THE EFFECTIVENESS OF PRE-DONATION WATER HYDRATION IN REDUCING VASOVAGAL REACTION AND ITS RISK FACTORS AMONG YOUNG BLOOD DONORS IN KELANTAN

BY

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DECLARATION

I hereby declare that this research has been sent to Universiti Sains Malaysia for the degree of Masters of Medicine in Transfusion Medicine. It is not to e sent to any other universities. With that, this research might be used for consultation and can be photocopied as reference.

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LIST OF ABBREVIATIONS

ADR	Adverse Donor Reaction
AMDI	Advanced Medical and Dental Institute
AMT	Applied Muscle Tension
BDRI	Blood Donor Reaction Inventory
BMI	Body Mass Index
BTS	Blood Transfusion Service
DVT	Deep Vein Thrombosis
EBV	Estimated Blood Volume
HUSM	Hospital Universiti Sains Malaysia
IHN	International Haemovigilance Network
ISBT	International Society of Blood Transfusion
LOC	Loss of Consciousness
MI	Myocardial Infarction
NBC	National Blood Center
TIA	Transient Ischaemic Attack
TMU	Transfusion Medicine Unit
TTI	Transfusion Transmissible Infection
USM	Universiti Sains Malaysia
VS	Versus
VVR	Vasovagal Reaction
WHO	World Health Organization

ABSTRAK

Latar belakang: Reaksi Vasovagal (VVR) merupakan kesan sampingan yang paling kerap berlaku ketika pendermaan darah di seluruh dunia. Reaksi vasovagal ini mempunyai kesan negatif terhadap usaha mengekalkan penderma darah. Tujuan kajian ini adalah mengkaji keberkesanan hidrasi dengan meminum air pra-pendermaan bagi mengurangkan kesan reaksi vasovagal pada penderma darah muda di Kelantan.

Kaedah: Kajian quasi intervensi telah dilakukan terhadap penderma – penderma darah di Kelantan yang menderma darah di tempat pendermaan darah bergerak yang dianjurkan oleh Unit Perubatan Transfusi, Hospital Universiti Sains Malaysia (HUSM) sepanjang tempoh lapan bulan Sebanyak 284 penderma darah muda yang layak menderma darah berumur diantara 17 hingga 23 tahun dibahagikan kepada dua kumpulan iaitu kumpulan hidrasi dan tiada hidrasi. Penderma darah kemudian dinilai menggunakan BDRI 4-Items pada 30 minit dan pada 48 jam pendermaan.

Keputusan: Kadar insiden reaksi vasovagal dikalangan penderma – penderma darah muda di Kelantan adalah sebanyak 25.4%. Secara statistik, kadar reaksi vasovagal di dalam kumpulan hidrasi adalah kurang secara signifikan berbanding kumpulan tiada hidrasi (15.5% vs. 32.5%) (nilai p = 0.0001). Usia muda, kurang berat badan dan kurang isipadu darah adalah risiko – risiko untuk reaksi vasovagal. Min skor BDRI 4-Items pada 30 minit dan pada 48 jam adalah lebih rendah secara signifikan bagi kumpulan hidrasi berbanding kumpulan tiada hidrasi (nilai p =

0.0001). Skor BDRI 4-Items pada 30 minit dan pada 48 jam menunjukkan korelasi yang positif pada kumpulan tiada hidrasi sahaja.

Kesimpulan: Hidrasi dengan meminum air pra-pendermaan adalah berkesan untuk mengurangkan kadar reaksi vasovagal, dikalangan penderma darah muda di Kelantan.

Kata Kunci: reaksi vasovagal, risiko reaksi vasovagal, Hospital Universiti Sains Malaysia (HUSM), hidrasi dengan meminum air pra-pendermaan, Skor BDRI

ABSTRACT

Background: Vasovagal Reaction (VVR) is the commonest adverse reaction that occurred among blood donors worldwide. It has negative effect towards donor retention. Therefore it is important to reduce the VVR incidence. The aim of this study was to determine the effectiveness of pre-donation water hydration in reducing VVR among young blood donors in Kelantan.

