ORIGINAL ARTICLE

von Willebrand Factor Profiles of the Different ABO Blood Groups Among the Malay Population

Rohaida Abdul Rahman[1], Faridah Md Afandi[2], Tun Maizura Mohd Fathullah[2], Rafeezul Mohamed[3]

[1] Medical Transfusion Unit, Pathology Department, Hospital Sultan Ismail, Johor Bahru, Malaysia.
[2] National Blood Center, Jalan Tun Razak, 50400, Kuala Lumpur, Malaysia.
[3] Regenerative Medicine Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, 13200 Kepala Batas, Pulau Pinang, Malaysia

ABSTRACT

Introduction: The National Blood Center, Kuala Lumpur interprets laboratory results for the von Willebrand factor (VWF) profile based on guidelines which were established based on the Caucasian population. The VWF profiles among the Malay population has not yet been established. The current study aims to determine the VWF profiles of the different ABO blood types among Malays and to evaluate their association with demographic characteristics and smoking habits. **Methods:** One hundred and forty Malay donors were involved. Factor VIII (FVIII:C), VWF antigen (VWF:Ag), and ristocetin cofactor (VWF:RiCof) levels and collagen binding activity (VWF:CBA) were measured by coagulometric clot detection, latex agglutination, and enzyme-linked immunosorbent assay. **Results:** The majority of donors (59.3%) were 30–49 years old, male (81.43%), non-smokers (74.3%), and overweight (71.4%). The Malay VWF:Ag were slightly higher than those of Caucasians, Indians, Thais, and Chinese, but the average ratios of VWF activity (i.e., VWF:RiCof level and VWF:CBA) to VWF:Ag were slightly lower than those of the other populations. The highest level of VWF:Ag was found among those with the B blood group, followed by types A and O. **Conclusion:** Malays with type O blood had lower values of the components of the VWF profile compared to subjects with non-O blood. The higher levels of these elements and lower VWF activity to VWF:Ag ratio in Malays compared to other populations suggest that ethnicity impacts the plasma VWF levels and their interaction with collagen and platelets.

Keywords: von Willebrand factor (VWF), ABO blood group, Malays

Corresponding Author:
Rafeezul Mohamed, PhD
Email: rafeezul@usm.my
Tel: +604-5622541

INTRODUCTION

von Willebrand factor (VWF) is a plasma protein that mediates the initial adhesion of platelets at the sites of vascular injury and also acts as a carrier for and stabilizer of blood clotting Factor VIII (FVIII:C) (1). As an acute phase reactant protein, the level of VWF in circulation varies. About 60% of this variance among individuals is due to genetic factors, of which 30% is due to the effect of ABO blood type (2). ABO blood type greatly influences the VWF plasma level, as subjects with type O blood have lower VWF levels than those with non-O type blood (3). A person's ABO blood type appears to strongly influence the clearance of VWF (4). Additionally, smoking and demographic characteristics of individuals can affect plasma VWF levels (5, 6, 7).

Von Willebrand disease (VWD) is the most common hereditary bleeding tendency disorder, and it occurs in almost 1% of the world's population (8). VWD is a highly heterogeneous disorder with bleeding events ranging from asymptomatic to very severe life threatening haemorrhage (9). Of the three types of VWD, Types 1 and 3 are due to quantitative defects, whereas Type 2 is due to qualitative defects of the VWF protein (10). Current available data reported that the VWD accounts for 6-13% of the hereditary bleeding disorders in developing countries (11). Of the 28 million people in Malaysia, only 0.002% have been diagnosed with VWD (12). Most patients were diagnosed as having VWD Type 1 (77.2%) (13). Sixty-three percent of patients diagnosed with VWD in Malaysia were Malay, and 60% were females (13).

Previous population studies reported that VWF levels were higher among African Americans than Caucasians (14). These racial differences in VWF level further complicate the issues surrounding the diagnosis of VWD (14). Another study conducted in South Africa with a distinct ethnic mixture of Africans, Caucasians, and Indians reported that the African had significantly higher VWF antigen (VWF:Ag) and Factor VIII (FVIII:C) levels than the other groups. The researchers suggested that the influence of ethnicity on VWF levels should be considered in the clinical and laboratory evaluation

of VWD (15). In the U.S. National Heart, Lung, and Blood Institute exome sequencing project, researchers found that some VWF missense variants, which were commonly or uncommonly present among certain ethnic groups, contributed to the phenotypic variation of VWF and FVIII:C in those groups (16).

