

**PRELIMINARY STUDY OF VON WILLEBRAND  
FACTOR PROFILES OF THE DIFFERENT ABO BLOOD  
GROUP AMONG MALAY POPULATION**

**BY  
DR. ROHAIDA BINTI ABDUL RAHMAN**

**DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF  
THE REQUIREMENT FOR THE DEGREE OF MASTER OF  
MEDICINE (TRANSFUSION MEDICINE)**

**ADVANCED MEDICAL AND DENTAL INSTITUTE (AMDI)**

**UNIVERSITI SAINS MALAYSIA**

**2016**

## **DECLARATION**

I hereby declare that this research has been sent to Universiti Sains Malaysia (USM) for the degree of Master of Medicine (Transfusion Medicine). It has not been sent to any other universities. With that, this research can be used for consultation and can be photocopied as a reference.

**DR. ROHAIDA BINTI ABDUL RAHMAN**

IPM0003/13

## ACKNOWLEDGEMENT

Bismillahirrahmanirrahim Alhamdulillahirabbila'lamin.

The highest gratitude of all goes to Allah the Almighty for His love and blessing, for giving me the opportunity to live in good health, giving me the guidance in making decisions, helping me to go through all the obstacles and difficulties during my study and finally completing my dissertation. Peace and blessing be upon Prophet Muhammad (pbuh) who had performed his duty to deliver the messages to all mankind. His patience had inspired me to face all the problems with confidence and looking forward to the future ahead with positive outlook.

I would like to take this opportunity to express my deepest gratitude to my supervisors, Dr.Rafeezul Bin Mohamed from Advanced Medical and Dental Institute and Dr.Tun Maizura Binti Mohd Fathullah from National Blood Centre, Kuala Lumpur for their guidance, supervision and comments. Without all that, it will be impossible for me to complete this dissertation. Special thanks to Dr.Rohayu Binti Hami, Dr. Noor Suzana Binti Mohd Shariff and Mr. Nizuwan Bin Azman from Advanced Medical and Dental Institute, for their contributions in the statistical analysis of this study.

Appreciation to the Director of National Blood Centre, Kuala Lumpur, Dr.Noryati Binti Abu Amin for giving me permission to perform this study at National Blood Centre. I am indebted to all the staffs at the haemostasis laboratory especially Cik Faridah Binti Afandi, Puan Mariana Binti Mohamed and Puan Sufiza Binti Jamaluddin as they had taught me a lot in the aspect of laboratory testing.

I would like to dedicate this thesis to my father, Haji Abdul Rahman Bin Yiacob who had always been the motivating factor behind me, giving me the best could he provided me with educations since my childhood and put his trust in me to succeed despite all odds.

To my beloved mom, Hajjah Rakhayah Binti Mohd Amin, no words can express my gratitude towards her, for her prayer and her encouragements. To my dear husband Khairul Saleh Bin Abdullah, thank you for your love and understanding that had kept me strong throughout the years. To my three lovely children, Farah Nur Alia, Muhammad Aniq Rayyan and Muhammad Aqeef Iman, I really hope that your patience of waiting for me to complete the journey of my study will be well worth. I wish to extend my sincere gratitude to all my friends, batch 2013 Transfusion Medicine Master Students. Thank you for all your help, support and advice towards the completion of the thesis.

## TABLE OF CONTENTS

|                             |       |
|-----------------------------|-------|
| Declaration.....            | ii    |
| Acknowledgement.....        | iii   |
| Table of Contents .....     | v     |
| List of Tables .....        | xii   |
| List of Figures .....       | xiv   |
| List of Abbreviations ..... | xv    |
| Abstrak .....               | xvi   |
| Abstract.....               | xviii |

### CHAPTER 1- INTRODUCTION

|  |    |
|--|----|
| 1.1 Overview .....                                   | 1  |
| 1.1.1 Von Willebrand Factor.....                     | 1  |
| 1.1.2 Von Willebrand Disease.....                    | 2  |
| 1.1.3 Von Willebrand Disease: epidemiology.....      | 7  |
| 1.1.4 Malaysia: Multiracial country.....             | 8  |
| 1.1.5 Ethnic variation in von Willebrand Factor..... | 9  |
| 1.1.6 vWF level influence by ABO blood group.....    | 10 |
| 1.2 List of definition.....                          | 11 |
| 1.3 Research Justification and Benefits .....        | 12 |

|                                |    |
|--------------------------------|----|
| 1.4 Research Objectives.....   | 13 |
| 1.4.1 General Objective .....  | 13 |
| 1.4.2 Specific Objectives..... | 13 |
| 1.5 Research Hypothesis .....  | 13 |
| 1.6 Conceptual Framework ..... | 14 |

## **CHAPTER 2- LITERATURE REVIEW**

|   |    |
|---|----|
| 2.1 The Malays.....                                   | 15 |
| 2.2 Malays and thromboembolic and bleeding event..... | 16 |
| 2.3 Malays and Von Willebrand Disease.....            | 16 |
| 2.4 Diagnosis of Von Willebrand Disease.....          | 17 |
| 2.5 VWF levels varies among population.....           | 21 |
| 2.6 ABO blood group and plasma VWF .....              | 23 |
| 2.7 Von Willebrand versus smoking habits.....         | 24 |
| 2.8 Von Willebrand versus gender.....                 | 25 |
| 2.9 Von Willebrand versus age group.....              | 25 |
| 2.10 Von Willebrand versus body mass index.....       | 26 |

## **CHAPTER 3- MATERIALS AND METHODS**

|                       |    |
|-----------------------|----|
| 3.1 Study Design..... | 27 |
|-----------------------|----|

|   |    |
|---|----|
| 3.2 Study location.....                               | 27 |
| 3.3 Study Variables.....                              | 28 |
| 3.3.1 Dependent Variables .....                       | 27 |
| 3.3.2 Independent Variables.....                      | 27 |
| 3.4 Subjects.....                                     | 28 |
| 3.4.1 Inclusion Criteria.....                         | 29 |
| 3.4.2 Exclusion Criteria.....                         | 29 |
| 3.5 Sample Size.....                                  | 30 |
| 3.6 Sampling Method.....                              | 34 |
| 3.7 Duration of study.....                            | 34 |
| 3.8 Research tools and materials.....                 | 34 |
| 3.8.1 Porforma.....                                   | 34 |
| 3.8.2 Laboratory Apparatus and Equipment.....         | 35 |
| 3.8.3 Chemicals and Reagents.....                     | 36 |
| 3.8.4 Kits and Consumable.....                        | 36 |
| 3.9 Flow chart of study.....                          | 37 |
| 3.10 Statistical Analysis .....                       | 38 |
| 3.11 Operational Definition.....                      | 38 |
| 3.12 Ethical Issues .....                             | 40 |
| 3.13 Blood Samples Preparation and Test Procedur..... | 41 |

|                                 |    |
|---------------------------------|----|
| 3.13.1 ELISA .....              | 41 |
| 3.13.2 ACL Top 500 .....        | 43 |
| i) Factor VIII.....             | 43 |
| ii) Von Willebrand Antigen..... | 44 |
| iii) RiCof.....                 | 45 |