Methods: A quasi experimental study was performed among blood donors in Kelantan who donate blood in blood drives conducted by Transfusion Medicine Unit, Hospital Universiti Sains Malaysia (HUSM) over a period of eight months. A total of 284 young blood donors age from 17 to 23 years old, were assigned equally into two groups, hydration and non hydration group. Donors then were assessed with BDRI 4-Items at 30 minutes and 48 hours post blood donation.

Results: The incidence of VVR among young blood donors in this study was 25.4%. Statistically, a hydration group showed significantly lower VVR incidence compared to non hydration group (1.5% vs. 32.5%)(p value = 0.0001). Younger age, lower weight, lower estimated blood volumes were associated with VVR incidence. The mean BDRI score at 30 minutes and at 48 hours were significantly lower in hydration group compared to non hydration group. BDRI 4-Items score at 30 minutes and at 48 hours showed positive correlation only in non hydration group.

Conclusions: This study showed that pre-donation water hydration was effective in reducing VVR incidence among young blood donors in Kelantan.

Key words: vasovagal reaction, vasovagal syncope, risk factors for VVR, Hospital Universiti Sains Malaysia (HUSM), pre-donation water hydration.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction of Adverse Donor Reaction

Adverse Donor Reaction (ADR) is any untoward event or complication experienced by the donor before, during or after the blood donation process. It is a part of haemovigilance programme (a set of surveillance procedures covering the entire blood transfusion chain, which is from the collection of blood from donors until transfusion of the blood to the patients, including their follow-up). All blood transfusion service (BTS) need to report any ADR incidence to the National Haemovigilance Programme in National Blood Centre (NBC). NBC later will compile all ADR cases and produce the national haemovigilance report which includes the incidence rate and current trends. This report is to assess the risk in term of incidence rate, factors influencing each ADR, the current trend and finally constructing strategies to manage the risk. Therefore it is a great significance for each BTS to report every ADR cases and submit it to NBC.

ADR can be divided into systemic, local symptoms, aphaeresis related complications, allergic reaction, major cardiovascular event, and others (ISBT/IHN definitions., 2014). Another classification method of ADR are acute or delayed, and categorization through the severity of the adverse event which is mild, moderate and severe (Gonçalez *et al.*, 2012; Yuan *et al.*, 2010). Table 1 showed the type of ADR and the examples of each type.

Table 1: Types of ADR and examples of each type (Gonçalez *et al.*, 2012)

Local complications	Generalized symptoms	Aphaeresis Related Complications	Major Cardiovascular Event
• Haematomas/ Bruises • Arterial puncture • Nerve irritation or injury • Inflammation • Thrombophle-bitis • Allergic reaction • Compartment syndrome	• Vasovagal reaction • Deep vein thrombosis (DVT) • Anaphylaxis	• Citrate reaction • Haemolysis • Air embolism	• Myocardial infarction (MI) • Stroke • Transient ischemic attack (TIA) • Cardiac arrest • Death

1.2 Overview of Vasovagal Reaction

Vasovagal reaction (VVR) is defined as a general feeling of discomfort and weakness with anxiety, dizziness and nausea, which may progress to loss of consciousness (faint) (ISBT/IHN definitions.,2014). It is the commonest adverse donor reaction. Many researchers tried to explain why vasovagal reaction (VVR) occurred after blood donation. Reflex bradycardia and hypotension that occurred after a blood donation played an important role during VVR. It can happen due to a wide variety of stimuli, for example, pain and anxiety. Combination of increased cardiovagal tone which results in bradycardia and a reduction in peripheral sympathetic activity which

then can lead to vasodilation and hypotension (van Dijk and Wieling, 2013). When brain perfusion substantially diminished, for example, systolic blood pressure of less than 75mmhg, the syncopal attack can occur.

The signs and symptoms of VVR include cold extremities/chills, hypotension, lightheadedness, dizziness, nausea/vomiting, pallor, slow or rapid pulse, sweating or weakness. More severe sign and symptoms include chest pain, difficulty in breathing, convulsion, or even loss of consciousness. It is important for every blood donation staff to identify the signs and symptoms to ensure donor will receive immediate management.

