Research Article

To check the abundance of specific ABO blood groups and its coordination with bleeding time and clotting time among individuals of different ethnicity in Quetta, Balochistan

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Abstract

Blood group, bleeding time and clotting time play very important role before any surgical procedure. ABO blood group system is an important factor in the field of transfusion medicine. The study was aimed to observe the abundance of specific ABO blood groups and its coordination with bleeding and clotting time of individuals of different ethnicity in Quetta, Balochistan, and also some other variables were taken under consideration such as gender, age, weight and blood pressure. Blood group was determined by antisera, bleeding time by duke's filter paper method and clotting time by Wright's capillary tube method. Chi-square test was applied and the P value of <0.05 was taken as statistically significant. As a result of study, B blood group was found to be most common whereas AB was least common and they had no significance with bleeding time and clotting time but bleeding time was observed to be significant with ethnicity and clotting time significant with ethnicity and gender. Bleeding time had negative correlation with clotting time, age, weight and systolic blood pressure. While clotting time had positive correlation with age and weight but it was also negatively correlated with systolic blood pressure. Finally, no exact abundance of ABO blood groups occurred based on four mentioned ethnic groups but bleeding time and clotting time differ among them and also bleeding time and clotting time increase with decreasing age. Females and low aged individuals lie in the normal range of blood pressure as compared to males and high aged individuals.

Keywords: Age; Bleeding time (B.T); Blood Group; Clotting time (C.T); Ethnicity; Gender

Introduction

Hematological parameters play an important role before initiating any

surgical procedure. Hence on routine bases the hospital blood tests are performed [1]. Group of blood, time of bleeding and time

of clotting are the most important and initial hematological parameters. The blood group diagnosis has an important role in blood transfusion. Austrian Biologist and Physician Karl Land Steiner discovered the ABO blood group system, according to which there are four blood groups (A,B,AB and O). Type A individuals have antigen A, type B have B antigen, type AB have both antigens A and B and type O have neither of the antigens [2]. The antigens named as A and B, are complex oligosaccharides that are different in structure at terminal sugar [3]. The von Willibrand factor (vWf) is a blood glycoprotein that maintains the hemostasis and carries antigen [4]. It is synthesized by the megakaryocytes and endothelial cells. There is a clear association between ABO blood groups and vWf [5, 6]. O blood group has less vWf than other blood groups [7]. That gene which codes for vWf is present on chromosome number 12p12. It was proved that the locus of gene for ABO groups of blood is on chromosome number 9q34 that has a main effect on the gene of vWf [4]. And that's why, the time of bleeding and clotting are affected by the ABO blood group system [8]. Many studies found out the association between bleeding disorders and clotting disorders with different ABO blood groups, and on the other hand many studies also show that there is no association between the bleeding tendencies and different ABO blood groups [7, 9].

In 1951 O'Brien defined the bleeding time as the duration of time between the administration of a small and standard cut and the moment when the flow of blood stops or the time taken for a standard skin wound to stop bleeding [10]. Upon injury of vessels, platelets stick to one another and form a haemostatic platelet plaque. The bleeding time is used to measure the ability of platelets to stop bleeding and therefore it can also measure the function and number of platelets. Commonly, the bleeding lasts for 3 to 4 minutes [11].

Clotting time factors' absence or defect cause prolonged clotting time [12]. Platelets are non-nucleated; which live for 7-10 days and found in the form of fragments called "formed elements". Bleeding time depends upon the formation of platelets and clotting time depends upon the mechanism of clotting effectiveness [12]. The vWf is responsible for the adhesion of platelets and platelet aggregation. This acts as a specific type of protein which is used as carrier for clotting factor VIII (anti-hemophilic factor A). Due to this, the vWf plays a very vital role to form a short-term plug caused by hemostasis and then formation of clot which is done by activating the mechanism of clotting. Normal value of clotting time is 5 to 8 minutes [12].

Bleeding and clotting time are influenced by several factors such as gender, age, skin characteristics, temperature and diseases or medications. Presence of more amount of the estrogen in female may cause prolonged bleeding time [13]. It has also been observed that female had high clotting time than male [14]. It was observed that platelet count decreases with age and also seen that thrombocytopenia was common in the elders and the thrombocytosis was more common in the younger people [15]. The fibrinogen level in the plasma increases with age that shortens the clotting time in elder people and this makes the person more prone to thromboembolism [16-18].