## **CHAPTER 4- RESULTS**

|   |    |
|---|----|
| 4.0 Introduction.....   | 47 |
| 4.1 Descriptive Analysis.....   | 47 |
| 4.1.1 Distribution of demographic characteristics and smoking<br>habit.....                               | 47 |
| 4.1.2 Distribution of blood group.....  | 48 |
| 4.1.3 Distribution of Von Willebrand profiles.....  | 48 |
| 4.2 Statistical Analysis (Univariate Analysis).....   | 51 |
| 4.2.1 Difference of distribution of vWF profiles between blood<br>group.....                              | 51 |
| 4.2.2 Association of demographic characteristics and smoking habit<br>among donors with vWF profiles..... | 52 |
| i) Association of smoking habits with vWF profiles.....   | 52 |
| ii) Association of gender with vWF profiles.....  | 53 |

|   |    |
|---|----|
| iii) Association of age group with vWF profiles.....  | 53 |
| iv) Association of BMI with vWF profiles.....         | 54 |
| 4.3 Statistical Analysis (Multivariate Analysis)..... | 55 |

## **CHAPTER 5- DISCUSSION**

|   |    |
|---|----|
| 5.0 Overview.....   | 57 |
| 5.1 Bleeding history .....                                      | 57 |
| 5.2 von Willebrand profiles in Malays.....                      | 58 |
| 5.2.1 Genetics variants among different ethnic group .....      | 59 |
| 5.2.2 Method of testing .....                                   | 60 |
| 5.2.3 Pre-analytical variables .....                            | 60 |
| 5.3 Prevalance of low von Willebrand profiles among Malays..... | 61 |
| 5.4 Ratio of vWF activity: vWF antigen.....                     | 62 |
| 5.5 ABO blood group and Von Willebrand Profiles.....            | 63 |
| 5.5.1 ABO and vWF antigen.....                                  | 63 |
| 5.5.2 ABO and Factor VIII.....                                  | 64 |
| 5.5.3 ABO and vWF RiCof.....                                    | 64 |
| 5.5.4 ABO and vWF CBA.....                                      | 65 |
| 5.6 Association of smoking habits with vWF profiles.....        | 68 |
| 5.7 Association of age group with vWF profiles.....             | 69 |

|  |    |
|--|----|
| 5.8 Association of gender with vWF profiles..... | 70 |
| 5.9 Association of BMI with vWF profiles.....    | 70 |
| 5.10 Conclusion.....                             | 71 |

## **CHAPTER 6- CONCLUSION, RECOMMENDATIONS AND LIMITATIONS**

|   |    |
|---|----|
| 6.1 Conclusion.....                         | 72 |
| 6.2 Limitations of the current study.....   | 72 |
| 6.3 Recommendation for Future Research..... | 72 |

|                       |           |
|-----------------------|-----------|
| <b>REFERENCE.....</b> | <b>74</b> |
|-----------------------|-----------|

|                        |           |
|------------------------|-----------|
| <b>APPENDICES.....</b> | <b>86</b> |
|------------------------|-----------|

|            |   |     |
|------------|---|-----|
| Appendix 1 | Approval from Medical and Ethics Committee, Ministry of Health..... | 87  |
| Appendix 2 | Annual Ethical Renewal for 2016.....                                | 89  |
| Appendix 3 | Approval from Human Research Ethics Committee (HREC).....           | 90  |
| Appendix 4 | Participants Information Sheet (English).....                       | 92  |
| Appendix 5 | Participants Information Sheet (Malay).....                         | 97  |
| Appendix 6 | Subject Consent Form (English).....                                 | 102 |
| Appendix 7 | Subject's Material Publication Consent (English).....               | 103 |
| Appendix 8 | Participant Consent Form (Bahasa Melayu).....                       | 104 |

|             |   |     |
|-------------|---|-----|
| Appendix 9  | Subject's Material Publication Consent (Bahasa Melayu)..... | 105 |
| Appendix 10 | Bleeding Tendency Questionnaire from NHLBI.....             | 106 |
| Appendix 11 | Blood Donor Registration Form (Bahasa Melayu).....          | 107 |
| Appendix 12 | Research PORFORMA.....                                      | 111 |
| Appendix 13 | Preventive Maintenance of ACL Top 500.....                  | 112 |
| Appendix 14 | Validation of ACL Top 500.....                              | 114 |
| Appendix 15 | Preventive Maintenance of Centrifuge Kubota.....            | 115 |
| Appendix 16 | Validation of Centrifuge Kubota.....                        | 116 |
| Appendix 17 | Preventive Maintenance of ELISA Reader.....                 | 117 |

## LIST OF TABLES

|           |  | Page |
|-----------|--|------|
| Table 1.1 | Classification of von Willebrand disease.  | 3    |
| Table 1.2 | Guideline to diagnose vWD<br>(National Heart, lung and Blood Institute-2008).                      | 5    |
| Table 3.1 | vWF Antigen Levels and Function in Healthy Thais.  | 30   |
| Table 3.2 | Sample size to determine vWF profiles of different ABO<br>blood group among Malays donors.         | 31   |
| Table 3.3 | Sample size to compare the vWF profiles of different ABO<br>blood group among Malays blood donors. | 32   |
| Table 3.4 | List of laboratory apparatus and equipment   | 35   |
| Table 3.5 | List of chemicals and reagents.  | 36   |
| Table 4.1 | Demographic characteristics and smoking habits among the<br>Malay donors.                          | 47   |
| Table 4.2 | Distribution of blood group between the regular Malay<br>donors.                                   | 48   |
| Table 4.3 | Distribution of vWF profiles in Malays.  | 48   |
| Table 4.4 | Distribution of vWF profiles and blood group correlation.  | 49   |
| Table 4.5 | The prevalence of low vWF profiles level (less than 50 IU/dl)<br>and blood group correlation.      | 49   |
| Table 4.6 | Ratio of vWF activity: vWF antigen in Malays   | 50   |
| Table 4.7 | Ratio of vWF activity:vWF antigen according to blood group<br>in Malays                            | 50   |

|            |  |    |
|------------|--|----|
| Table 4.8  | Difference of distribution of vWF profiles between blood group | 51 |
| Table 4.9  | Association of smoking habits with vWF profiles.               | 52 |
| Table 4.10 | Association of gender with vWF profiles.                       | 53 |
| Table 4.11 | Association of age group with vWF profiles.                    | 53 |
| Table 4.12 | Association of BMI with vWF profiles.                          | 54 |
| Table 4.13 | Multivariate analysis between blood group and vWF profiles.    | 55 |

## **LIST OF FIGURES**

|   |    |
|---|----|
| Figure 1.1 Conceptual Framework.                                | 14 |
| Figure 3.1 Study flow chart.                                    | 37 |
| Figure 3.2 ELISA Reader Infinite F50.                           | 46 |
| Figure 3.3 Automated Coagulation Analyser ACL TOP 500.          | 46 |
| Figure 5.1 ABO blood group and risk of thrombosis and bleeding. | 67 |

## LIST OF ABBREVIATIONS

|          |  |
|----------|--|
| vWF      | von Willebrand Factor.   |
| ADAMTS13 | A Disintegrinlike And Metalloprotease domain (reprolysin type) with thromboSpondin type 1 motif, member 13]. |
| vWD      | von Willebrand Disease.  |
| FVIII    | Factor VIII.   |
| APTT     | Activated Partial Thromboplastin Time test.  |
| PAI-1    | Plasmin Activator Inhibitor-1.   |
| vWF:Ag   | von Willebrand antigen.  |
| RiCof    | Ristocetin Cofactor Assay.   |
| CBA      | Collagen Binding Assay.  |
| ACL      | Automated Coagulometer.  |
| BMI      | Body Mass Index.   |
| AMDI     | Advance Medical and Dental Institute.  |
| USM      | Universiti Sains Malaysia.   |
| NBCKL    | National Blood Centre Kuala Lumpur.  |
| ANOVA    | Analysis of Variance.  |
| MANOVA   | Multivariate Analysis of Variance.   |
| MOH      | Ministry of Health.  |
| WHO      | World Health Organization.   |
| NHLBI    | National Heart, Lung, and Blood Institute.   |