1.3 VVR Classification

VVR can be classified into two categories; immediate or delayed. In immediate VVR, the reactions or symptoms develop before, during and after blood donation before leaving the donation site. As for delayed VVR, the reaction or symptoms occur after donors had left the donation site. The duration of delayed VVR is not defined in a consistent manner. International Standard for Blood Transfusion (ISBT) and International Haemovigilance Network (IHN) did not define specifically the total duration of VVR after donor leaving donation area to be considered as delayed VVR (ISBT/IHN definitions.,2014). A study in France, defined delayed VVR as symptoms that occur outside the donation site until 24 hours post blood donation (Narbey *et al.*, 2016). However there is another study in which the researcher made a phone call to donors after three

weeks post donation to assess VVR and donors experienced up to 48 hours post- donation. For this study, as the VVR symptoms still can persist up to 48 hours; we use it as the duration for delayed VVR (Morand *et al.*, 2016)

Another classification of VVR is through the severity of the symptoms which can be graded into mild, moderate and severe. The grading is based on patient symptoms, signs and duration of the event (Gonçalez *et al.*, 2012; Yuan *et al.*, 2010). Table 2 below showed grading of the severity of the adverse reaction.

Table 2: VVR severity grading (Gonçalez *et al.*, 2012)

Grading/ Description	Mild	Moderate	Severe
Symptoms	Lightheadedness Weakness Nausea Pallor diaphoresis	Bradycardia Hypotension Vomiting Any mild symptoms persist > 15 minutes.	Fainting Seizure Any moderate reaction occur > 15 minutes

1.4 VVR Incidence in Blood Transfusion Centers in Malaysia and in Other Countries

VVR is the commonest ADR reported in National Blood Centre (NBC). The incidence of VVR reported by NBC was 472 cases (0.39%) in 2017. In Hospital Universiti Sains Malaysia, Kelantan, the reported VVR incidence in 2017 was 20 cases (0.18%). The

incidences were recorded through donor observation by the donation staff or donor complaint in both centres. There are possibilities of under-reported VVR cases, as donor might not complain of mild VVR, or the staffs are not able to assess the mild VVR signs accordingly. This may result in a lower VVR rate. Currently, the incidence in Malaysia was lower when compare it with another country which varies from 2.2% to 7.0% (Gonçalez *et al.*, 2012; Newman, 2004; Wiersum-Osselton *et al.*, 2014; Wong *et al.*, 2013).

1.5 Risk Factors for VVR Development

Many risk factors had been identified as a predictor for VVR. Age of blood donors is one of the most vital predictor: Young blood donors, who are at the age of 17 to 18 years old have the greatest risk (OR, 2.8; 95% CI, 2.59-2.98) while those who are 18 to 24 years old also has a significant risk of VVR (OR, 2.39; 95% CI, 2.23-2.56) (Wiltbank *et al.*, 2008). A data from the American Red Cross Whole Blood Donation Database showed that 54% of donors who had VVR are younger than 23 years old. The other significant risk factor for VVR among blood donors is having a low estimated blood volume (EBV). Those who have less than 3500 ml estimated blood volume, the incidence of VVR are 3.49% in comparison with those who are 3500 ml – 4000ml and 4000ml-4750ml group, 2.34% and 1.44% respectively (Wiltbank *et al.*, 2008). Donating for the first time also was identified as an important risk factor for VVR (Eder *et al.*, 2008a; Wiltbank *et al.*, 2008).

