VON WILLEBRAND FACTOR (vWF) ANTIGEN LEVELS AND FUNCTION IN HEALTHY THAIS

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Abstract. Type I von Willebrand disease (vWD) is very common in caucasians. Its genetic basis is possibly heterogenous, lying both within and out of the vWF gene locus. We sought to investigate vWF levels in the Thai population, to compare with those of western countries. The vWF antigen and activity were measured using ELISA and Collagen Binding Assay (CBA), respectively, in 311 healthy Thai volunteers. The mean age was 32.3, ranging from 18 to 75 years. Fifty-four percent were female. Low vWF antigen and activity (below 50 U/dl) were found in 3.5% and 10.2%, respectively. Around 75% and 20% of these cases had O and A blood groups, respectively. Three (0.96%) had definitely low levels of vWF (vWF antigen level below 35 U/dl), suggesting the diagnosis of vWD. Similar to previous studies, vWF levels were lowest in subjects with group O blood. We found that subjects with blood group A had higher vWF levels than group O subjects, but significantly lower vWF levels than those with group B. The average ratio between the vWF activity and antigen was 0.96, ranging from 0.66 to 1.66. These ratios were inversely correlated with age (p=0.047), suggesting a decline in vWF activity per vWF protein with advancing age. Low levels of vWF are common in healthy Thais. Clinicians should be aware of vWD in bleeding patients and beware low levels of vWF in therapeutic plasma products, especially from blood groups O and A.

INTRODUCTION

Type I von Willebrand disease (vWD), the partial quantitative defect of von Willebrand factor (vWF), is the most common congenital bleeding disorder in humans with a reported incidence of up to 1% in the general western population (Rodeghiero *et al,* 1987, 1990; Werner *et al,* 1993). The definitive diagnosis of the disease requires the presence of a mutation in the vWF gene. This can be demonstrated only in some cases with very low vWF levels, in conjunction with strong personal and familial bleeding symptoms (Laffan *et al,* 2004). However, vWF gene mutations have not been found in a large proportion of patients with modestly low levels of vWF with mild or questionable bleeding tendencies. Several lines of evidence show that bleeding symptoms in these subjects are significantly influenced by other factors outside the vWF gene locus. For example, vWF levels in the general population vary greatly, depending largely on ABO blood types. Individuals with blood group O have lower vWF levels than those with non-O blood groups (Gill *et al,* 1987). However, the diagnosis of vWD according to the blood-group-specific normal range is not helpful, because bleeding symptoms depend on vWF levels, regardless of the ABO blood types (Nitu Whalley *et al,* 2000). In addition, vWF levels are elevated by female hormone, physical exertion, even mental stress (Laffan *et al,* 2004). Furthermore, bleeding manifestations in cases with similar levels of vWF also depend on the platelet glycoprotein polymorphisms that contribute to platelet function (Kunicki *et al*, 2004). Therefore, low vWF levels, in contrast to vWD, should be regarded as a risk factor for bleeding, in the same way as other continuous variables, such as cholesterol and blood pressure, are risk factors of atherosclerosis (Sadler, 2003). High vWF levels have been reported to be predictive for coronary heart diseases (Thompson *et al,* 1995; Whincup *et al,* 2002). Nevertheless, a direct causal role of vWF in thrombosis has not been clearly demonstrated. High vWF levels may contribute to increased platelet adhesiveness, resulting in arterial thrombosis. Alternatively, it may merely be a marker of endothelial injury in subjects with atherosclerosis.

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vWF can be measured by its antigen or its function. The antigen levels are determined using an ELISA method. There are 3 main vWF function: collagen binding, platelet glycoprotein Ib binding, and factor VIII stabilization. These can be measured by collagen binding assay (CBA), ristocetin cofactor activity (RiCof), and factor VIII assay, respectively. RiCof, was shown to be more sensitive than vWF antigen ELISA for the screening of vWD in the general population (Rodeghiero *et al,* 1990). Therefore, both vWF antigen and function measurements are needed for the diagnosis of vWD. High molecular weight multimers of vWF are more active in binding to glycoprotein Ib and collagen. Therefore, RiCof and CBA are more sensitive than the antigen test in detecting type II vWD, in which the large vWF multimers are decreased or absent. The disproportionate reduction in vWF functions compared to antigen levels is suggestive of this condition. Compared to RiCof, CBA was shown to be more useful in the classification of vWD subtypes, using low vWF function: antigen ratio to diagnose type II vWD (Dean *et al,* 2000). Furthermore, CBA, based on an ELISA method, is less labor-intensive and more reproducible (Favaloro *et al,* 1993) than RiCof, and requires a platelet aggregometer.