## ABSTRAK

**Latar Belakang:** Di kalangan 28 juta penduduk Malaysia pada tahun 2010, sebanyak 0.002% pesakit von Willebrand dilaporkan dengan 63% daripadanya berbangsa Melayu. Disebabkan kepelbagaian genetik pada gene vWF dan faktor-faktor lain, paras vWF di dalam darah dan presentasi penyakit berbeza antara individu. Objektif kajian ini adalah untuk mendapatkan data profil vWF di kalangan Melayu yang berkumpulan darah ABO yang berbeza dan untuk melihat hubungkait demografi data dan status merokok, dengan paras profil vWF.

**Kaedah:** Seramai 140 penderma darah Melayu yang mempunyai kumpulan darah ABO yang berbeza, terlibat dalam kajian keratin rentas yang dijalankan di Pusat Darah Negara, Kuala Lumpur. Paras FVIII diukur dengan kaedah 'coagulometric clot detection', vWF antigen dan RiCof, dengan kaedah 'latex particles agglutination' dan CBA dengan kaedah 'ELISA'.

**Keputusan:** Majoriti penderma (59.3%) berumur 30-49 tahun, lelaki (81.43%), bukan perokok (74.3%) dan, mempunyai berat badan berlebihan atau obesiti (40.7%). We found the vWF profiles were higher in B blood group, followed by A and O. Didapati paras profil vWF tertinggi adalah pada kumpulan darah B diikuti oleh A dan O. Paras (IU/dL) Faktor VIII, antigen vWF, RiCof dan CBA di kalangan kumpulan darah A ialah  $138.77 \pm 37.74$ ,  $143.30 \pm 45.20$ ,  $100.97 \pm 24.47$ ,  $95.80 \pm 32.55$ , kumpulan darah B ialah  $144.43 \pm 31.69$ ,  $151.37 \pm 40.79$ ,  $107.93 \pm 25.95$  dan  $103.78 \pm 31.74$ , manakala kumpulan O ialah  $115.10 \pm 29.65$ ,  $104.96 \pm 40.11$ ,  $83.26 \pm 21.63$  dan  $87.86 \pm 24.31$ . Prevalens vWF antigen dan CBA yang rendah (<50 IU/dL) adalah jarang pada Melayu iaitu 1.4% dan 0.7%. Kesemua mereka berkumpulan darah O dan A. Tiada subjek yang mempunyai vWF antigen <30 IU/dL. Seorang subjek mempunyai RiCof <30 IU/dL tetapi parameter ujian

lainnya adalah normal. Dalam kes ini, perlu diulangi ujian RiCof dan ujian lanjut mungkin juga perlu bagi memastikan beliau tiada penyakit von Willebrand Jenis 2. Nisbah purata RiCof: antigen di kalangan kumpulan A, B dan O adalah  $0.70 \pm 0.56$ ,  $0.71 \pm 0.51$  dan  $0.79 \pm 0.64$ , manakala nisbah CBA: antigen adalah  $0.67 \pm 0.66$ ,  $0.68 \pm 0.58$  dan  $0.84 \pm 0.66$ . Didapati juga paras CBA mempunyai hubungkait yang signifikan dengan kumpulan umur. Kajian juga mendapati paras vWF antigen di kalangan Melayu sedikit tinggi tetapi nisbah vWf aktiviti: antigen rendah berbanding populasi kulit putih, India, Cina dan Thailand.

**Kesimpulan dan cadangan:** Seperti kajian sebelumnya, subjek berkumpulan O mempunyai paras profil vWF yang rendah berbanding bukan O. Adalah dicadangkan untuk mengambil nisbah aktiviti vWF: antigen  $<0.6$  sebagai aras untuk meningkatkan pengesanan vWD varian di kalangan Melayu. Bagi kajian yang akan datang, dicadangkan untuk membuat ujian molekular di kalangan pesakit von Willebrand berbangsa Melayu bagi mengenalpasti kewujudan varian pada gene vWF yang mungkin menyebabkan paras dan aktiviti vWF protein yang berbeza di kalangan bangsa tersebut berbanding dengan populasi lain. Dicapangkan juga untuk membuat kajian yang melibatkan populasi yang lebih besar dengan penyertaan pelbagai kaum di Malaysia dan subjek berkumpulan darah AB kerana saiz sampel yang kecil menjadikan limitasi pada kajian kali ini.

**Kata Kunci:** Profile Faktor von Willebrand, Kumpulan darah ABO, Melayu

## ABSTRACT

**Background:** In the year 2010, 0.002% von Willebrand disease patients were reported among 28 million of Malaysia population, with 63% of them from the Malay ethnicity. Due to multiple genetic makeup on vWF gene and other factors, the vWF level in the bloodstream and disease presentation vary among different individuals. The objective of this research is to obtain the data of vWF profiles of the different ABO blood type among Malays and to observe the association of demographic characteristic and smoking habit with the profiles.

**Methodology:** One hundred and forty (140) of the different ABO blood group Malay donors were involved in the cross sectional study administered in the NBCKL. FVIII, vWF antigen and RiCof, and CBA levels were measured by coagulometric clot detection, latex particles agglutination and ELISA methods respectively.

**Results:** Majority of the donor (59.3%) were aged between 30-49 years, male (81.43%), non-smoker (74.3%) and, overweight and obese (71.4%). We found that the vWF profiles were higher in B blood group, followed by A and O. The levels (IU/dL) of FVIII, vWF antigen, RiCof and CBA in A blood group were  $138.77 \pm 37.74$ ,  $143.30 \pm 45.20$ ,  $100.97 \pm 24.47$ ,  $95.80 \pm 32.55$  respectively, in B blood group were  $144.43 \pm 31.69$ ,  $151.37 \pm 40.79$ ,  $107.93 \pm 25.95$  and  $103.78 \pm 31.74$  respectively and, in O blood group were  $115.10 \pm 29.65$ ,  $104.96 \pm 40.11$ ,  $83.26 \pm 21.63$  and  $87.86 \pm 24.31$  respectively. The prevalence of low (<50 IU/dl) vWF antigen and CBA were rare among the Malays which were 1.4% (n=2) and 0.7% (n=1) respectively. All of them were in the O and A blood groups. None of the subject had vWF antigen <30 IU/dL. One subject had RiCof <30 IU/dL but the other test parameters were normal. In this case, RiCof test should be repeated and further investigation may require to exclude vWD Type 2. The average ratio of RiCof: antigen

among A, B and O groups were  $0.70 \pm 0.56$ ,  $0.71 \pm 0.51$  and  $0.79 \pm 0.64$  whereas the ratio of CBA: antigen were  $0.67 \pm 0.66$ ,  $0.68 \pm 0.58$  and  $0.84 \pm 0.66$ . It was also observed that the level of CBA was significantly-interrelated with the age group. The vWF antigen in Malays were slightly higher but the average ratio of vWF activity: antigen was slightly lower compared to Caucasians, Indian, Chinese and Thais.