Female donors are also identified as a risk factor. About 10% of the female donors had a reaction while 6.4% of male donors had a reaction in a study done in young whole

blood donor (Reiss *et al.*, 2009). A recent study in Japan demonstrated that 30% of donors who experienced VVR had less than 6 hours sleep, in comparison to 13% of donors who did not experience VVR (Takanashi *et al.*, 2012). Fear has been found to be associated with a higher VVR rate. According to a study, experienced donors who expressed fear had a higher rate of positive BDRIs compared to those who did not express fear, 48% and 18% respectively (France *et al.*, 2012). There were also studies suggested that different ethnic group had a risk for VVR. In one study showed that black African-American blood donors had a lower incidence of VVR than that in Caucasian blood donors (Newman, 2002; Newman *et al.*, 2005). In term of donation site, there was no significant difference in the rate of VVR among donors donating in the hospital premise or mobile blood donation camp (Agnihotri *et al.*, 2012). The temperature of the mobile site has not been confirmed as a factor that will affect the rate of VVR (Callahan *et al.,* 1963).

1.6 VVR Evaluation Tools among Blood Donors

Currently, there are few tools to evaluate VVR among blood donors. The AABB Donor Biovigilance and International Standard in Blood Transfusion's Committee had developed useful categories to identify donor who had VVR through observation by staff at the collection site. The data can provide valuable information such as the site of reaction, duration of reaction, the symptoms and signs and also the presence of any injury. However this tool has its limitation. It is unable to detect a mild VVR as it might not be obvious or recorded. Written interview and donor self-assessment may provide a more accurate assessment of donor reactions. Therefore, post three weeks interview (Newman *et*

al., 2003) or post donation surveys shows a significantly higher VVR rates in the general donor population.

The Blood Donor Reaction Inventory (BDRI) is one of the donor self-assessment tools. It is a powerful self-survey technique in which can acquire more detailed data from the blood donor, usually after the donation period. The donor will grade the severity of VVR symptoms, from 0 for no reaction to 5, the most severe reaction symptoms. The donor will answer the question themselves and it will reflect even the slightest of VVR symptoms (Meade *et al.*, 1996). To simplify the process further, 11 question BDRI had been reduced into a 4 question BDRI. It had been shown to have good results in evaluating VVR. It allows researchers to acquire more detailed data from a small group of donors to a much larger subjects for analysis which can be more significance and relevance (France *et al.*, 2008). In conclusion, combinations of both observational method and donor survey can result in a higher sensitivity in detecting even the mildest VVR.

1.7 Impact of VVR to Blood Transfusion Service

The VVR has a negative impact on blood donors return rate. Moderate to severe VVR post donation reduce blood donor return rate by 50% while light VVR reduces 20% and 33% of return rate for first time and experienced blood donor respectively (France *et al.*, 2005). Another study showed that a reduction in retention for both female and male blood donor after VVR post blood donation (van Dongen *et al.*, 2013). A study in China also confirms that donor who had VVR are less likely not to donate again 4 times more than those who do not have any reaction (Wong *et al.*, 2013).

Another negative impact of VVR is, it can increase blood discard rate due to suboptimal volume collected. In Tanzania, the reported whole blood discard rate was 1.76% (1,169/66,255) (Kanani *et al.*, 2017) although it didn't state the reason for under volume. In Malaysia, a study regarding blood discard rate reveals that underweight was the main reason for discard of whole blood amounting 52% of the total cause of discarded whole blood unit (338 units) (Morish *et al.*, 2012). The study also mentioned that one of the reasons for underweight blood collection was due to VVR incidence.

Furthermore, VVR may also cause injury and possibly serious injury if donors happened to develop delayed VVR with loss of consciousness while doing critical work or driving. One study in the United States showed that injury could occur from starting of donation process (filling up form until after donor leave the donation site). The highest incident of injury can occur at phase 3A, which is the period of 4 minutes post phlebotomy ends until the donor leave the donation site and at phase 3B which is after the donor had left the donation site. The injury cases reported was 47 at phase 3A and 16 cases at 3B phase (Bravo *et al.*, 2011). Therefore preventive measures to reduce VVR incidence is very crucial to prevent such incidence.