To date, most studies of VWF have been conducted among Caucasians, and numerous studies have been recently conducted on Chinese, Japanese, Korean, Singaporean and Saudi. The goals of the current study were to provide preliminary data on the components of the VWF profile in the Malay population and to investigate the influence of the ABO blood group, smoking habits, and demographic characteristics (gender, age, and body mass index (BMI)) on the VWF profiles.

MATERIALS AND METHODS

Subjects

One hundred and forty healthy Malay regular blood donors at the National Blood Center, Kuala Lumpur (NBCKL) were recruited for this study. They were aged 18 years or above; male or female; had type A, B, or O blood; not on any medication; and had no history of tendency to bleed. A bleeding tendency questionnaire adopted from U.S. National Heart, Lung, and Blood Institute was used to exclude donors with increased risk of bleeding events. The AB blood group donors were excluded as the AB group is the least common among the Malay population (17). Consent was obtained from all donors. This research was approved by the Universiti Sains Malaysia Human Research Ethics Committee (USM/JEPeM/15080280) and the Ministry of Health Malaysia Ethics Committee (NMRR-15-857-26266).

All of the demographic data (ABO blood type, age, weight, and gender) were extracted from the Blood Donor Registration Form, and each donor's height in meters was measured by a seca® height and weight machine (Chino, California, USA). Each participant's smoking status was identified by asking whether he or she was a smoker or non-smoker. A smoker was defined as someone who, at the time of the study, smoked any tobacco product either daily or occasionally, whereas a non-smoker was someone who never smoked or stopped smoking for more than 6 months (18).

Blood sample preparation for analyses

Blood samples for analyses were taken during the donation (from the diversion pouch) and placed into four 3.2% trisodium citrate tubes each containing 2.7 ml of blood. The samples were centrifuged for 10 minutes at 3000 rpm at room temperature within 4 hours of collection to separate the platelet-poor plasma. Samples were stored at –80°C until tested. Prior to testing, all samples were thawed by incubating them in a water bath at 37°C.

The VWF collagen binding assay (VWF:CBA) was conducted using the enzyme-linked immunosorbent assay (ELISA) method with a Technozym® kit (Technoclone GmbH, Vienna, Austria) according to manufacturer's instructions. Briefly, diluted calibrators, samples and control plasmas were pipetted into the test wells. The test wells were covered with film and incubated for 45 minutes at room temperature. Then the samples-containing wells were washed with washing buffer. After the last washing, wells were aspirated thoroughly by turning them upside down. To remove the last remnant, gentle tapped the test wells on a blotting paper. Then, the conjugate working solution was pipetted into the wells and covered with test strip with film. Then stopping solution was pipetted into the wells. The samples were shake for 10 seconds and the absorbance were measured within 10 minutes with Tecan® Infinite F50 ELISA reader machine (Tecan Group Ltd, MΔnnedorf, Switzerland) at 450 nm.

The FVIII:C level was measured using the coagulometric clot detection method by using the modified 1 stage APTT substitution test based on manufacturer's guidelines. Briefly, 50 µL of 1:10 diluted donor plasma in factor diluent solution and 50 µL of APTT-2 reagent, was added to 50 µL of FVIII-depleted plasma. The mixture was incubated at 37 °C for 4 minutes. Then 50 µL of a 0.025 mol/L calcium chloride solution was added to the mixture. The ACL TOP 500 coagulation analyser was used to measure the FVIII:C activities.

VWF:Ag kit contains of latex particle which enhanced the immunoturbidimetric assay. The coated latex particles agglutinate when VWF containing plasma were mixed with latex reagent and the reaction buffer. The concentration of VWF:Ag were proportionate to the degree of agglutination which measured by the decrease of the transmitted light. By using the calibration curve, the amount of VWF:Ag were determined by ACL TOP 500 coagulation analyser.

VWF:RiCof levels were measured by the latex particle agglutination method. Briefly, monoclonal antibody to the receptor on the VWF were coated with the latex particles. The latex will enhanced the immunoturbidimetric assay to quantify the VWF:RiCof activity. The agglutination of latex reagent produces the increase in turbidity. The VWF:RiCof activity were quantified by measuring the decrease of light transmission caused by the aggregate by using an ACL Top 500 Automated Coagulation Analyser All reagents were obtained from the Instrumentation Laboratory Company.