The pressure that the blood exerts on the blood vessels is called blood pressure (BP). The normal blood pressure is 120/80 mmHg, where 120mmHg is called as systolic and the blood pressure 90mmHg is diastolic. When the blood pressure increases from 120 mmHg to 139mmHg, then it is said to be "Prehypertensive" and if it increases from this range, then it is called "hypertensive" [19].

The aim of this study is to find out which blood group is more abundant among different ethnicity and within each ethnic group and if there is any coordination of

blood groups with bleeding time, clotting time and gender. Also the coordination of bleeding time, clotting time and Systolic blood pressure is to be checked with gender, ethnicity, age and with each other if there is any or whether they are correlated.

Materials and methods

This study was conducted on four different ethnic groups residing greatly in Quetta, Balochistan. Among these four, were Hazaras, Pathans, Balochs and Punjabis. Fifty (50) individuals from each ethnic group were selected that involved twenty-five (25) males and twenty-five (25) females, regardless of their age. Initially, they were told about the purpose and output of the study and the type of experiment used was explained to them. Then a questionnaire was filled by each individual that included some personal information about the person like; name, father's name, age, gender, contact number, address and ethnicity. Later on, the individual's blood group, bleeding time, clotting time, weight and blood pressure were checked and filled in the questionnaire in order to observe their coordination with each other.

Blood group was determined by collecting the blood samples and mixing them with standard antisera. First, the finger-tip was cleaned with cotton swab immersed in a solution of 75% alcohol. Under aseptic conditions, a minute incision was made on the finger-tip by an adjustable lancing device (from diabetes care club) and the finger was pressed to bleed then three drops of blood were collected on clean glass slides (from SAIL BRAND China). And these blood drops were mixed with antisera i.e. Anti-A serum, Anti-B serum and Anti-D serum (from ImuMed ANTITOXIN GmbH; 69245 Bammental, Germany) and after 1-2 minutes, the blood groups were checked by the presence or absence of agglutination in the blood samples. Blood group A was obtained if agglutination occurred in blood drop mixed with Anti-A serum and blood group

B was obtained if agglutination occurred in blood drop mixed with Anti-B serum. The appearance of agglutination in both blood drops blended with Anti-A and Anti-B sera displayed blood group AB. Along with these demonstrations, the presence of agglutination in blood drop mixed with Anti-D serum exhibited positivity (e.g. blood group A+ive) and its absence exhibited negativity (e.g. blood group A-ive). But O+ive blood group was acquired by the occurrence of agglutination in only that blood drop which was mixed with Anti-D serum and if none of them agglutinated, then it was said to be the O-ive blood type which is usually very rare [20].

Bleeding time was checked by Duke's filter paper method. A roundly cut piece of filter paper was taken and marked with time interval of thirty seconds. The finger-tip was sterilized by a piece of cotton swab dipped in 75% alcohol. Then it was pricked with a sterile lancet by the adjustable lancing device and the time was noted by starting a stopwatch when the first drop of blood was marked on the filter paper. After every thirty seconds, the filter paper was marked by the blood coming out of the incision till the flow of blood stopped. The bleeding time was calculated simply by observing the time interval marked by blood drops on the filter paper or by multiplying the number of blood drops with time (30 sec). The bleeding time by Duke's filter paper method generally lies in the range of 1-5 minutes [20].

The Capillary tube method was used to determine the clotting time. The finger-tip was cleaned with 75% alcohol and then lanced with the needle in the lancing device. The finger was squeezed in order to obtain enough blood to fill the glass capillary tube or the micro haematocrit capillary tube (from ISO LAB, VITREX MEDICAL A/S Vasekaer6-8 Denmark and MARIENFELD laboratory glassware, Germany). When the capillary tube was filled with blood then it was kept in

horizontal position for about two minutes. Stopwatch was used to check the time duration accurately. Then the glass capillary tube was held between the thumb and the fingers of both the hands so that it could be broken easily. This was broken after every thirty seconds with the distance of about 1 cm away from the end. The time was noted by the appearance of fibrin thread across the distance between the broken ends of the capillary tube and this time duration was taken as the blood clotting time. The estimated clotting time by this method normally falls in the range of 3-6 minutes [21].