1.8 Interventions to Reduce VVR

To improve donor safety, there are several methods explored which can reduce VVR among blood donors. Providing donors pre-donation water ingestion had shown promising results in some of the study (Ando *et al.*, 2009; France *et al.*, 2010; Monnard and Grasser, 2017). The pressor response to water relied on the volume and it is the result of gastric distention increasing sympathetic nerve activity by reflex mechanisms (Rossi *et al.*, 1998). The net effect of pressor response by the water ingestion are increase in donor's blood pressure, increase in total peripheral resistance and blunting heart rate response to orthostatic stress (Lu *et al.*, 2003). There is a study evaluating the effect of ingestion of 500ml of water pre-donation in both with chronic dehydrated and well hydrated donors before donation resulted in 47% reduction of total donation related symptoms (Hanson and France, 2004). In Japan, a study to find the impact of 300ml of water ingestion pre-donation showed a reduction in VVR rate in high risk donors. The authors also postulated that the effect of water hydration to prevent VVR are optimum at 15 to 20 minutes after water drinking (Ando *et al.*, 2009). However, in a randomised controlled trial where donors were assigned to an intervention of applied muscle tension, 500 ml of water ingestion before phlebotomy start, a combination of both intervention and no treatment at all showed no significant difference between water ingestion group and no treatment group (France *et al.*, 2010).

In view of the significance VVR incidence and its negative impact to blood donation, the aim of the study is to determine the effectiveness of pre-donation water

ingestion in reducing VVR incidence among young blood donors in Kelantan, Malaysia. This study will also determine the incidence rate of VVR among young blood donors. The correlation between donor's ages, gender, number of donation, weight, estimated blood volume, blood volume collected, sleeping hours, systolic blood pressure and ethnic group with VVR incidence will be determined.

We hypothesized that pre-donation water hydration could reduce VVR incidence among young blood donors in Kelantan. Young age, lower weight, lower estimated blood volume, female gender, first time donor status and higher blood volume collection is associated with higher incidence of VVR.

1.9 Problem Statement

VVR is considered a threat and it is the most common adverse donor reaction in major blood bank globally. It is associated with reduction in donor retention and contribute to many negative impacts towards blood transfusion service such as underweight blood collection that can lead to reducing the shelf life of the red cell or worse, will be discarded. It will also increase the time and work of the staff during blood donation to manage donors' adverse reaction and also may convey the perception that blood donation is an unsafe procedure to the public. Therefore, with all of the risks, mitigation steps are crucial.

1.10 Justification/ Rationale of Study

The BTS aim to improve donor safety and increase in donor retention as regular donors are considered as the safest donors in term of risk for transfusion transmitted infection (Song *et al.*, 2014). This was the first comparative interventional study in Malaysia studying the effect of pre-donation water hydration. Malaysian population is different from other countries due to the difference in body weight, weather, ethnic groups and the volume of blood collected compared to western countries. The results will be evaluated and if it is proven to reduce the VVR incidence, it later can be proposed as a standard measure to be implemented in all blood transfusion service in Malaysia as it is cheap and easy to be implemented.

Another reason why this study is important is that potentially there will be an improvement in donor haemovigilance data particularly on VVR cases. Currently, many centre use data collected through the observational method. This may result in under reported VVR cases and does not reflect the true incidence of VVR in Malaysia. Therefore, this study will use both methods, observational and survey using BDRI 4 items. It can determine the actual VVR incidence rate among blood donors, especially in high risk group (young blood donor). The association of risk factors such as younger age, female gender, low estimated blood volume, higher blood volume collected, lack of sleeping time and lower systolic blood pressure with VVR incidence also can be determined in this study.

This study also will be able to evaluate method used by (Ando *et al.*, 2009) by providing water less than 20 minutes before the start of phlebotomy can be beneficial as it prevents hypotension caused by volume collected through blood donation.

1.11 Conceptual framework

Many factors can contribute to VVR. All of the factors are shown in Figure 1.1. The intervention by giving pre-donation water hydration can reduce the incidence of VVR among young blood donors.