Statistical analysis

The data were analysed using Statistical Package for the Social Sciences (SPSS) Version 23.0 (IBM, New York, NY, United States). Results were presented as number, percentage, and mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to

compare the mean VWF profile data (VWF:Ag, FVIII:C, and VWF:RiCof and VWF:CBA) with the different ABO blood groups and post-hoc Bonferroni used to provide specific information on which means are significantly different from each other. Independent t-test were used to evaluate the association of the demographic data and smoking habits with the VWF profiles. One-way multivariate analysis of variance (MANOVA) was used to evaluate the association of different ABO blood group types with different VWF profiles. $p < 0.05$ was considered to be statistically significant.

RESULTS

Table I shows the smoking, demographic, and ABO blood group characteristics of the 140 subjects. The majority of them were 30–49 years old, male, overweight/obese, and non-smokers. Forty-eight participants had type O blood, 46 had type A blood, and 46 had type B blood.

Table I: Demographic, smoking and ABO blood group characteristics of regular Malay donors

Characteristics	Number (percentage)
	n (%)
Number of patients, N	140
Age (years)	
≤ 29	47 (33.6)
30–49	83 (59.3)
50–69	10 (2.1)
Gender	
Male	114 (81.4)
Female	26 (18.6)
Body Mass Index (kg/m²)	
< 25	40 (28.6)
≥ 25	100 (71.4)
Smoking status	
Yes	36 (25.7)
No	104 (74.3)
Blood Group	
A	46 (32.9)
B	46 (32.9)
O	48 (34.2)

The mean ± SD values for FVIII:C, VWF:Ag, VWF:RiCof, and VWF:CBA among the Malay subjects were 132.51 ± 35.33, 132.81 ± 46.50, 97.19 ± 26.07, and 95.70 ± 30.20 IU/dL respectively. Table II shows the distribution of the VWF profile components among the ABO blood groups. Subjects with type B blood had the highest level of VWF profile elements, followed by A and O. Table III shows the prevalence of low values of the VWF profile components (< 50 IU/dl) among the blood groups. The prevalence of low VWF:Ag, VWF:RiCof, and VWF:CBA was 1.4%, 3.6% and 0.7% respectively and most of these subjects had type O blood. Table IV shows the ratio of VWF activity to VWF:Ag. The ratio of VWF:RiCof to VWF:Ag in all blood groups was > 0.7 and that of VWF:CBA to VWF:Ag was > 0.6.

Table II: Distribution of vWF profile factors among the ABO blood groups

Blood group	vWF profiles			
	FVIII Ag (mean ± s.d.)	vWFAg (mean ± s.d.)	vWF RiCof (mean ± s.d.)	vWF CBA (mean ± s.d.)
A	138.77 ± 37.74	143.30 ± 45.20	100.97 ± 24.47	95.80 ± 32.55
B	144.43 ± 31.69	151.37 ± 40.79	107.93 ± 25.95	103.78 ± 31.74
O	115.10 ± 29.65	104.96 ± 40.11	83.26 ± 21.63	87.86 ± 24.31

Table III: The number of subjects (out of 140) with low levels of vWF profile factors (< 50 IU/dl) among the ABO blood groups

	vWF Ag	vWF RiCof	vWF CBA
A	0	2	0
B	0	1	0
O	2	2	1
Prevalence	1.4%	3.6%	0.7%

Table IV: Ratio of vWF activity: vWF antigen (Ag)

Blood group	vWF RiCof: vWF Ag	vWF CBA: vWF Ag
A	0.70 ± 0.56	0.67 ± 0.66
B	0.71 ± 0.51	0.68 ± 0.58
O	0.79 ± 0.64	0.84 ± 0.66

Table V shows the different distributions of VWF profiles among blood groups. Post-hoc Bonferroni analysis revealed significant differences ($p < 0.017$) between blood group A donors and B donors in comparison with blood group O for all VWF factors except for VWF:RiCof ($p > 0.017$).