The weight of the person was checked by a weighing machine (from TANITA, Model no. HA540, Lot no. 890312) and recorded in kilograms. Each of the individuals were told to stand in straight upright position on the weighing machine without holding anything or having something along with himself or herself which could weigh or interfere in the original weight of the individual. And the person was also not allowed to be supported or attached with any wall or by any pillar etc. This reading was then entered as weight of the individual in the questionnaire.

The blood pressure was checked by the blood pressure apparatus i.e. the mercurial sphygmomanometer (from Kenzmedico Co. Ltd, Japan. Model no. 600) along with the stethoscope (from Green Star Medical Corporation). The inflatable cuff was wrapped around the upper arm of the individual normally (not very tight) above the elbow in such a way that the two tubes lie on the inner side of the arm. The sensitive part of stethoscope was placed on the inner side of the elbow where the pulse could be felt. The screw near the rubber bulb was tightly closed and it was pumped to a pressure of about 180 mmHg or a little more that the pulse disappeared. Then the screw was slightly opened to let the pressure decrease slowly so that the pulse reappeared and was continued to decrease till it gets weaker and weaker and finally disappears. The point where the pulse

appeared first was noted as systolic pressure and the point where it disappeared was noted as diastolic pressure. The cuff was squeezed to relax and then unwrapped from the individual's arm. The blood pressure of a normal person is generally 120/80 mmHg.

Results

Blood group

Table 1 shows comparison of ABO Blood groups with bleeding time (taken in seconds), clotting time (taken in minutes), gender and the ethnic groups (Baloch, Hazara, Pathan and Punjabi). Majority of the participants (140/200) had bleeding time less than or equal to thirty seconds and clotting time (122/200) less than five minutes but no association was observed between blood groups with bleeding time ($P = 0.284^{ns}$) and clotting time ($P = 0.289^{ns}$). Comparison of blood groups with gender ($P = 0.513^{ns}$) and ethnicity ($P = 0.652^{ns}$) also did not show statistically significant difference.

By the comparison of bleeding time and the blood groups, those with bleeding time below and equal to 30 sec were 140 individuals that included the blood group A (26), B (57), AB (18), and O (39). Further, those who had bleeding time greater than 30 sec were 60 in numbers and the blood groups included their number were A (12), B (17), AB (7), O (24). Bleeding time and blood group had no association ($p = 0.284$) and were non-significant.

Clotting time of those with <5 min were 122 individuals distributed as A (20), B (50), AB (17), O (35) and ≥ 5 were A (18), B (24), AB, (8), O (28). The clotting time and blood group were non-significant ($p = 0.289$).

By comparing the blood group with gender, blood group A had 18 females and 20 males, blood group B had 35 females and 39 males, blood group AB had 16 females and 9 males, and blood group O had 31 females and 32 males out of the total 200 individuals. No association was observed between them ($p = 0.513$).

Blood group and ethnicity were compared (50 participants of each ethnic group) and then it was observed that among the total 50 Baloch participants 10 individuals had blood group A, 16 with B, 8 with AB and O with 16 individuals. The Hazaras with A blood group had 10 individuals, B with 19 individuals, AB with 3 individuals and O with 18 individuals. Pathans with A blood

group had 8 individuals, B with 23 individuals, AB with 8 individuals and O with 11 individuals. Panjabis with A blood group had 10 individuals, B with 16 individuals, AB with 6 individuals and O with 18 individuals. Blood group with ethnicity were non-significant ($p = 0.652^{ns}$).