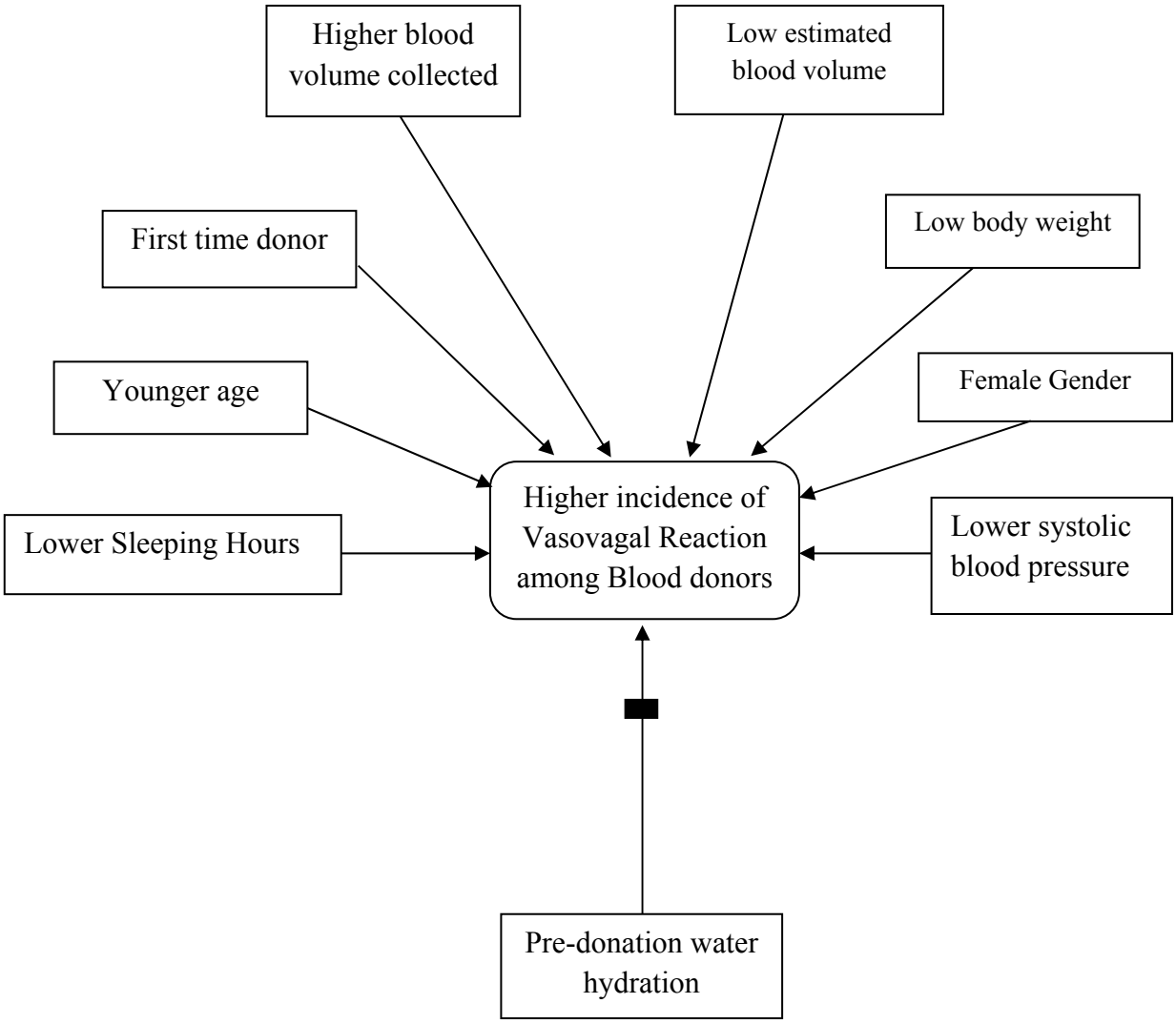


Figure 1: Factors associated with higher incidence of VVR among blood donors and the effect of pre-donation water hydration to reduce VVR incidence

CHAPTER 2: OBJECTIVES

2.1 General Objective:

To determine the effectiveness of pre-donation water hydration in reducing vasovagal reaction and to determine the risk factors for vasovagal reaction among young blood donors in Kelantan.