Tables VI show the association between smoking habits, gender, age, and BMI and VWF profiles. No significant associations were found for any of these factors except for age group with VWF:CBA. Multivariate analysis (Table VII) revealed a statistically significant difference in VWF profiles based on blood group among donors, except blood group was not statistically different for VWF:CBA ($F(2,137) = 3.376$; $p > 0.0125$).

DISCUSSION

To the best of our knowledge, there are no published data regarding the VWF profiles among the Malaysian population. Thus, this study provides preliminary data for Malays, who were studied because they represent the majority (68.6%) of the country's population (19), make up the largest ethnic group (63.0%) among VWD patients in Malaysia (13), and constitute 49.5% of the donors at the NBCKL (20).

The mean FVIII:C, VWF:Ag, and VWF:RiCof and VWF:CBA of the participants in this study were slightly higher than those of previous studies conducted among Caucasians, Indians, Chinese Singaporean, and Thais (15, 21, 22). In contrast, in a few previous studies among different ethnicities, the levels of VWF in Indians (15), Thais (22), Japanese (23), and American Chinese (24) were comparable to that of Caucasians. The higher

Table V: Difference distributions of vWF profile factors among blood groups

vWF profiles	Blood group	Mean ± s.d.	95% Confidence Interval		p-value	Post-hoc analysis (Bonferroni)	p-value
			Lower bound	Upper bound			
FVIIIAg	A	138.77 ± 37.74	127.565	149.983	< 0.001*	A*O	0.002**
	B	144.43 ± 31.69	135.015	153.837		B*O	< 0.001**
	O	115.10 ± 29.65	106.487	123.708			
vWFAg	A	143.30 ± 45.20	129.883	156.726	< 0.001*	A*O	< 0.001**
	B	151.37 ± 40.79	139.258	163.485		B*O	< 0.001**
	O	104.96 ± 40.11	93.313	116.604			
RiCof	A	100.97 ± 24.47	86.139	105.470	0.037*	B*O	0.031
	B	107.93 ± 25.95	94.349	113.203			
	O	83.26 ± 21.63	80.800	94.917			
CBA	A	95.80 ± 32.55	93.707	108.241	< 0.001*	A*O	0.001**
	B	103.78 ± 31.74	100.222	115.634		B*O	< 0.001**
	O	87.86 ± 24.31	76.983	89.542			

One-Way ANOVA: *significant at p < 0.05; **Significant Bonferroni at p < 0.017

Table VI: Association of smoking status and demographic characteristic with vWF profile

Variables	FVIII			vWF antigen			CBA			RiCof		
	Mean	Std. Deviation	p-value	Mean	Std. Deviation	p-value	Mean	Std. Deviation	p-value	Mean	Std. Deviation	p-value
Smoking												
Yes	130.15	35.80	0.643	134.97	43.60	0.747	100.50	26.27	0.270	101.75	19.97	0.157
No	133.33	35.31		132.06	47.64		94.04	31.39		95.61	27.79	
Gender												
Male	133.47	36.13	0.507	134.34	46.07	0.416	95.99	29.55	0.810	98.54	26.10	0.200
Female	128.34	31.93		126.09	48.71		94.41	33.49		91.26	25.61	
Age												
< 29	124.72	32.09	0.179	127.87	46.29	0.643	96.30	34.53	0.041*	90.47	27.39	0.507
30–49	136.33	37.12		135.82	47.23		96.66	28.49		99.37	24.69	
50–69	137.48	31.37		131.02	43.63		84.94	21.28		110.62	25.17	
BMI												
< 25	132.03	40.35	0.919	134.09	50.14	0.837	98.96	35.23	0.468	95.07	30.80	0.587
≥ 25	132.71	33.34		132.30	45.22		94.40	28.02		98.03	24.05	

Independent-t test, *Significant if p-value <0.05

BMI < 25: Underweight and normal > 25: Overweight and obese

Table VII: Multivariate analysis between blood group and vWF profiles

Independent variable	Dependent variables	Between subject effect			Multivariate test			
		F (df)	[b]p-value	Partial Eta-squared (η^2)	Wilks' Lambda	F (df)	[a]p-value	Partial Eta-squared (η^2)
Blood group	FVIII Ag	10.409 (2,137)	< 0.001	0.132	0.777	4.514 (8,268)	< 0.001	**0.119**
	vWF Ag	16.433 (2,137)	< 0.001	0.193				
	vWF CBA	3.376 (2,137)	0.037	0.047				
	vWF RiCof	13.206 (2,137)	< 0.001	0.162				

One-way MANOVA: [a]significant at p < 0.05; [b]significant at p < 0.0125

levels of components of the VWF profile found in Malays relative to other populations may be unique to the Malay population. However, a large population-based study is needed because the small sample size is an obvious limitation of the current study. Nevertheless, these preliminary findings suggested that ethnicity may impact VWF levels.