Table 1. Distribution of blood group on the basis of bleeding time, clotting time, gender and ethnicity

Variables	Categories	Total	Blood groups				P value
			A	B	AB	O	
Bleeding time (sec)	≤ 30	140	26	57	18	39	0.284 ^{ns}
	> 30	60	12	17	7	24	
Clotting time (min)	< 5	122	20	50	17	35	0.289 ^{ns}
	≥ 5	78	18	24	8	28	
Gender	Female	100	18	35	16	31	0.513 ^{ns}
	Male	100	20	39	9	32	
Ethnicity	Baloch	50	10	16	8	16	0.652 ^{ns}
	Hazara	50	10	19	3	18	
	Pathan	50	8	23	8	11	
	Punjabi	50	10	16	6	18	

P^{ns}= non-significant

Bleeding time

Table 2 shows the association of bleeding time with clotting time, gender, ethnicity, age and systolic blood pressure. This table shows that bleeding time has no association with clotting time, gender, age and systolic blood pressure but a significant result is obtained by comparing the bleeding time with ethnicity ($p = 0.004^*$).

From total 200 individuals, majority of them (i.e.140) had bleeding time less than and equal to 30 seconds out of which 82 individuals' clotting time were less than 5 minutes and 58's were greater than and equal to 5 minutes and only 60 of them had bleeding time more than 30 seconds where 40 individuals had clotting time less than 5 minutes and 20 of them had clotting time greater than and equal to 5 minutes. There wasn't any statistical significant difference between the two factors ($p = 0.282^{ns}$).

The association of bleeding time was also checked with gender, equal numbers of female and male individuals were selected (i.e. 100 female and 100 male). Out of 100 females, 72 of them had bleeding time less than and equal to 30 seconds and 28 of them had bleeding time more than 30 seconds and from males 68 individuals' bleeding time were less than and equal to 30 seconds and 32's were more than 30 seconds. No significant difference was observed between these two variables ($p = 0.537^{ns}$).

As this study is based on different ethnicity, so the bleeding time of 50 individuals from each ethnic groups were checked in which the people with bleeding time less than and equal to 30 seconds were; Baloch = 31, Hazara = 43, Pathan = 38, Punjabi = 28 and people with bleeding time more than 30 seconds were 19,7,12,22 respectively. Significant result was obtained between these variables ($p =$

0.004*) as shown in the (Table 2) where Hazaras have comparatively lower bleeding time than other ethnic groups. The other variable whose association had to be compared with the bleeding time was the age of the individuals. Two age groups were formed which are ≤ 22 years and > 22 years of age (Table 3). Both the groups had same number of individuals ($n = 70$) whose bleeding time were less than and equal to 30 seconds and with bleeding time more than 30 seconds there were 37 individuals with ages less than and equal to 22 years and 23 individuals with ages greater than 22 years. The result gained from their comparison was statistically non-significant ($p = 0.130^{ns}$). As the ranges of systolic blood pressure was divided into 3 groups, majority of the

participants ($n = 108$) had normal blood pressure, no significant result was found by comparing it with bleeding time ($p = 0.716^{ns}$). Bleeding time less than and equal to 30 seconds contained 74 individuals with normal blood pressure, 49 being prehypertensive and 17 being hypertensive, 34 individuals with normal blood pressure, 21 with prehypertensive blood pressure and only 5 with hypertensive blood pressure had bleeding time higher than 30 seconds (Table 2). So, in both groups of bleeding time, number of individuals based on their systolic blood pressure had a descending order as normal $>$ Prehypertension $>$ Hypertension and the same order was observed overall among total participants.

Table 2. Distribution of bleeding time on the basis of clotting time, gender, ethnicity, age groups and systolic blood pressure

Variables	Categories	Total	Bleeding time (sec)		P value
			≤ 30	> 30	
Clotting time (min)	< 5	122	82	40	0.282^{ns}
	≥ 5	78	58	20	
Gender	Female	100	72	28	0.537^{ns}
	Male	100	68	32	
Ethnicity	Baloch	50	31	19	0.004*
	Hazara	50	43	7	
	Pathan	50	38	12	
	Punjabi	50	28	22	
Age groups	≤ 22	107	70	37	0.130^{ns}
	> 22	93	70	23	
Systolic B.P groups (mmHg)	< 120	108	74	34	0.716^{ns}
	120-139	70	49	21	
	> 139	22	17	5	

P^{ns} = non-significant

P^* = significant

Table 3. Distribution of participants based on age groups

Gender		Age groups		Total
		≤ 22	> 22	
	Female	71	29	100
	Male	36	64	100
Total		107	93	200

Clotting time

Table 4 shows the comparison of clotting time with gender, ethnicity, age and

systolic blood pressure. As the total samples were 200 having 100 males and 100 females from the four ethnic groups

(Baloch, Hazara, Pathan and Punjabi), among these 200 individuals 122 of them have clotting time less than 5 minutes that includes 54 females and 68 males while the 78 have clotting time greater and equals to 5 minutes that have 46 females and 32 males. Significant difference was observed between them ($p = 0.042^*$). Table 3 shows distribution of participants based on age groups of ≤ 22 , >22 female and male respectively.