**Conclusion and recommendation:** Similarly with the previous studies, subjects in O group had lower vWF profiles compared to non-O. We recommended to set up cut-off 0.6 for the ratio of vWF activity: antigen to improve detection of vWD variance in Malays. Molecular study among Malay vWD patients is suggested to find out the existence of variant on vWF genes, which may result in a different vWF levels and activities in Malays. It is also suggested that a bigger scale population-based study should be administered with the participation of multiple ethnics in Malaysia and for those with AB blood type as smaller sample size has been a limitation in this study.

**Keywords:** von Willebrand Factor profiles, Malays, ABO blood group

# CHAPTER 1

## INTRODUCTION

### 1.1 OVERVIEW

#### 1.1.1 von Willebrand Factor (vWF)

vWF is a plasma protein that mediates the initial adhesion of platelets at the sites of vascular injury. It also acts as a carrier for blood clotting Factor VIII (FVIII) and stabilizes the factor, hindering it from proteolysis in the circulation (Sadler, 1998). Therefore, any defect in vWF can cause bleeding due to impaired platelet adhesion capability or by reducing the concentration of FVIII.

vWF is synthesized by the endothelial cells (EC) throughout the body and stored in weibel-palade bodies. The small amount of the factors is synthesized by megakaryocytes and stored in the alpha granule of the platelets (Sadler, 1998). vWF is a large multimer glycoprotein, ranging in size from 500,000 to more than 20 million daltons with more than 2 micrometres in length (Mohanty and Shetty, 2014). It is coded by a gene located at a short arm of chromosome 12 (Ginsburg *et al.*, 1985). ADAMTS13 is a plasma metalloprotease that cuts the vWF protein at the peptide bond Tyr1605-Met1606 (Doldan-Silvero *et al.*, 2008). It will rapidly reduce the size of the vWF multimer at the time it is secreted into the plasma thus determining the vWF's specific activity. The interaction of vWF with the platelet and collagen depends on the size of its multimers. The most effective size to assist wound healing are 5000–10 000 kilo daltons, under conditions of high shear stress (Stockschlaeder *et al.*, 2014).

As an acute phase reactant protein, the levels of vWF in circulation varies. It is increased during acute infectious illnesses and dropped after the recovery period (Pottinger *et al.*, 1989). The level also varies among normal individuals or when measured repeatedly among the same individuals (Werner *et al.*, 1995). Orstavik (1985) reported that most (60%) of the reasons for the variance among individuals were due to genetic factors in which 30% of that, was due to the effect of ABO blood type (Orstavik *et al.*, 1985).

The fate of the vWF in circulation depends on the size of the protein multimer, the interaction with the platelet or other cells, the exposure to ADAMTS13 for proteolysis and the rate of clearance in the liver or spleen (Sadler, 2009).

### **1.1.2 von Willebrand Disease (vWD)**

vWD is the commonest hereditary bleeding disorder worldwide. The disease is a highly heterogeneous disorder with bleeding event, ranging from asymptomatic or very minimal bleeding symptom to very severe life threatening haemorrhage (Mohanty and Shetty, 2014). It is caused by the decreased amount or abnormal function of the vWF protein. There are 3 types of vWD that have been reported. vWD Type 1 and 3 are due to quantitative defect of vWF protein, whereas Type 2 is caused by qualitative defects of the protein. In vWD Type 1, there is a minimal deficiency of plasma vWF whereas in vWD Type 3, the level of the factor in circulation is very low or almost nil.

The mild quantities deficiency of vWF in vWD Type 1 leads to mild bleeding tendency while in Type 3 leads to life threatening bleeding events. Table 1.1 showed the classification of vWD as adapted from (Gill, 2004).

Table 1.1 Classification of von Willebrand disease (Gill, 2004).

| <b>vWD type</b> | <b>Prevalence</b>                         | <b>Pathophysiologic characteristics</b>  | <b>Diagnostic vWF Assays</b>   |
|-----------------|---|--|--|
| Type 1          | 1–2% of general population; 70–80% of vWD | Decreased amount of normal vWF.  | Proportionally decreased vWF RiCof and vWF Ag with normal multimers; Factor VIII C variably decreased.   |
| Type 3          | 1:250,000                                 | Complete absence of vWF.   | Absent vWF RiCof, vWF Ag, and vWF multimers; Factor VIII C 3–5%.   |
| Type 2A         | 10% of diagnosed vWD                      | Failure of mutant vWF to multimerize or increased proteolysis of mutant vWF.   | Decreased vWF RiCof, normal or decreased vWF Ag, absent high and intermediate molecular weight multimers.  |
| Type 2B         | Rare 20% of vWD Type 2                    | Adsorption of high molecular weight mutant vWF multimers from plasma due to increased binding to platelet glycoprotein 1b (GP 1b). | Decreased vWF RiCof, normal or decreased vWF Ag, absent high molecular weight multimers, increased RIPA at low-dose ristocetin, increased vWF binding to normal platelets, thrombocytopenia.   |
| Type 2M         | Rare                                      | Abnormal vWF binding site for platelet GP 1b.  | Decreased vWF RiCof, normal or decreased vWF Ag, normal vWF multimer structure, decreased vWF binding to normal platelets.   |
| Type 2N         | Rare                                      | Abnormal vWF binding site for Factor VIII.   | Normal vWF RiCof, normal vWF: Ag, normal vWF multimer structure, decreased Factor VIII C, decreased Factor VIII binding to vWF.  |
| Platelet-type   | Rare                                      | Adsorption of high molecular weight vWF multimers from plasma due to increased binding to mutant platelet GP 1b.                   | Decreased vWF RiCof, normal or decreased vWF antigen, absent high molecular weight multimers, increased RIPA at low-dose ristocetin, normal vWF binding to normal platelets, thrombocytopenia. |

vWF: von Willebrand Factor, vWF Ag: von Willebrand Factor antigen, vWF RiCof: von Willebrand Factor Ristocetin Cofactor Assay, RIPA: Ristocetin-induced platelet aggregation, Factor VIII C : Coagulation Factor VIII

The heterogeneous of the disease presentations and the laboratory findings which may overlap with normal subjects, make it challenging for the clinicians to establish the diagnosis of vWD. The molecular study may correspond to the specific variants, but has a wide range of genetic mechanisms (Fernández and de Alarcón, 2014).

Currently, diagnosis of vWD is based on an array of laboratory tests (vWF antigen, Factor VIII, vWF Ristocetin Cofactor Assay and VwF Collagen Binding Assay) together with the history of increased bleeding tendency, which usually is also present in the family members. The blood investigation should be carried out in a dedicated laboratory that is qualified to perform all the tests correctly and providing the patients with a balanced view of their bleeding risks. In Malaysia, the National Blood Centre in Kuala Lumpur is one of the accredited laboratories to perform all of these tests.

Family and patient's bleeding history are very important in determining the diagnosis of vWD and serve better prediction of future bleeding than depending only on plasma vWF level. Further testing on subjects with the vWF level of 30 to 50 IU/dL will not identify the clinically significant patients whereas testing on the bleeding patients will identify only the relatively small number of patients with clinical severity of vWD Type 1, 2 and 3, and larger numbers with a moderately low vWF level. (Sadler, 2009). However, the high prevalence of mild bleeding symptoms even in a normal individual requires the usage of a standardized questionnaire and bleeding score for the identification of patients who require further laboratory evaluation for vWD (Rodeghiero *et al.*, 2005). In the current study, we used the standardized questionnaire extracted from Guidelines from the National Heart, Lungs, And Blood Institute (NHLBI) 2008 (Nichols *et al.*, 2008) as a screening tool to exclude any bleeding tendencies in donors and their family members.