Genetic variants of the VWF gene in different ethnic groups may result in different levels and functions of the VWF protein. Van Schie et al. (2011) reported that four VWF gene variants affected the vWF profiles, namely single nucleotide polymorphisms rs7306706, rs1063857, rs216318, and rs4764478 (25). Johnsen et al. (2013) found that certain types of VWF variants were associated with higher or lower VWF levels (16). For example, they found that p.Arg2185Gln was associated with lower VWF and FVIII:C levels, whereas p.Thr789Ala or p.Asp1472His amino acid substitutions were associated with higher VWF levels. They concluded that the VWF missense mutation causes differences in phenotypes of VWF and FVIII:C among African Americans (16). Different testing methods might also play a role in the different outcomes of different studies. For example, the previously mentioned studies of Thais and Caucasians used the ELISA technique to quantify the VWF:Ag (15, 22), whereas we used the latex particle agglutination (immunoassay) technique. However, the immunoassay technique has a good correlation with the ELISA method in quantitative VWF:Ag testing (26). Chen et al. (2011) also reported that VWF:RiCof testing using the immunoassay technique has diagnostic accuracy comparable to that of the ELISA technique (27).

The cut-off level of 50 IU/dL was chosen as normal because less than that showed increased bleeding risk with a relative risk of 2.0-3.9 (28). However, by using this value as a definitive diagnosis of VWD would place 2.5% of the US population at risk of being diagnosed as having VWD (28). The prevalence of low VWF:Ag level and activity was common among Caucasians and Thais in previous studies but not among the Malays in the current study. All of the Malay subjects with low VWF:Ag level and VWF:CBA in this study had type O blood, and most subjects with low VWF:RiCof level had types O and A blood. These findings for particular constituents of the VWF profile were consistent with results of the studies conducted among Caucasians and Thais (15, 22). None of the subjects in the current study had VWF profile values < 30 IU/dL, except one male blood type B subject whose VWF:RiCof value was < 30 IU/dL. His ratio of VWF activity: antigen was 0.17. For this subject, the VWF:RiCof analysis should be repeated and bleeding history should be reevaluated. Further testing may be needed to rule out VWD Type 2.

The qualitative defects of the VWF protein can be suggested by evaluating the ratio of the VWF activity to the VWF:Ag. The lower ratio suggested of VWD Type 2. In the current study, the ratios of VWF:RiCof: VWF:Ag for groups A, B, and O were 0.70 + 0.56, 0.71 + 0.51, and 0.79 + 0.64, respectively, whereas the ratios of VWF:CBA: VWF:Ag for blood types A, B, and O were 0.67 + 0.66, 0.68 + 0.58, and 0.84 + 0.66, respectively. The ratios were slightly higher among O group compared to non-O group subjects. In contrast, mean FVIII:C and VWF levels (VWF:Ag, VWF:CBA, and VWF:Rcof) were significantly higher in non-O than in O type subjects ($p < 0.05$ for all comparison) of Chinese population as recently reported by Wang et al. (2017) (29). Furthermore, the average ratio of VWF:RiCof: VWF:Ag in Malays was slightly lower than that reported for Chinese Singaporeans (0.77 vs. 1.37) (21). The average ratio of VWF:CBA: VWF:Ag also was lower in Malays compared to Caucasians, African Americans, Indians, Chinese, and Thais (0.72 vs. 1.05, 1.03, 1.05, 1.18 and 0.92 respectively) (15, 22). These differences may suggest ethnic variation in the interaction of VWF with collagen and platelets. A previous study conducted among Chinese Singaporeans participants found that the reduction of VWF:CBA was greater than that of VWF:RiCof in blood type O subjects compared to non-O subjects (53% vs. 27%), which resulted in a lower VWF:CBA:VWF:Ag n ratio, whereas the VWF:RiCof:VWF:Ag ratio was unaffected (21). Recently, mean FVIII:C and VWF levels (VWF:Ag, VWF:CBA, and VWF:Rcof) were significantly higher in non-O than in O type subjects ($p < 0.05$ for all comparison).In the Malay population, the levels in O group subjects were not so different from those in non-O subjects (12% vs. 20%). These findings indicate the need for further study of other major races in Malaysia, especially Chinese and Indian.