The clotting time was also checked in different ethnic groups, the result shows that among total 50 Baloch participants, there were 19 individuals with clotting time less than 5 minutes and 31 with

clotting time greater than and equal to 5 minutes. Among Hazaras ($n=50$), there were 44 participants with clotting time less than 5 minutes and 6 with clotting time greater than and equal to 5 minutes. Among total 50 Pathan participants, there were 30 individuals with clotting time less than 5 minutes and 20 with clotting time greater than and equal to 5 minutes. Among Punjabis, there were 29 participants with clotting time less than 5 minutes and 21 with clotting time greater than and equal to 5 minutes. The result obtained was statistically significant and that the two variables have association with each other ($p = 0.000^*$).

Table 4. Distribution of clotting time on the basis of gender, ethnicity, age and systolic blood pressure

Variables	Categories	Total	Clotting time (min)		P value
			<5	≥ 5	
Gender	Female	100	54	46	0.042*
	Male	100	68	32	
Ethnicity	Baloch	50	19	31	0.000*
	Hazara	50	44	6	
	Pathan	50	30	20	
	Punjabi	50	29	21	
Age groups	≤ 22	107	70	37	0.169^{ns}
	>22	93	52	41	
Systolic B.P groups (mmHg)	<120	108	64	44	0.859^{ns}
	120-139	70	44	26	
	>139	22	14	8	

^{ns}= non-significant

^{*}= significant

The clotting time was then compared with age, from total 200 participants, there were 107 individuals with ages less than or equal to 22 years, among whom 70 were with clotting time less than 5 minutes and 37 were with clotting time greater than and equal to 5 minutes. While 93 individuals were with ages greater than 22 years that had 52 individuals with clotting time less than 5 minutes and 41 with clotting time greater than and equal to 5 minutes. There was no association found between the two variables ($p = 0.169^{\text{ns}}$).

Clotting time from 50 individuals of each ethnic group were checked, in which the

people with clotting time less than 5 minutes include 54 females and 68 males and the individuals with clotting time equal to and greater than 5 minutes include 46 females and 32 males. The result was obtained was significant ($P = 0.042^*$).

When the association of clotting time was checked with four ethnic groups then it was observed that the individuals with clotting time less than 5 minutes included 19 Baloch, 44 Hazara, 30 Pathan and 29 Punjabi and those having clotting time equal to and greater than 5 minutes included 31 Baloch, 6 Hazara, 20 Pathan

and 21 Punjabi. The result was statistically significant ($p = 0.000^*$).

By comparing clotting time with age, it was observed that they had no association or significant difference with each other ($p=0.169^{ns}$). Majority of the participants had clotting time less than 5 minutes regardless of their ages.

When Systolic Blood Pressure was compared with clotting time again the same descending order was observed as normal > prehypertension > hypertension. The number of participants with clotting time less than 5 minutes were 64 having normal blood pressure, 44 being prehypertensive and 14 being hypertensive, while the number of participants with clotting time equal to or greater than 5 minutes were 44 being normal, 26 being prehypertensive and only 8 being hypertensive. The result obtained did not show any significant difference ($p = 0.859^{ns}$).

Blood pressure

Normal, prehypertensive and hypertensive are the groups for systolic blood pressure.

The hypertension includes two stages known as stage 1 and stage 2 hypertension [19] but no division of these stages have been taken under consideration in this study as all those individuals were included in hypertensive range whose systolic B.P crossed 139 mmHg.

By comparing the systolic blood pressure (SBP) with gender, ethnicity and age; the results show: As shown in Table 5, three SBP groups were made, the 1st group that has SBP less than 120mmHg contains 79 females and 29 males. This group contains 29 Baloch, 30 Hazara, 23 Pathan and 26 Punjabi and there are 73 participants with ages less than and equal to 22 years and 93 persons with ages greater than 22 years in this group.