Most of vWF level variations among races are due largely to ethnic differences in ABO blood groups. However, there are studies that show that within the same blood group, caucasians show lower vWF levels than Africans, but have similar levels to Indians (Miller *et al,* 2001; Sukhu *et al,* 2004). There is much less reported information on vWF levels in the Asian population. Hospital-based studies in China (Zhang *et al*, 2003) and Thailand (Chuansumrit *et al*, 2004) have shown that the prevalence of vWD is rare. However, population-based studies are still lacking. The objective of this study was to acquire preliminary data on vWF antigen and functional levels in the apparently healthy Thai population.

MATERIALS AND METHODS

Population

Three hundred eleven subjects were included in the study, including 220 healthy blood donors at the National Blood Center, Thai Red Cross Society, and 91 volunteers who were healthy medical students and medical personnel at Chulalongkorn University Hospital. Information regarding age and sex were recorded. ABO blood typing was performed at the National Blood Center on the blood donor group, and performed at our laboratory for the volunteer group. All subjects were asked for, and all denied any known history of, bleeding disorders. Blood was collected in the morning and immediately anticoagulated in 3.2% buffered sodium citrate. Plasma was separated within 3 hours of collection and frozen at -80ºC until tested.

Methods

The vWF antigen ELISA method was performed according to Mannucci *et al* (1999). Briefly, ELISA plates were coated with rabbit anti human vWF (DAKO A 0082; Dako, Glostrup, Denmark) before incubation with diluted plasma. Captured vWF was then reacted sequentially with peroxidase-conjugated rabbit anti-human vWF (DAKO P 0226), OPD substrate (Sigma P 6912, Sigma, St Louis, MO, USA) and H_2O_2.OD at 495 nm was then measured and vWF levels calculated from the standard curve generated from serially diluted International Standard Plasma (Dade Behring, Newark, DE, USA). One international normal control (control N) and one abnormal control (control P) were used in each run (Dade Behring). Both values needed to be within the company-defined ranges to assure that the test results were acceptable. Each sample and each control was tested in duplicate, using 2 different dilutions. The intra-assay coefficients of variation (CV) for normal and abnormal controls were 3.99% and 6.26%, respectively. The respective inter-assay CVs were 7.11% and 2.57%, respectively.

The vWF collagen-binding assay (CBA) method was performed, similar to the vWF antigen ELISA, except 25 µg/ml bovine collagen (ICN 193492, ICN Pharmaceuticals, Costa Mesa, CA, USA) was used to coat the plate instead of captured antibody. The collagen was comprised of 95% type I, and 5% type III collagen. These sources and concentrations of collagen were recommended by Favaloro *et al* (2000) for good overall utility, and the ability to discriminate between type I and II vWD. The results correlated strongly with vWF antigen in the normal popula-

tion (correlation coefficient 0.942). The intra-assay and inter-assay CVs were 4.29% and 5.25%, respectively.

The data were expressed as means ± standard deviations (SD) for descriptive purposes. The kurtosis and skewness of the data set were expressed as values ± 2 times standard error (2xSE). The Mann-Whitney was used for comparison of the means. Pearson correlation coefficients were utilized to examine continuous data. All statistical calculations were performed using SPSS 9.0 for Window Software.

RESULTS

The mean age of the subjects was 32.2 ± 10.9 years, ranging from 18 to 75 years. Forty-six percent were male. vWF antigen was measured in all of them, but the collagen binding assay (CBA) was performed in 226. Blood group information was available in 301.The means of vWF antigen, CBA and CBA/antigen ratio of the participants were 100.1 ± 38.7 U/dl, 92.5 ± 36.8 U/dl and 0.96 ± 0.13, respectively.

To examine the distribution of vWF levels in the Thai population, kurtosis and skewness values of the data were calculated. In normal distribution, both values are equal to zero. The values plus and minus 2 times standard errors that do not cross zero suggest non-normal distribution. Similar to previous reports in western countries, vWF antigen levels and activity levels showed a non-normal distribution with positive kurtosis (a high proportion of extreme values), and positive skewness (a high proportion of lower values), as shown in Fig 1. To investigate whether this was due to variations in the ABO blood group, kurtosis and skewness of each blood group were calculated separately. Interestingly, vWF levels of subjects with blood group O and AB were normally distributed, but those in blood groups A and B were not. vWF levels in blood groups A and B still displayed positive kurtosis and skewness (Fig 1A and B).