2.2 Specific Objectives :

i. To determine the incidence, severity and type of vasovagal reaction (VVR) among young blood donors in Kelantan for both hydration and non hydration group.

ii. To compare the incidence, severity and type of VVR for both hydration and non hydration group.

iii. To determine the risk factors for VVR developments.

iv. To compare the BDRI 4- Items score at 30 minutes and 48 hours for hydration and non hydration group.

v. To determine the correlation between BDRI score at 30 minutes and 48 hours in hydration and non hydration group.

2.3 Research Hypothesis

2.3.1 Null hypothesis

i. Pre-donation water hydration is not effective in reducing vasovagal reaction and its severity among young blood donors in Kelantan.

ii. Younger age, female, lower weight, lower estimated blood volume, higher blood volume collected and first time donors, lower sleeping hours and lower systolic blood pressure do not have higher incidence of VVR.

2.3.2 Alternative hypothesis

i. Pre-donation water hydration is effective in reducing vasovagal reaction and its severity among young blood donors in Kelantan.

iii. Younger age, female, lower weight, lower estimated blood volume, higher blood volume collected and first time donors, lower sleeping hours and lower systolic blood pressure have higher incidence of VVR.

CHAPTER 3: METHODOLOGY

3.1 Study Background

Hospital Universiti Sains Malaysia (HUSM) is the second largest blood collection center in Kelantan after Hospital Raja Perempuan Zainab, Kota Bharu. There are two collection site, static blood donation site which is located in Transfusion Medicine Unit (TMU), HUSM and blood donation mobiles which is conducted mainly in Kota Bharu, Pasir Mas, Jeli, Bachok, Machang, and some of the blood drives were held in Besut, Terengganu. The blood donation mobiles usually were held in higher institutions, government and private offices, schools, and malls. The total blood collection in 2017 was 10817 units. There are 20 (0.18%) VVR cases that were recorded through blood bank staffs observation. The current practice is to allow donors to drink and have some meals before donation. There is no specific requirement of drinking 500ml of mineral water before donation. Therefore this study aim to determine the VVR incidence rate through donor self assessment and interview and to study the effectiveness of pre-donation water hydration among young blood donors which is at higher risk of developing VVR.

3.1.1 Study Design

This was a comparative interventional study among young blood donors who donate blood in Kelantan which is conducted by Hospital Universiti Sains Malaysia Transfusion Medicine Unit from January 2017 to December 2018.

3.1.2 Study Location

This study was done during blood drives conducted by Transfusion Medicine Unit, Hospital Universiti Sains Malaysia in Kelantan area. Five mobile blood drives were selected by purposeful selection and all were from the higher educational institutions around Kelantan. The institutions include Kolej Politeknik Pasir Mas, Universiti Malaysia Kelantan (2 mobile blood drives), Kolej Polimas Kota Bharu and Kolej Kemahiran Mara Kota Bharu.