The 2nd group that has SBP ranging from 120mmHg to 139mmHg contains 20 females and 50 males. This group has 13 Baloch, 15 Hazara, 22 Pathan and 20 Punjabi. This group also contains 28 individuals with ages less than and equal to 22 years and 42 persons with ages greater than 22 years.

Table 5. Distribution of systolic blood pressure based on gender, ethnicity and age

Variables	Categories	total	Systolic B.P (mmHg)			P value
			<120	120-139	>139	
Gender	Female	100	79	20	1	0.000*
	Male	100	29	50	21	
Ethnicity	Baloch	50	29	13	8	0.449 ^{ns}
	Hazara	50	30	15	5	
	Pathan	50	23	22	5	
	Punjabi	50	26	20	4	0.000*
Age groups	≤22	107	73	28	6	
	>22	93	35	42	16	

P^{ns}= non-significant

P* = significant

The 3rd group that has SBP greater than 139mmHg contains only one female and 21 male. This group has 8 Baloch, 5 Hazara, 5 Pathan and 4 Punjabi and 6 participants with ages less than and equal to 22 years and 16 participants with ages greater than 22 years.

The result shows that greater number of females (n=79) have a SBP of less than 120mmHg and only one female has SBP greater than 139mmHg while the greater number (n=50) of males have SBP ranging from 120mmHg to 130mmHg and 21 have SBP greater than 139mmHg.

The results shows that majority (n=108) have normal SBP group i-e <120mmHg and the minority (n=22) have SBP >139mmHg that are hypertensive. From the Baloch participants (n=50) there were 8 individuals with SBP greater than 139mmHg (hypertensive) which is more in number than the other ethnic groups. The majority were with normal SBP and aged less than 22 years and there were only 6 persons with the SBP greater than 139mmHg and majority (n=42) of those with ages above 22 years have SBP ranging from 120mmHg to 139mmHg.

By the comparison of systolic B.P groups with gender (male and female), it was obtained that from the total of 200 participants, systolic B.P less than 120mmHg contained 79 females and 29 males. The systolic B.P group ranging from 120mmHg to 139mmHg has 20 females and 50 males and the third group having systolic B.P greater than 139mmHg has only one female and 21 male. Significant association was obtained ($p=0.000^*$).

The systolic blood pressure was then compared with ethnicity, the result shows that 1st group, having systolic B.P less than 120mmHg contains 29 Baloch, 30 Hazara, 23 Pathan and 26 Punjabi. The 2nd group, having SBP ranging from 120mmHg to 139mmHg contains 13 Baloch, 15 Hazara, 22 Pathan and 20 Punjabi. And the 3rd

group, having SBP greater than 139mmHg has 8 Baloch, 5 Hazara, 5 Pathan and 4 Punjabi and their result showed no association with each other ($p=0.449^{ns}$).

When systolic blood pressure was compared with age, the result showed that the 1st SBP group having SBP less than 120mmHg contained 73 individuals of ages less than 22 years and 35 having ages greater than 22 years. The 2nd SBP group having SBP ranging from 120mmHg to 139mmHg contained 28 persons that were aged less than 22 years and 42 with ages greater than 22 years. The 3rd SBP group, having SBP greater than 139mmHg, contained 6 individuals with ages less than 22 years and 16 with ages greater than 22 years. Their association comes out to be significant ($p=0.000^*$) a positive correlation was found between systolic blood pressure and age ($r=0.394$) which shows that one factor increases with the increase of the other factor.

Correlation

From the study, the correlation was checked between bleeding time and clotting time, bleeding time and age, bleeding time and weight, bleeding time and systolic blood pressure, clotting time and age, clotting time and weight and clotting time and systolic blood pressure, age and weight, age and systolic blood pressure, weight and systolic blood pressure, are given in the (Table 6).