The Table 1.2 is the guideline from the NHLBI, United States of America (USA) to diagnose vWD. Currently National Blood Centre, KL followed the NHLBI guideline and the treatment given to the patient were depended on the severity of the bleeding and the type of the disease.

Table 1.2 Guideline to diagnose vWD (National Heart, Lung, And Blood Institute-2008).

| <b>Condition</b> | <b>Description</b>  | <b>FVIII<br/>(IU/dL)</b> | <b>vWF Ag<br/>(IU/dL)</b> | <b>RiCof<br/>(IU/dL)</b> | <b>RiCof/<br/>vWF Ag</b> |
|------------------|---|--------------------------|---------------------------|--------------------------|--------------------------|
| Type 1           | Partial quantitative vWF deficiency (75% of symptomatic vWD).                                       | ↓ or normal.             | <30                       | <30                      | >0.5-0.7                 |
| Type 2A          | Low vWF-dependent platelet adhesion with selective deficiency of high-molecular-weight multimer.    | ↓ or normal.             | <30-200                   | <30                      | <0.5-0.7                 |
| Type 2B          | Increase affinity for platelet GP 1b.   | ↓ or normal.             | <30-200                   | <30                      | Usually<br><0.5-0.7      |
| Type 2M          | Low vWF-dependent platelet adhesion without selective deficiency of high-molecular-weight multimer. | ↓ or normal.             | <30-200                   | <30                      | <0.5-0.7                 |
| Type 2N          | Markedly decrease binding affinity for FVIII.   | ↓↓                       | 30-200                    | 30-<br>200               | >0.5-0.7                 |
| Type 3           | Virtually complete deficiency of vWF (severe, rare).  | ↓↓↓<br>(<10IU/dl).       | <3                        | <3                       | Not<br>applicable        |
| Low vWF          |   | Normal.                  | 30-50                     | 30-<br>50                | >0.5-0.7                 |
| Normal           |   | Normal.                  | 50-200                    | 50-<br>200               | >0.5-0.7                 |

vWF: von Willebrand Factor, vWF Ag: von Willebrand Factor antigen., vWF RiCof: von Willebrand Factor Ristocetin Cofactor Assay, FVIII: Factor VIII

Currently, the National Blood Centre, KL followed the NHLBI guidelines and the treatment given to the patients are dependent on the severity of the bleeding and the types of the disease.

The NHLBI chose the vWF antigen and RiCof of less than 30 IU/dL as the level for a definitive diagnosis of vWD because of the high prevalence of the O blood group in the United States of America (USA) which is associated with low vWF antigen levels, the absence of genetic abnormality in patients with mild to moderate level of RiCof and the presence of a significant number of individuals with a bleeding history but no underlying disease detected.

The absent of vWF (vWD Type 3) and many qualitative defects (vWD Type 2) are straightforward diagnosis but not in vWD Type 1 due to a broader distribution of normal vWF level, the high prevalence of mild bleeding symptoms even in a normal person and the weak relationship between vWF level and bleeding event. Therefore, none of the vWF level can isolate patients into a group with clearly different clinical features (Sadler, 2003). Consequently, many vWD Type 1 patients do not have the specific bleeding disease at all which limits the value of the diagnosis. False positive diagnosis of vWD Type 1 also causes many patients to be subjected to risky, expensive and ineffective treatments, while the actual causes of symptoms are overlooked and untreated. Many patients had to change their lifestyles due to fear of bleeding and some have even been denied of insurance coverage.

Recent studies of vWD Type 1 patients have defined several pathophysiologic mechanisms that determine the vWF plasma concentration but the relationship between the vWF levels and the probability of bleeding remains inconclusive and the difference between 'normal' and 'low' is undistinguishable. These problems might be resolved by

an epidemiologic approach to vWF and other risk factors for the bleeding event (Sadler, 2009).

The treatment of vWD is dependent on the severity of the bleeding symptoms, the type of the disease and the type of surgical procedure that the patient plans to proceed with. The treatments include drugs to induce the releasing of vWF and FVIII into the blood circulation to prevent lysis of the blood clots, to control heavy menstrual bleeding in women and to replace the vWF.

While inherited vWD is the commonest inherited bleeding disorder, acquired vWD is a rare bleeding disorder. It is associated with other diseases such as lymphoproliferative or myeloproliferative disorders, malignancies, immunologic diseases, various congenital cardiac defects or other drug related ailments. The production and function of vWF in this type of diseases are normal, but the factor is rapidly eliminated from circulation by autoantibodies, absorption of the vWF onto the cancer cell clones or loss of high-molecular-weight vWF multimers under the high shear stress (Federici, 2006).

### **1.1.3 von Willebrand disease: Epidemiology**

Previously, vWD is classified as a rare disease (Office of Rare Diseases of the National Institutes of Health, US). However, Rodeghiero (1987) revealed that the prevalence of the Italian population with vWD was between 0.57%-1.15% (Rodeghiero *et al.*, 1987). Meanwhile, Werner (1993) showed that the prevalence among the USA population was at 1.3% (Werner *et al.*, 1993). In 2010, Bowman reported that there were relatively low prevalence of medically significant bleeding patients attending primary health clinics and a low prevalence of symptomatic vWD in the primary care setting which was at least 1 in 1000. They suggested that further investigations needed to be carried out to investigate the discrepancies of these prevalent findings (Bowman *et al.*, 2010).

The disease shows no geographical nor ethnic preferences. As an autosomal disorder, both genders inherit the mutant vWF alleles equally, but women show frequent bleeding symptoms by almost 2:1 probably because of the excessive per vaginal bleeding during their reproductive age (Lillicrap, 2013).

Data on the vWD epidemiology in developing countries are very limited. Although there is no accurate data on the estimation of vWD prevalence in those countries, the available data suggests that the diagnosed cases were less than the actual number, accounting for only 6% to 13% of patients with hereditary bleeding disorders. The number with severe diseases tend to be much higher, particularly in certain parts of the world where consanguineous marriage is common (Srivastava and Rodeghiero, 2005). In Malaysia, only 0.002% (n=464) of the diagnosed cases were reported among the 28 million of the population (World Federation of Hemophilia, 2010). Most of the patients were diagnosed to have vWD Type 1 (77.2%) (Periayah *et al.*, 2016). This corresponds with the estimated prevalence in some countries where the symptomatic patients that was seen at haemostasis clinics ranging from 23 to 110 per million population (0.0023–0.01%) (Nichols *et al.*, 2008). In another study conducted by Hassan (2012), it is found that vWD were common among menorrhagia patients who attended the gynaecology clinic in Hospital Universiti Sains Malaysia, Kelantan, which accounted up to 13.3%. They suggested that vWD testing should be provided to complete the diagnostic work-up for menorrhagia (Hassan *et al.*, 2012). It was similar with other studies which reported that the frequency of vWD in menorrhagia ranges from 5% to 20% (Kujovich, 2005).