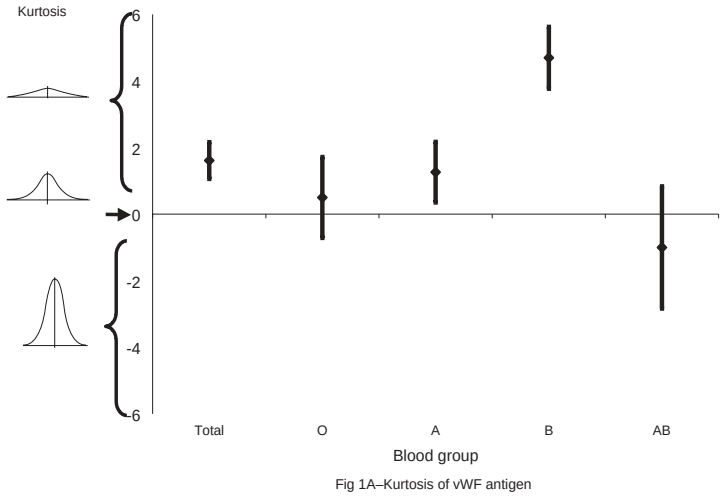

Fig 1A–Kurtosis of vWF antigen

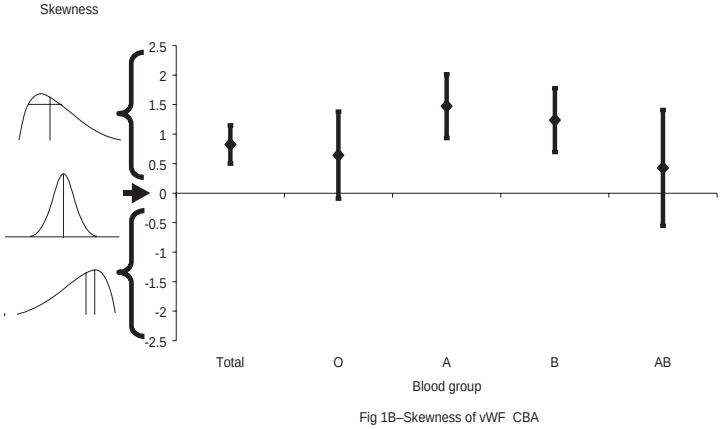

Fig 1B–Skewness of vWF CBA

Fig 1–Data distribution. A. Kurtosis of vWF antigen of the whole population and each blood group. The error bars represent ± 2 times standard errors of kurtosis. The bars crossing zero points suggest normal distribution (the graph in the middle). Positive values suggest flat-topped, wide-based curve (upper graph) and negative values suggest pointed-topped, narrow-based curve (lower graph). B. Skewness of vWF: Collagen binding assay of the whole population and each blood groups are shown. The error bars represent ± 2 times standard errors of kurtosis. The bars crossing zero points suggest normal distribution (the graph in the middle). Positive values suggest skew to the left (upper graph) and negative values suggest skew to the right (lower graph)

The means and standard deviations of the vWF antigens levels and function levels in the total subjects and categorized by ABO blood

Table 1
vWF levels in Thais compared with a previous report in Americans.

Blood group	% of total	vWF Ag (Thai)	VWF Ag (US)[a]	vWF CBA	vWFCBA: vWF Ag
O	36.9	81.6 ± 31.0	74.8	74.1 ± 31.1	0.95 ± 0.12
A	19.9	103.2 ± 38.0	105.9	92.3 ± 36.8	0.95 ± 0.10
B	35.2	114.3 ± 36.1	116.9	107.0 ± 31.9	0.97 ± 0.15
AB	8.0	115.7 ± 50.0	123.3	106.9 ± 43.9	0.96 ± 0.14

[a]Data from Gill *et al* (1987).

Table 2
The prevalence of low vWF levels and blood group correlation.

Blood group	VWF Ag < 50 U/dl (N=10)	CBA < 50U/dl (N=23)	VWF < 35 U/dl (N=3)	Total population
O	80%	74%	2/3	36.9%
A	20%	21.7%	1/3	19.9%
B	0%	4.3%	0	35.2%
AB	0%	0%	0	8.0%
Prevalence	3.5%	10.2%	0.96%	

groups are shown in Table 1. The values were comparable with previous reports in the US population (Gill *et al,* 1987). Subjects with blood group O had lower vWF antigen levels and function levels than those in the non-O blood group ($p < 0.001$). Notably, cases with blood group A had vWF antigen levels and function levels intermediate between groups O and B. vWF antigen levels and CBA of group A were significantly higher than group O, $p<0.001$ and $p=0.005$, respectively. In addition, the vWF antigen level and CBA in subjects with blood group A were significantly lower than those of group B ($p=0.036$ and $p=0.013$, respectively). vWF levels in blood groups B and AB were similar.