In the Malay subjects in this study, the levels of components of the VWF profile were higher in subjects with type B blood, followed by those with blood types A and O. Previous studies also reported similar findings (22, 30). Post-hoc Bonferroni analysis revealed significantly higher levels of VWF profile elements in non-O compared to O blood group subjects, except for VWF:RiCof level. Sweeney et al. (1989) also reported that the VWF profile were affected by ABO blood group but VWF:RiCof did not reached significant association (31). MANOVA detected a statistically significant difference in each VWF profile component based on blood group, except for VWF:CBA. Previous studies reported that the level of VWF:Ag was highest in the AB blood group, followed by B, A, and O (22, 32). In the current study, we did not include AB group subjects, but the finding of higher VWF levels in subjects with type B followed by types A and O blood was similar to results of those studies. All findings in the current study confirmed that the VWF profile was influenced by the ABO blood group.

Significant evidence from *in vivo* and *in vitro* cell culture systems indicates that smoking can result in endothelial

cell (EC) damage, and damaged EC may release VWF into the circulation (33). A study conducted among Arab subjects found that VWF levels were higher in smokers compared to non-smokers (34). However, VWF level was not associated with smoking status in Malays, as was also reported in several other studies (6, 35). However, subjects in the current study were not classified by frequency of smoking, amount of sticks per day smoked, and type of cigarettes consumed, but all of these factors may influence the results. Kumari et al. (2000) reported that only subjects who smoked 21 sticks per day had significantly higher levels of VWF compared to lighter smokers (36).

Some researchers reported that the level of VWF:Ag increased with age (6, 7, 32). For example, Kadir et al. (1999) reported that with each decade increase of age, the levels of VWF:Ag increased by 0.17 U/ml and VWF activity by 0.15 U/ml (7). However, those findings were contradicted by other studies that found no significant effect of age on VWF level (30, 37). In the current study of Malays and another of a Thai population (22), VWF:CBA decreased with advancing age. Among Malays, the highest VWF:CBA occurred in subjects < 29 year old and the lowest in subjects 50–69 years old. Coppola et al. (2003) found that 51% of centenarians had a reduced relative proportion of high molecular weight multimers compared to younger subjects, which suggested that the number of large multimers decreases with increasing age (38). This was consistent with the finding that the highest VWF:CBA among Malays occurred in the youngest subjects and the lowest in the oldest subjects.
In general, the incidence of VWD is equal among males and females, although the incidence of bleeding symptoms is twice as high in females, possibly due to excessive vaginal bleeding during their reproductive years (39). Conlan et al. (1993) reported that mean levels of VWF and FVIII:C were lower in men than in women (6). However many other researchers reported no differences in VWF level based on gender (24, 30, 32, 40). In the current study of Malays, no significant association between gender and VWF profile was detected.

Folsom et al. (1992) reported that the levels of FVIII:C and VWF increased with increasing BMI (41). However, no significant associations between VWF profile and BMI of < 25 or > 25 were found in the current study, possibly due to the small sample size.

CONCLUSION

Results of this study showed that the levels of components of the VWF profile were influenced by ABO blood group, as the highest values were found among participants with type B blood, followed by types A and O. Low VWF:Ag and activity values were not common in Malays. The levels of plasma VWF among Malays were slightly higher than those reported for Thais, Caucasians, Indians, and Chinese. However, the average ratio of VWF activity to VWF:Ag was lower in Malays than in other populations. These findings suggest that ethnicity may impact the plasma VWF levels and ethnic variation in the interaction of the VWF protein with collagen and platelets.

The association of the VWF profile components with demographic data, such as the decrease of VWF:CBA with age, may help clinicians predict the bleeding risk in Malay patients, especially the elderly. A larger scale population study should be conducted and include participants from multiple ethnic groups in Malaysia. Molecular studies to identify VWF gene variants among Malay patients are also suggested, as such variants might impact the VWF levels and activities.