#### **1.1.4 Malaysia is a multiracial country**

An interesting fact about the Malaysian society is the diversity of its ethnic composition. It is the result of large population movements in the nineteenth and early twentieth

centuries (Schafgans, 1998). Currently, Malaysia has a population of 31.2 million, consisting of 15.3 million of women and 16.4 million of men where 68.6% of them are Malays, 23.4% are Chinese, and 7% are Indians and 1% of other ethnic groups (<https://www.statistics.gov.my/>). Therefore, we have decided to perform the study among the Malays because it represents the majority of the country's population (68.6%) and the majority (63.0%) of the vWD patients diagnosed in Malaysia (Periayah *et al.*, 2016). In the National Blood Centre, KL the majority (49.5%) of donors were also Malays (Blood Bank Information System [BBIS] National Blood Centre Kuala Lumpur 2015).

The distribution of the ABO blood group among our blood donors, have been reported by Musa in 2012. They revealed about 34.5 % of Malays were in the O group, 27.5% were in group B, 30.5% were in group A and 7.5% were in group AB (Musa *et al.*, 2012). In addition, Manoharan (2013) also reported that 39% of Malays were in group O, 32% were in group B, 23% were in group A and 13% were in group AB as the subjects of the study were students at Asia Metropolitan University of Malaysia. In the current study, we included the blood group O, A and B of Malay donors, but excluded group AB as it is less prevalent among our population (Manoharan *et al.*, 2013; Musa *et al.*, 2012)

#### **1.1.5 Ethnic variation in von Willebrand Factor**

In previous population studies, they found that vWF were higher among African Americans than Caucasians. These racial differences in vWF further complicated the issues surrounding a diagnosis of vWD (Miller *et al.*, 2001). Another study performed in South Africa with a distinct ethnic mixture of Africans, Caucasians and Indians reported that the African Americans had significantly higher vWF antigen and Factor VIII levels when compared to others. However, they found that the Indians had comparable levels of vWF with Caucasians. They suggested that the influence of ethnicity on the vWF levels

should also be considered in the clinical and laboratory evaluation of vWD (Sukhu *et al.*, 2003). This is contradictory to the earlier study conducted by Werner (1993), who found that there were no significant differences in vWF activity via ethnicity but they only carried out the study focusing on the age group of paediatric subjects (Werner *et al.*, 1993). Later, Johnsen (2013) in the NHLBI exome sequencing project found that some vWF missensed variants, which were commonly or uncommonly present among certain ethnicity had contributed to the phenotypic variation of the vWF and Factor VIII (Johnsen *et al.*, 2013).

Even when they had different ethnicity than Caucasians, Rojnuckarin (2005) reported that the vWF profiles among Thais were comparable with the earlier reports in the USA population (Rojnuckarin *et al.*, 2005).

#### **1.1.6 von Willebrand Factor level influence by ABO group**

ABO blood groups greatly influence the plasma level of vWF as the O blood group subjects have a lower vWF levels compared to non O blood group (Franchini *et al.*, 2007). Rojnuckarin (2005) found that AB blood group subjects had the highest level of vWF followed by B, A and O (Rojnuckarin *et al.*, 2005). The ABO blood group appeared to strongly influence the clearance of vWF, but not its protein synthesis or its release from EC (Gallinaro *et al.*, 2008). In a study conducted by Orstavik (1985) revealed that Factor VIII was dependent on vWF antigen levels and 30% of the genetic variance of vWF antigen were due to the effect of ABO blood group (Orstavik *et al.*, 1985). They also found that the concentration of plasma vWF and Factor VIII were the lowest in O, higher in A2, and highest in A1 and B group subjects (Orstavik *et al.*, 1985). For the diagnosis of vWD among the different ABO blood group, the National Heart, Lung, and Blood Institute chose the less than 30 IU/dL plasma vWF level for a definitive diagnosis of vWD

because there is a high prevalence of O blood group in the USA which is associated with ‘low’ vWF levels. They did not use different reference ranges for diagnosing vWD among group O individuals. In this current study, we focus on blood group A, B and O as AB group is less prevalence among our population, our blood donors and Malay donors at NBCKL.

## 1.2 List of definitions

1.2.1 Body mass index: Is a measure of body fat based on height and weight that applies to adult men and women. BMI categories:

|                 |               |
|-----------------|---------------|
| Underweight =   | <18.5         |
| Normal weight = | 18.5–24.9     |
| Overweight =    | 25–29.9       |
| Obesity =       | 30 or greater |

(<http://www.nhlbi.nih.gov/>)

1.2.2 vWF antigen: Test to measures the quantity of a vWF in the circulation.

1.2.3 ADAMTS13: A Disintegrin like And Metalloprotease domain (reprolysin type) with ThromboSpondin type 1 motif, member 13]. A plasma protein that cleaves multimeric vWF.

1.2.3 RiCof: Ristocetin Cofactor Activity; test to evaluate the capability of vWF to bind platelet glycoprotein 1b (GP 1b) and promote platelet plug formation.

1.2.4 CBA: Collagen Binding Assay; test to evaluate the capability of vWF to bind to collagen, mimicking the interaction with the subendothelial matrix at the site of vascular injury.

(<http://practical-haemostssis.com/>)

1.2.5 Rare disease: Disease that affects less than 200,000 people in the US population.

(<https://www.genome.gov/>)

### **1.3 Research Justification and Benefits**

vWD is the commonest hereditary bleeding tendency disorder encountered in almost 1% of the worldwide population (Kouides and Kreuz, 2009). However, the diagnosis of vWD Type 1 is very challenging due to the broad distribution of normal vWF levels, the high prevalence of mild bleeding symptoms even in the normal population and the weak relationship between vWF levels and the bleeding events.

In the National Blood Centre, Kuala Lumpur (NBCKL), the interpretation of laboratory results for vWF profiles follows the guidelines from the NHLBI. However, the normal range that is being used by the NHLBI was established according to the Caucasians population. There are several studies found that the levels of vWF and Factor VIII were significantly higher in blacks compared to the white population (Fleming, 2003; Gomperts *et al.*, 1976; Kadir *et al.*, 1999; Miller *et al.*, 2001) proposed that the reference ranges of laboratory haemostasis investigations, cannot be used as a reference across the world as the range is largely based on the results obtained from the Caucasians.

Up to the best of our knowledge, the normal reference range of the vWF profiles in the Malaysian population is not established yet. This study will serve as preliminary data of the vWF profiles among the Malay population in our country. If the data obtained from this study is largely deviated from the NHLBI 2008 data, we then have to conduct a larger scale population-based study in the future to obtain our own normal vWF profiles reference range. It may include the 3 major races in Malaysia (Malays, Chinese and Indians), including all ABO blood groups.

There are limited studies on vWF levels among the Asian compared to the western population. It is hope that this study will serve as a preliminary data on vWF antigen and activity levels in the healthy Malay population in Malaysia.

## **1.4 Research Objectives**

### **1.4.1 General Objectives**

To study von Willebrand Factor profiles of the different ABO blood group among Malay donors at NBCKL in 2015.

### **1.4.2 Specific Objectives**

- 1) To determine the von Willebrand Factor profiles (Factor VIII, von Willebrand antigen, RiCof and Collagen Binding Assay) of the different ABO blood group among Malay donors at NBCKL in 2015.
- 2) To compare the von Willebrand Factor profiles (Factor VIII, von Willebrand antigen, RiCof and Collagen Binding Assay) with the different ABO blood group among Malay donors at NBCKL in 2015.
- 3) To assess the influence of demographic data (gender, age group and BMI) and smoking habit with the von Willebrand Factor profiles among Malay donors at NBCKL in 2015.