The prevalence of cases with low vWF was then analyzed (Table 2). Eleven subjects (3.5%) showed vWF antigen levels lower than 50% of the normal plasma level (50 U/dl). Eight of them (72.2%) had blood group O; Two (18.2%) were group A and no blood group data was available in one. All but one of these subjects also had a vWF CBA below 50 U/dl. This one had a CBA of 52 U/dl. Twenty-three of 226 (10.2%) had vWF CBA lower than 50 U/dl. Seventeen of them (70.8%) had blood group O, 5 (20.8%) had group A and 1 (4.2%) had group B. To estimate the prevalence of vWD, the arbitrary cut-off point of 35 U/dl was used. Three cases (0.96%) had vWF antigen below this point. Two had blood group O and the other had blood group A. Two of them showed vWF function levels below 35 U/dl and the other had a vWF CBA of 37 U/dl. On the other extreme, six subjects (1.93%) had vWF antigen levels above 200 U/dl. Four had blood group A and 2 had blood group B.

The vWF CBA to antigen ratio ranged from 0.66 to 1.66. Only one case showed a ratio below 0.7 (0.66). She was a blood group A subject with an vWF antigen level and activity level of 0.56 U/ml and 0.37 U/ml, respectively. There was no difference in the ratios in the blood groups and between the sexes. Interestingly, there was a negative correlation between the vWF function to antigen ratios and age, with a correlation coefficient of -0.164 ($p = 0.047$).

DISCUSSION

vWF levels in the general population varied highly, with positive kurtosis and a positive (leftward) skew. One of the causes is the difference in the ABO blood groups. Subjects with blood group O show lower vWF levels resulting in a positive skew in the whole population. When cases with each blood group were analyzed

separately, groups O and AB becomes normally distributed. However, those with blood groups A and B still showed positive kurtosis and skew. This may be due to their variation in A/B or H expression on group A and group B vWF. Cases with AO or BO genotypes that express lower A or B antigens and a higher H antigen have lower vWF levels than those with AA or BB genotypes (Shima *et al*, 1995, Souto *et al,* 2000). On the other hand, blood groups O and AB are genotypically homogenous, expressing similar levels of A/B and H antigens.

This study confirms previous reports in the caucasian population, that vWF levels were lower in subjects with blood group O. Around 75% of cases with low levels of vWF in our study had blood group O, although group O constituted only 37% of the whole population. Furthermore, we found that cases with blood group A showed vWF levels intermediate between those of groups O and B. A similar finding was also reported by Gill *et al* (1987), but no statistical analysis was performed. Consistent with this finding, the proportion of cases with low vWF antigen levels and function levels in blood group A was 20%, roughly equal to the proportion of group A in the general population. On the other hand, group B subjects with low vWF levels were very rare, although blood group B is more common than blood group A in our population. vWF has been found to express A and B antigens that may play roles in its adhesive functions (Sarode *et al*, 2000). One study has shown that vWF levels were inversely correlated with H antigen expression that is higher in blood group O and lower in blood group A (O'Donnell *et al,* 2002). In addition, vWF from blood group O was found to be more sensitive to proteolysis by ADAMTS13, explaining the lower levels of vWF level in this blood group (Bowen *et al*, 2003). However, in the same study, group B vWF is more sensitive to proteolysis than group A vWF. This cannot explain the lower vWF in blood group A than B found in our study. The mechanism underlying lower vWF levels in blood group A remains to be determined.

As a continuous variable with non-normal distribution, the normal range of vWF should be determined by clinical outcomes. An equivalent example is the serum cholesterol, for which the 'normal range' was obtained using the levels that do not increase the risk of coronary events. Notably, the 'normal range' for serum cholesterol has been decreasing over the past few years. For the vWF levels, values below 50 U/dl were shown to increase the risk of bleeding with a relative risk of 2.0-3.9 (Sadler, 2003). Therefore, this cut-off level was chosen. A low vWF level was common in Thais, especially when vWF CBA was used. Consistent with this finding, the vWF functional assay was shown to be more sensitive in detecting vWF in an epidemiological screening (Rodeghiero *et al*, 1990). The cut-off point for vWF CBA for predicting bleeding still needs further study.

An arbitrary vWF level of 35 U/dl was utilized to estimate the prevalence of vWD to represent the definitely low level. The prevalence of vWD of 0.96% was similar to reports in the Western population. A personal or familial history of bleeding was unavailable in our study. Consequently, a definite diagnosis of vWD cannot be made. However, the sensitivity of having a bleeding history for detecting mild vWD in cases who have never undergone surgery or trauma is probably low. In this study, we also found that the vWF CBA to vWF antigen ratio was declining with advancing age. Because large vWF multimers are more active in binding collagen, this finding suggests a decrease in large multimers with aging. Consistent with our data, a recent report has shown that about half of healthy centenarians showed reduced high-molecular-weight vWF multimers (Coppola *et al*, 2003). In this study, we demonstrated a progressive decline before that age.

In conclusion, vWF levels in Thais were comparable to previous reports in caucasians. Low levels were found to be common. Because plasma products are still used to treat vWD patients in developing countries, low levels of vWF in these products should be of concern, especially in subjects with blood group O and some with group A.

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