ACKNOWLEDGEMENTS

Appreciation to the Director of National Blood Center, Kuala Lumpur, Dr. Noryati Binti Abu Amin for approved budget to perform this study at National Blood Center. Special thanks to Mariana Binti Mohamed and Sufiza Binti Jamaluddin for their technical assistances.

REFERENCES

1. Sadler JE. Biochemistry and genetics of von Willebrand factor. Annu Rev Biochem. 1998; 67: 395-424.
2. Orstavik K, Magnus P, Reisner H, Berg K, Graham J, et al. 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.
3. Franchini M, Capra F, Targher G, Montagnana M, & Lippi G. Relationship between ABO blood group and von Willebrand factor levels: from biology to clinical implications. Thromb J. 2007; 5: 1.
4. Gallinaro L, Cattini MG, Sztukowska M, Padrini, R, Sartorello F, 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: 3540-3545.
5. Pittilo M. Cigarette smoking, endothelial injury and cardiovascular disease. Inter J Exp Pathol. 2000; 81: 219-230.
6. Conlan MG, Folsom AR, Finch A, Davis C, Sorlie P, et al. Associations of factor VIII and von Willebrand factor with age, race, sex, and risk factors for atherosclerosis. The Atherosclerosis Risk in Communities (ARIC) Study. Thromb Haemost. 1993; 70: 380-385.
7. Kadir RA, Economides DL, Sabin CA, Owens D, Lee CA. Variations in coagulation factors in women: effects of age, ethnicity, menstrual cycle and combined oral contraceptive. Thromb Haemost. 1999; 82: 1456-1461.
8. Kouides P, Kreuz W. Diagnosis and management of inherited von Willebrand disease in the next