## **1.5 Research Hypothesis**

- 1) There are differences in the von Willebrand Factor profiles in different ABO blood groups among Malay donors at NBCKL.
- 2) There are association between demographic data (gender, age group and BMI) and smoking habit, and the von Willebrand Factor profiles among Malay donors at NBCKL.

## 1.6 Conceptual Framework

There are a few factors that may influence the levels of plasma vWF. Some of them are stated in the figure below. In the current study, we examined the effect of ABO blood group and donors' demographic data (gender, age group and BMI) and smoking habit on the plasma vWF profiles.

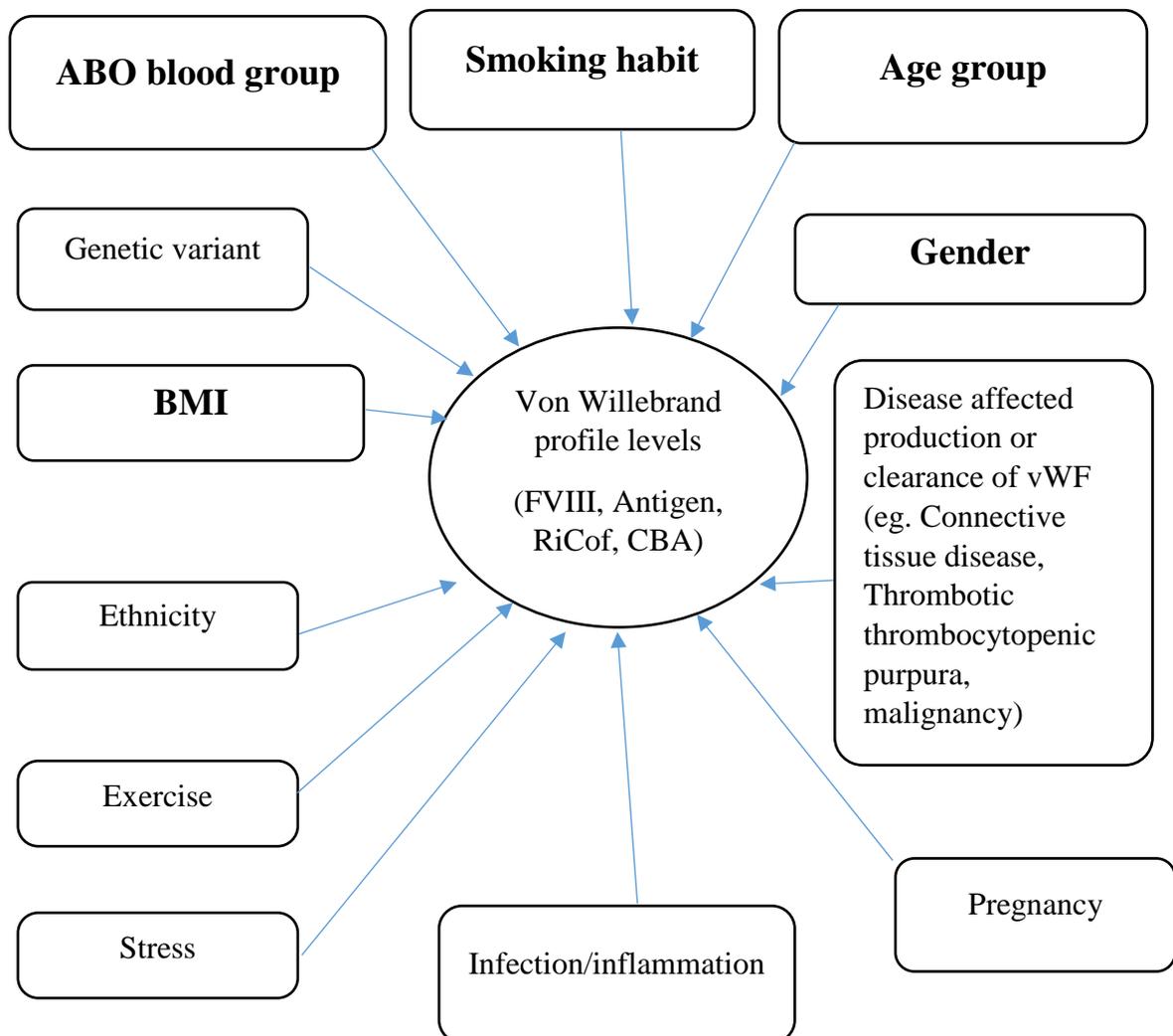


Figure 1.1 Factors may affecting the levels of vWF

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 The Malays

The Malays is the race of people who live mainly in Peninsular Malaysia and a portion of the adjacent island of Southeast Asia, including the coast of Borneo, the east coast of Sumatra and small islands that lie between these areas (<http://sabrizain.org/malaya/>). Based on their migrations a few centuries ago, there are various sub-ethnic groups in the Malay population present currently, which are believed to have different ancestral origins. The major sub-ethnics are Melayu Jawa, Melayu Minang, Melayu Bugis, Melayu Kelantan and Melayu Kedah (Hatin *et al.*, 2011). Some of the present day Malays are mixed with modern Chinese, Indian, Arab and Thai blood (<http://sabrizain.org/malaya/>). Most of the Malays in Malaysia are practising Islam as their religion, speak Malay language and practice Malay customs (*adat*) and cultures.

The Malays is one of the unique ethnicity in the world with different genetic variances compared to others. In the fields of population genetics and forensics, the human X chromosome has been focused on by many researchers in recent years. Samejima (2012) has carried out a genetic study on the X-chromosomal short tandem repeats (X-STRs) and found 12 X-STRs in the Malay population that differed from East Asian, European, or African populations (Samejima *et al.*, 2012). In the genetic study for drug metabolism, Teh (2001) found that the genetic polymorphism of CYP2D6 in Malays was different from the Chinese and Far Eastern races. These variances result in ethnic differences in the metabolism of CYP2D6 drugs (Teh *et al.*, 2001). The genetics differences are also present among sub-ethnic group as previously reported by Hatin (2014) and that there is a genetic mixture among sub-ethnic Malays in Peninsular Malaysia (Hatin *et al.*, 2011).

These genetic variants may increase risk among the Malays to inherit certain diseases or resistance to certain drugs.

## **2.2 Malays and thromboembolic and bleeding event**

Up to the best of our knowledge, there are very limited published studies on thromboembolic or bleeding event among the Malays. Nawawi (2002) reported that the prevalence of the coronary risk factors among rural Malays in Malaysia was high according to the Global Risk Assessment. Apart from genetic predisposition, the high prevalence was probably due to the rapid socioeconomic development at the rural areas (Nawawi *et al.*, 2002). Loo (2012) had described a few studies on the prevalence of stroke (bleeding or ischaemic) in different states in Malaysia where they found about 86.1% of the patients were Malays and 13.9% were Chinese in Kelantan whereby 55.7% were Chinese, 28.9% were Malays and 14.2% were Indians in Pulau Pinang (Loo and Gan, 2012). This may reflect upon the local population as many Malays reside in Kelantan compared to the majority of Chinese located in Pulau Pinang especially on the island. However, these also may reflect the differences of genetic among the ethnicity which results in different risks of thromboembolic or bleeding incident. In 2004, Kandasami had conducted a study to look into the prevalence of bleeding events (peptic ulcer disease) among different ethnicity in Malaysia and found over presented symptoms in the Chinese but similar to the ethnic distribution in the Malays and Indians (Kandasami *et al.*, 2004).

