In blood group O students, 81.8% showed below 6 minutes and 18.2% were more than 6 minutes. Sri Lankan students with blood group A 100% and blood group B 50% had CT less than 6 minutes. Majority of blood group O students 81.8% had less than 6mins, only 18.2% had CT >6 minutes.

Table 4: Distribution of blood group and Bleeding Time (<4min and >4min) and clotting Time (<6min and > 6mins) in Sri Lanka

Variable	Blood group	Sri Lanka			
		<4min (%)	>4min (%)	Total	P value
Bleeding Time	A	9 (100)	0 (0)	9	-
	B	8 (100)	0 (0)	8	-
	AB	2 (100)	0 (0)	2	-
	O	21 (95.5)	1 (4.5)	22	0.0001†
	Total	40 (97.6)	1 (2.4)	41	0.0001†
		<6min (%)	>6min (%)		P value
Clotting Time	A	9 (100)	0 (0)	9	-
	B	4 (50.0)	4 (50)	8	1×
	AB	1 (50.0)	1 (50.0)	2	1×
	O	18 (81.8)	4 (18.2)	22	0.0001†
	Total	32 (78.0)	9 (22.0)	41	0.0001†

† p<0.01, statistically significant
× p>0.05, statistically not significant
- p value cannot be calculated

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Table 5: Bleeding Time and clotting time distribution in (mean±SD) different countries according to the blood group

Blood group		Mean ±SD Country			P value
		Nepal	India	Sri Lanka	
Bleeding Time	A	163.85±52.80	154.29±37.09	133.33±42.72	0.217×
	B	162.97±55.47	166.43±50.11	142.5±61.59	0.513×
	AB	171.43±37.61	158.57±48.11	150±0.00	0.758×
	O	172.66±60.39	158.18±39.48	163.64±63.96	0.594×
	Total	167.11±55.29	161.08±44.41	152.20±57.86	0.265×
Clotting Time	A	276.15±93.15	328.57±110.01	270±77.94	0.114×
	B	276.49±102.99	324.29±126.46	348.75±162.69	0.130×
	AB	304.29±97.10	312.86±113.39	345±106.06	0.892×
	O	273.33±76.01	287.72±83.66	301.36±101.62	0.436×
	Total	276.80±89.98	315.65±112.20	305.85±110.88	0.016*

* p<0.05, statistically significant
× p>0.05, statistically not significant

Table 5 shows that Nepalese students with blood group A had a higher bleeding time (163.85 sec) when compared to Indian (154.29 sec) and Sri Lankan (133.33 sec). Indian Students of blood group B had a slightly higher value for bleeding time 166.43 sec, when compared to Nepalese and Sri Lankan students (162.97sec and 142.5 sec respectively). For AB blood group, Nepalese students had higher values 171.42 sec, whereas Indian and Sri Lankan students had lower values (158.57 sec and 150 sec respectively). O Blood group students followed a similar pattern to AB group, except in Sri Lankan students, which came to around 163.64 sec. Indian students having Blood group A had higher clotting time (328.59 sec) followed by Nepali (276.15 sec) and Sri Lankan students (270 sec). Blood group B students had clotting time almost equal to the A blood group, but Sri Lankan students had the highest 348.75 sec. For blood group AB, Sri Lankan students showed a similar pattern, 345 sec, which was more than Indian (312.86) and Nepali students (304.29 sec). Students from all countries with Blood group O showed relatively less clotting time values.

Table - 6: Gender wise Distribution of Bleeding Time

Bleeding Time			
Nepal	<4mins	>4mins	Total
Male	62	2	64
Female	58	6	64
Total	120	8	128
P value	0.137×		
India			
Male	43	0	43
Female	46	3	49
Total	89	3	92
P value	0.147×		
Sri Lanka			
Male	12	0	12
Female	28	1	29
Total	40	1	41
P value	0.707×		

× p>0.05, statistically not significant

Table 6 explains that among the Nepalese males (64), only 2 showed bleeding time values of more than 4 minutes, but in case of females it was more. Among 64 females, 6 had bleeding time more than 4 minutes. In the Indian male students, all males had bleeding time below 4 minutes, but among the 49 female students, 3 showed BT more than 4 minutes. One Sri Lankan female student had more than 4 minutes, but the remaining 28 students had values below 4 minutes. All 12 males had BT below 4 minutes.

Table – 7: Gender wise Distribution of clotting time

Clotting Time			
Nepal	<6mins	>6mins	Total
Male	60	4	64
Female	52	12	64
Total	112	16	128
P value	0.029*		
India			
Male	34	9	43
Female	34	15	49
Total	68	24	92
P value	0.207×		
Sri Lanka			
Male	11	1	12
Female	21	8	29
Total	32	9	41
P value	0.176×		

* p<0.05, statistically significant
× p>0.05, statistically not significant

Table 7 shows that among the 64 Nepalese males, 4 showed CT higher than 6 minutes, whereas 12 females had CT more than 6 minutes. Among the 43 Indian males, 9 showed CT higher than 6 minutes, and 15 females out of 49 showed higher clotting time of more than 6 minutes. Among the Sri Lankan students out of 12 male, only one showed CT more than 6 minutes. In females, 21 students had values below 6 minutes and 8 students had CT more than 6 minutes.