Table 6. Correlation of bleeding time, clotting time, age and weight with different variables

Variables		Correlations
Bleeding time	Clotting time	-0.048
	Age	-0.064
	Weight	-0.055
	Systolic B.P	-0.114
Clotting time	Age	0.031
	Weight	0.039
	Systolic B.P	-0.048
Age	Weight	0.414
	Systolic B.P	0.394
Weight	Systolic B.P	0.451

The correlation between bleeding time and clotting time indicated negative correlation ($r = -0.048$) which means they are inversely proportional to each other, the correlation between bleeding time and age, weight and systolic blood pressure also indicated negative correlation ($r = -0.064$, $r = -0.055$, $r = -0.114$ respectively).

The correlation between clotting time and age, weight indicated weak correlation ($r = 0.031$, $r = 0.039$ respectively), which means there is weak relationship between them because the correlation value is near zero. The clotting time and systolic blood pressure show negative correlation ($r = -0.048$).

The correlation of age with weight indicated the moderate correlation ($r = 0.414$), and the age and systolic blood pressure also showed the moderate correlation ($r = 0.394$) which means there is moderate relationship between them because the value lies in the middle i.e. neither zero nor 1.

The weight and systolic blood pressure show the moderate correlation between them ($r = 0.451$) because this value also lies in between 0 and 1.

Discussion

The present study was carried out on 200 individuals, who were of different ages including both male and female equally. Over all, when the blood groups were observed then it was seen that those people with O blood group have prolonged bleeding time as greater than 30 seconds, which was also observed in an article Kaur *et al.* [22] that O blood group has prolonged bleeding time, AB blood group individuals had bleeding time less than 30 sec or equal to 30 sec which was seen in a research paper by Roy *et al.* [23] that AB blood groups' bleeding time was less as compared to the other blood group individuals.

Clotting time was taken in two categories, those with clotting time less than 5 min and those with clotting time greater or equal to 5 min, in this study; it was found that the clotting time of most of the

individuals were less than 5 which was also seen in the in the article by Roy *et al.* [23] which stated that 87.5% Nepalese students, 76.2% Indian students, 78% Sri Lankan students clotting time was below 6 min. This study also shows that the two variables are non-significant ($P = 0.289^{ns}$). The clotting time was prolonged in blood group O that is observed in this study and was also observed in an article by Mirdha and Jena [24]. This is because individuals with non O group have high levels of vWF.

Smita [25] found that the bleeding time and clotting time were prolonged in those individuals having B blood group. Regarding Mahaptra, Sasekala and Saikumar [26, 27] said that clotting time was significantly prolonged in the blood group B individuals, but the bleeding time was prolonged in the individuals of AB blood group this was due to the percentage of blood group in different studies, may be due to sampling error, genetic factors, natural selection which was effected by traditions and habits.

From this study, it was found that the B blood group individuals were more common than the other blood groups individuals, B blood group with 37% followed by sequence of O blood group 31.5%, A blood group 19%, AB blood group 12.5% ($B > O > A > AB$). As, it was also seen in another article Kaur *et al.* [22].

In the present study the ethnicity was also compared with the ABO blood group, 50 individuals from each ethnic group were present and by the result, B blood group was most common and Pathans had B blood group in majority that is 23 out of 50 total individuals then Hazaras with 19 out of total 50 had B blood group, Punjabis and Balochs had O blood group in majority that were 19 and 19 respectively. In a book "The Arians: A Historial Perspective" [28], it was said that Pathans and Punjabis have B blood group in majority and Balochs have O blood group in majority, and this study presents

that Hazaras have blood group O more common among them.

According to this study, the result of coordination between bleeding and clotting time were non-significant ($P = 0.282^{ns}$) and they had a negative correlation with each other where ($r = -0.048$). This shows that as bleeding time increases, clotting time will decrease or vice versa. But, Ambreen and Muqet [29], in their study has showed that bleeding time and clotting time are significant and interdependent on each other or these variables are directly proportional to each other. This difference in the results can be due to the different ages of individuals involved in the study for sample collection as in the mentioned study individuals of same age groups participated (i.e. 17-20 years) but in the present study the participants were of different ages ranging from 15 years up to 68 years of age. A study by Feusner James [30] supports these results where he showed that there are differences in the bleeding time of children (3.4 min) and adults (2.8 min) and his study also showed a good correlation between decreasing platelet count and increasing bleeding time.