## **2.3 Malays and von Willebrand disease**

Apart from collecting, processing and distributing of blood and its components to hospitals, the National Blood Centre, Kuala Lumpur also provides expert medical services and groundwork for research in the field of haematology. Based on the recent update from the National Blood Centre, the effect of vWD was quite low among

Malaysians due to under reporting even after many conferences, campaigns, awareness and colloquia that have been organized. Only 545 cases were reported from the year 1979 to 2013 (Periayah *et al.*, 2016). The prevalence was highest in the Malays (63%) followed by Indians (15.2%) and lowest in Chinese (5.5%) Most of the patients were diagnosed to have vWD Type 1 and about 40% of them were males and 60% were females (Periayah *et al.*, 2016).

#### **2.4 Diagnosis of von Willebrand disease**

vWD is a heterogeneous disorder and a very complex disease (Sadler and Gralnick, 1994). It is very difficult to establish the diagnosis of vWD especially in Type 1 disease due to the wide distribution of the normal vWF level, high prevalence of mild bleeding symptoms even in a normal population and the weak relationship between vWF levels and bleeding events. In Type 1, the vWF level is not obviously low, but usually near to the lower end of the normal vWF range. The wide distribution of vWF levels in a normal population, makes the situation more complicated. Almost 95% of the plasma vWF levels lie between 50 to 200 IU/dL among the 300 million of the USA population and around 7.5 million people who had the vWF levels less than 50 IU/dL would be at risk for the diagnosis of vWD Type 1 (Nichols *et al.*, 2008). 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 (Sadler, 2003).

Nitu-Whalley (2000) had conducted a retrospective study to investigate the difficulties in making a diagnosis of vWD Type 1 in one of the Hemophilia Centre in United Kingdom (Nitu-Whalley *et al.*, 2000). They found that among previously diagnosed vWD Type 1, 41% of them were in group O and had between 30-50 IU/dL of vWF levels, with or without a history of increase in bleeding tendencies. Those groups of patients might

require reclassification as 'not vWD' and searching for alternative diagnosis for the bleeding symptoms might be needed.

In view of the bleeding history, the standardized questionnaire is extremely important to screen bleeding risks among the population. Rodeghiero (2005) proposed the use of a standardized questionnaire and bleeding score to identify the patients who need laboratory evaluation for the vWD (Rodeghiero *et al.*, 2005). Friberg (2006) observed that about 23% of Swedish girls reported 3 or more haemorrhagic symptoms when using a self-reported questionnaire (Friberg *et al.*, 2006), whereas Rodeghiero (2005) revealed that by using a standardized questionnaire, they found less than 1% of normal control had 3 or more of the symptoms (Rodeghiero *et al.*, 2005).

Apart from the bleeding history, the diagnosis of vWD is also based on an array of laboratory tests to investigate the amount and function of the protein. The tests looked at the amount of vWF (vWF antigen) and determining the activity of the protein; to carry and stabilize the Factor VIII (Factor VIII Assay), to bind to the glycoprotein 1b on the platelet surface (vWF RiCof) and to bind to the collagen (vWF CBA). The primary site of platelet binding is in the A1 domain of vWF, whereas collagen binding is in the A3 domain. The interaction of platelets with vWF is forced *in vivo* by shear stress, which causes a conformational change of the protein and allows its binding to the platelet glycoprotein 1b (Sadler, 1998). *In vitro*, the interaction is aggravated by the antibiotic ristocetin, in the lack of shear stress condition (Scott *et al.*, 1991).

Similar to the vWF RiCof, vWF CBA relies on the size of the vWF multimeric in which the larger size will bind more avidly than the smaller forms. However, Dean (2000) conducted a study to compare the vWF CBA and vWF RiCof and found that the vWF CBA was most helpful in the classification of vWD Type 2 variants, using a low vWF

activity: vWF antigen ratio (Dean *et al.*, 2000). Similarly, Casonato (2001) observed that in disease Type 2A and 2B, even when both vWF CBA and vWF RiCof were decreased, the vWF CBA was more constant and they suggested to include vWF CBA to the test panel for diagnosis of vWD (Casonato *et al.*, 2001). A ratio of vWF CBA: vWF antigen of less than 0.5 is consistent with types 2A and 2B disease, whereas a normal ratio (0.5 or more) are associated with types 2M and 2N disease (Popov *et al.*, 2006). Flood (2013) found that vWF CBA provides a sensitive method to capture the variant of vWD especially by using the lowest (0.6) cut-off of vWF CBA: vWF antigen ratio (Flood *et al.*, 2013).

In early 2000, Favaloro reported that the ability to discriminate the subtype of vWD was dependant on the type of collagen used in CBA in which Type III or a mixture of Type I/III showed highest sensitivity (Favaloro *et al.*, 2000). In the current study, we used collagen Type III to accurately discriminate the subtype if found. Riddell (2002) reported that even if vWF CBA is a sensitive method to detect functional variants related to the loss of high molecular weight multimers, it's incapable to detect defective platelet-binding vWD variants in the presence of normal high molecular weight multimers but vWF RiCof does. They concluded that the vWF CBA should be used in association with vWF RiCof rather than as a replacement for it (Riddell *et al.*, 2002). A guideline from the UK Hemophilia Centre Doctors' Organization, 2014 also recommended to use both vWF RiCof and CBA to increase the ability to detect Type 2 variants and clear definition of vWD Type 1 (Laffan, M.A *et al.*, 2014).

An alternative to vWF RiCof, some laboratories offer vWF-Activity (vWF-Ac) to assess the function of vWF to bind to the platelet receptors without the presence of ristocetin. Geisen (2014) in a comparison study between vWF activity and vWF RiCof on

aggregometer showed a good correlation between the two methods (Geisen *et al.*, 2014). Other laboratory tests such as Ristocetin Induced Platelet Aggregation (RIPA) and multimeric analysis are also essential in order to differentiate the different subtypes of vWD.

Apart from that, molecular study is also one of the methods to distinguish vWD and its variants. vWD is highly heterogeneous due to the molecular mechanisms that produce various clinical presentation and laboratory findings. However, the requirement for the study in vWD is variable because the usefulness of genetic testing varies for different vWD subtypes. In case of vWD Type 1, the picture of the disease is not really clear as the causative molecular defect is unidentified in a significant number of cases, and even in those cases in which the causative mutation is known. The association of molecular pathology is not necessarily understood (Keeney *et al.*, 2008). Although genetic analysis is not mandatory to diagnose vWD or to define a classification type, it may be useful in isolated situations (Favaloro *et al.*, 2010).

Nowadays, various tests are available to diagnose vWD, however NHBLI in their guidelines suggested carrying out three initial tests namely vWF antigen, vWF RiCof and Factor VIII (Nichols *et al.*, 2008). If one or more results is noted to be abnormal, further test such as vWF CBA, RIPA, Factor VIII binding, platelet vWF studies, multimer distribution, ratio of vWF RiCof: vWF antigen and molecular study are recommended to distinguish the type of vWD and its variants. In NBCKL, vWD patients are normally classified based on the 3 essential laboratory investigations namely Factor VIII, vWF antigen and vWF CBA (Periyah *et al.*, 2016). However, in the current study, we performed a full investigation panel of vWF profiles including vWF RiCof.