Table – 8: Logistic regression table:

Gender		
Country	Bleeding Time	Clotting Time
Nepal	3.207(0.622,16.53)×	3.462(1.052,11.389)*
India	-	1.667(0.642,4.324)×
Sri Lanka	-	4.190(0.463,37.938)×
Total	4.432(0.952,20.64)×	2.453(1.248,4.822)†

† p<0.01, statistically significant
* p<0.05, statistically significant
× p>0.05, statistically not significant
- p value cannot calculate

Table 8 shows that female students are at 3.207 times greater risk of developing bleeding time greater than 4 minutes when compared to males. Nepalese female students have 3.462 times greater risk of developing clotting time greater than 6 minutes compared with male

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students. On a total, girls are 4.432 times more prone for bleeding time greater than 4 minutes and 2.453 times for clotting time value above 6 minutes as compared to males.

Discussion

Country wise distribution of blood group:

In Nepalese students, majority of them were O blood group, followed by A and B blood group. AB blood group was very rare among these students. Our data is similar to another study done by Pramanik et al on blood group distribution of different ethnic groups in Kathmandu valley[16]. The frequency of distribution of A, B, AB and O blood group was 28.5%, 27.3%, 8.7% and 35.5% respectively in his study. Among the Indian medical students B is the most prevalent, followed by O and A blood group. AB blood group prevails in very less population. It is similar to other study conducted by Abhishekh et al[17,18]. O group was prominent in Sri Lankan students. Percentage of A and B blood group students were almost same in all the counties and AB group was relatively less.

Blood group and diseases:

Much research has been carried out all over the world regarding diseases and their correlation with blood groups. It has been observed by Reddy et al that among the Caucasian patients with epistaxis, 50.44 per cent were blood group O compared with 45.10 percent of the control group[13]. In our study O group was more prevalent among the Nepalese students 35.2%, and Sri Lankan students, 53.7%. Indian students it is little less i.e. 23.9%.

Bleeding time is normal in hemophilia; the main cause of this is platelet adhesion and aggression[19]. O blood group people were more vulnerable to infection in an epidemic of gastrointestinal infections, which was due to Escherichia coli O157 in Scotland in 1996. A, B, and AB blood group people have more vulnerability to arterial and venous thrombotic disease compared to O group people[20]. Persons having any other blood group except O having more risk of venous thromboembolism (VTE) comparing with O group individuals. Non O group individuals have higher levels of von Willebrand factor (vWWF) and factor VIII (FVIII) [20, 21]. There is a close association between Norovirus infection and the expression of ABH and Le antigens in the gastrointestinal tract. Noroviruses are the most frequent cause of acute gastroenteritis in humans estimated to account for 60-85% of all gastroenteritis outbreaks in developing countries [22].

Gender wise distribution of BT and CT

In our study, we found that females of all countries had higher BT and CT when compared to males. This might be due to the presence of estrogens in females which increase the CT and decrease level of fibrinogen of blood plasma[5]. Among the 64 Nepalese students, only 2 males and 6 females showed BT more than 4 minutes. Among Indian students, all had BT below 4 minutes, but among the 49 female students, 3 showed BT more than 4 minutes. Only one female student of Sri Lanka had a BT value of more than 4 minutes.

Experiments done by Mahapatra et al states that CT was prolonged in blood group B compared to O group and BT was significantly higher in AB group[17]. In our study, we found higher CT values for Indians as compared to other countries. A study done by Mahapatra et al stated that there was no gender wise significant difference in BT and CT. But our study has contradicted that finding, showing that females have relatively higher values of bleeding time > 4min and clotting time > 6 minutes as compared to males.

Future scope of the study

A multicentre Study is recommended to verify the above mentioned findings. Further research work is recommended in order to investigate if there is any relationship of gender with Bleeding Time and Clotting Time which was related with our study outcome.

Conclusion

Our study suggests that O blood group is predominant in Nepalese students, which is a predisposing factor in developing gastrointestinal infections and epistaxis[23]. Most of the places in Nepal comprise of hilly region away from primary health care facilities, sanitation, purified drinking water etc. so it will increase the risk of infection, mortality and morbidity in Nepalese population.

Nepal Government should implement some screening tests for various disorders. As there are some relationship between blood group and various disorders like epistaxis, gastrointestinal disorders, arterial and venous thrombotic disorders. Thus preventive measures could be adopted before the onset of such disorders. Screening for Hypoprothrombinemia and Thrombocytopenia in females could be done in patients with higher BT and CT.

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Authors contributions

BR and MM done the experiment, interpreted the data, drafted the manuscript, and revised it. BR conceived the study with IB, acquired the data, conducted the data analysis, interpreted the data, and revised the manuscript. CGS improved the manuscript; BS participated in data analysis, interpreted the data, and revised the manuscript.

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