decade: a clinical perspective. Thromb Res 2009; 124: S1.
9. Mohanty D, Shetty S. Von Willebrand disease: An update. J Blood Disorders Transf. 2014; 5: 2.
10. Neff AT. Current controversies in the diagnosis and management of von Willebrand disease. Ther Adv Hematol. 2015; 6: 209-216.
11. Srivastava A, Rodeghiero F. Epidemiology of von Willebrand disease in developing countries. Proceedings from Seminars in Thrombosis and Hemostasis. 2005.
12. World Federation of Hemophilia Report on the ANNUAL GLOBAL SURVEY. Quebec, World Federation of Hemophilia. 2010.
13. Periayah MH, Halim AS, Saad AZM, Yaacob NS, Karim FA. Report on von Willebrand Disease in Malaysia. Open Access Maced J Med Sci. 2016; 4: 112.
14. Miller CH, Dilley A, Richardson L, Hooper WC, Evatt BL. Population differences in von Willebrand factor levels affect the diagnosis of von Willebrand disease in African-American women. Am J Hematol. 2001; 67: 125-129.
15. Sukhu K, Poovalingam V, Mahomed R, Giangrande P. Ethnic variation in von Willebrand factor levels can influence the diagnosis of von Willebrand disease. Clin Lab Haematol. 2003; 25: 247-249.
16. Johnsen JM, Auer PL, Morrison AC, Jiao S, Wei P, et al. Common and rare von Willebrand factor (VWF) coding variants, VWF levels, and factor VIII levels in African Americans: the NHLBI Exome Sequencing Project. Blood 2013; 122: 590-597.
17. Musa RH, Ahmed SA, Hashim H, Ayob Y, Asidin NH, Choo PY, Al-Joudi FS. Red cell phenotyping of blood from donors at the National blood center of Malaysia. Asian J Transfus Sci. 2012; 6: 3.
18. Raihan K, Azmawati MN. Cigarette smoking and cardiovascular risk factor among male youth population. Mal J Public Health Med. 2013; 13: 28-36.
19. Department of Statistics Malaysia, 22 August 2016, Government of Malaysia, https://www.statistics.gov.my/ [22 August 2016]
20. Blood Bank Information System, Ministry of Health Malaysia, 2007, http://bbis/ [3 February 2017]
21. Chng WJ, Yip CY, Baliwag MB, Liu TC. Differential effect of the ABO blood group on von Willebrand factor collagen binding activity and ristocetin cofactor assay. Blood Coagul Fibrinolysis 2005; 16: 75-78.
22. Rojnuckarin P, Akkawat B, Intragumtornchai T. von Willebrand factor (VWF) antigen levels and function in healthy Thais. Southeast Asian J Trop Med Public Health 2005; 36: 1292.
23. Iso H, Folsom AR, Wu KK, Finch, A, Munger RG, et al. Hemostatic variables in Japanese and Caucasian men plasma fibrinogen, Factor VIIc, Factor VIIIc, and von Willebrand Factor and their relations to cardiovascular disease risk factors. Am J Epidemiol. 1989; 130: 925-934.
24. Lutsey P, Cushman M, Steffen L, Green D, Barr R, et al. Plasma hemostatic factors and endothelial markers in four racial/ethnic groups: the MESA study. J Thromb Haemost. 2006; 4: 2629-2635.
25. van Schie MC, de Maat MP, Isaacs A, van Duijn CM, Deckers JW, et al. Variation in the von Willebrand factor gene is associated with von Willebrand factor levels and with the risk for cardiovascular disease. Blood 2011; 117: 1393-1399.
26. Werfen Group Instrumentation Laboratory, July 2007, Werfen Group IVD, http://www.werfen.com/ [7 October 206]
27. Chen D, Tange J, Meyers B, Pruthi R, Nichols W, Heit JA. Validation of an automated latex particle–enhanced immunoturbidimetric von Willebrand factor activity assay. J Thromb Haemost. 2011; 9: 1993-2002.
28. Sadler JE. Von Willebrand disease type 1: a diagnosis in search of a disease. Blood 2003; 101: 2089-2093.
29. Wang Z, Dou M, Du X, Ma L, Sun P, Cao H, Ye S, Jiang P, Liu F, Lin F, Zhang R, Li C. 2017. Influences of ABO blood group, age and gender on plasma coagulation factor VIII, fibrinogen, von Willebrand factor and ADAMTS13 levels in a Chinese population. PeerJ 2017, 5:e3156.
30. Asuquo JI, Okafor IM, Usanga EA, Idongesit I. von Willebrand factor antigen levels in different ABO blood groups in a Nigerian population. Inter J Biomed Lab Sci. 2014; 3: 4-28.
31. Sweeney JD, Labuzetta JW, Hoernig LA, Fitzpatrick JE. Platelet function and ABO blood group. Am J Clin Pathol. 1989; 91: 79-81.
32. Gill JC, Endres-Brooks J, Bauer PJ, Marks WJ, Montgomery RR. The effect of ABO blood group on the diagnosis of von Willebrand disease. Blood 1987; 69: 1691-1695.
33. Pittilo M. Cigarette smoking, endothelial injury and cardiovascular disease. Inter J Exp Pathol. 2000; 81: 219-230.
34. Al-Awadhi AM, Jadaon MM, Alsayegh FA, Al-Sharrah SK. Smoking, von Willebrand factor and ADAMTS-13 in healthy males. Scand J Clin Lab Invest. 2012; 72: 614-618.
35. Rumley A, Lowe G, Sweetnam P, Yarnell J., Ford R. Factor VIII, von Willebrand factor and the risk of major ischaemic heart disease in the Caerphilly Heart Study. Br J Haematol. 1999; 105: 110-116.
36. Kumari M, Marmot M, Brunner E. Social determinants of von Willebrand factor the Whitehall ii Study. Arterioscler Thromb Vasc Biol. 2000; 20: 1842-1847.
37. Miller C, Haff E, Platt S, Rawlins P, Drews C, Dilley A, Evatt B. Measurement of von Willebrand factor activity: relative effects of ABO blood type and race. J Thromb Haemos. 2003; 1: 2191-2197.
38. Coppola R, Mari D, Lattuada A, Franceschi C. von Willebrand factor in Italian centenarians.

Haematologica 2003; 88: 39-43.
39. Lillicrap D. Von Willebrand disease: advances in pathogenetic understanding, diagnosis, and therapy. Blood 2013; 122: 3735-3740.
40. Yuan Z, Chen Y, Zhang Y, Liu H, Liu Q, et al. Changes of plasma VWF level in response to the improvement of air quality: an observation of 114 healthy young adults. Annals Hematol. 2013; 92: 543548.
41. Folsom AR, Conlan MG, Davis C, Wu K, et al. Relations between hemostasis variables and cardiovascular risk factors in middle-aged adults. Ann Epidemiol. 1992; 2: 481-494