Generally, the comparison between bleeding time and clotting time with gender shows that females have higher bleeding time and clotting time as compared to males which might be due to soft tissue and hormonal differences in male and female as describe by Bain and Forster [31]. Females have higher levels of estrogen and lower levels of fibrinogen in their blood plasma which may be the cause for differences in their bleeding time and clotting time [13], but in this study, it was found that majority of the females had lower bleeding times as compared to males. Out of 100 females, 72 of them had bleeding time less than and equal to 30 seconds while out of 100 males, 68 of them were with this group of bleeding time which shows that females have slightly lower bleeding time than males and

statistically they were non-significant. The same condition was observed in Monika [32] study that bleeding time was higher in males than females where the mean bleeding time was 2.54 minutes in females and 2.56 minutes in males and again was not statistically significant. The present study is also similar with the other study by Mahapatra and Mishra [26] that there was no gender wise significant difference in bleeding time and clotting time. This can be because of age difference as majority of the females in the present study ($n = 71$) are in the ages of less than 23 years. Some researchers have also shown that bleeding time decreases by increasing age and also food, skin characteristics, temperature and medications etc have their effects. Valeri, Siriraj, Sutor, Macpherson and Berge [33-37] But in this study, the males have relatively higher clotting time than females; males (68%) had clotting time greater than 5 compared to the females (54%) which is statistically significant.

This study shows that bleeding time increases with decreasing age and have negative correlation with each other (-0.064) as given in the tables 2 and 5 where 37 individuals' bleeding time were greater than 30 seconds whose ages were below and equal to 22 years while individuals with ages greater than 22 years were only 23. There is an inverse relationship between length of bleeding time and clotting time with age which supports the results of the present study by Gerrard [38]. Macpherson [36] also mentioned that bleeding time was shorter in people who were older than 50 years than those who were younger.

From the designated study, the association between clotting time and ethnicity were observed significant ($P = 0.000^*$). This might be due to the genetic variation in von Willebrand Factor (vWF) which is responsible for the adhesion of platelet to the site of damage. Blood groups have some related diseases like hemophilia, in which bleeding time remains normal but

the platelet adhesion and aggregation is the main cause. Study by Zucker [39].

The positive correlation between increasing age and high blood pressure proves that the arteries become hardened and less active. And the same case is with the increasing weight, the more a person weights the more pressure is needed to supply oxygen to blood because greater pressure is needed to move blood around the body as describe by Kannel [40].

Conclusion

From this study, it was concluded that overall B blood group was the most common and AB blood group was the least common. According to different ethnic groups among Balochs, blood groups B and O were abundant while AB was rare, among Hazaras also blood groups B and O were common but AB blood group was very rare and their bleeding time was lower as compared to other ethnic groups here. Among Pathans, B blood group was very abundant while A and AB blood groups were rare and among Punjabis, blood group O was most abundant whereas AB blood group was least abundant and they had higher bleeding time comparatively. Majority of the individuals had bleeding time less than and equal to 30 seconds but no significant result was observed between blood groups and bleeding time, it was also observed that majority of the participants had clotting time less than 5 minutes with no significance with their blood groups although the same percentage of abundance of bleeding and clotting time is shown by the blood group B since it was the most common. A weak significance was shown between the bleeding time and ethnicity as mentioned, Hazaras have comparatively lower bleeding time while Punjabis having higher bleeding time. Gender wise bleeding time was lower in females than males but clotting times were higher in males than females and are significant, also they are negatively correlated to each other. Ethnicity and clotting time were also significant, with

Hazaras having lower clotting time and Baloch having higher clotting time. The study also showed that bleeding time and clotting time increase with decreasing age. Significant result was observed by comparing systolic blood pressure with gender and age, the study showed that comparatively lower and normal blood pressure were acquired in females as compared to males and also in individuals with lower ages. Positive correlation was found between systolic blood pressure and age. While, there was no coordination of blood pressure with bleeding time, clotting time and ethnicity.

Authors' contributions

Conceived and designed the experiments: Farzana, NA Yawar & MK Taj, Performed the experiments: Farzana, NA Yawar & I Taj, Analyzed the data: H Ali & U Zafar, Contributed materials/ analysis/ tools: HA Ahmed, Z Bibi & T Hameed, Wrote the paper: Farzana, NA Yawar, N Rafiq & MK Taj.

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