3.1.3 Study Duration

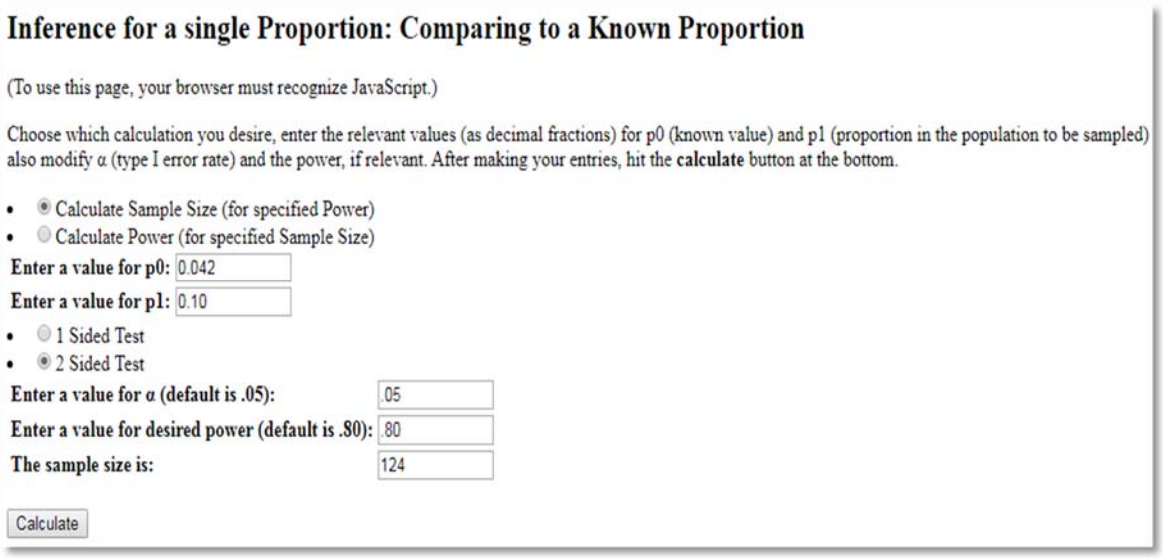
This study was conducted for two years which started from 1st January 2017 till 31st December 2018. Data collection was carried out for eight months which was from 1st August 2017 till 31st March 2018.

3.2 Sample Size

The sample size calculation was done in accordance with the objectives. Calculation was done using Power and Sample Size Calculations Software Version 3 except for the first objective which used https://www.stat.ubc.ca/~rollin/stats/ssize/b1.html, which is an online sample size calculator.

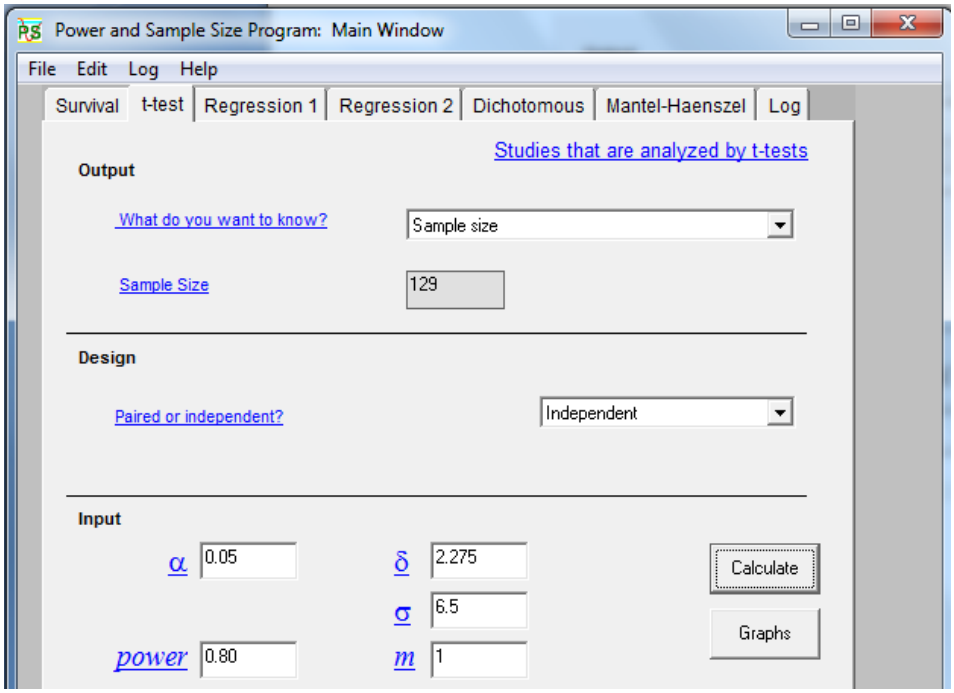
3.2.1 Objective 1 :

The sample size for the first objective was calculated using single proportion formula. Confidence interval was set at 95%, power of the study of 80%, proportion of VVR in a normal population (Po): 0.042 and inferential VVR in the study population (P1): 0.10, yielding 137 subjects in each group which equate 274 subjects in both group including 10% dropout rate (Wong *et al.*, 2013).



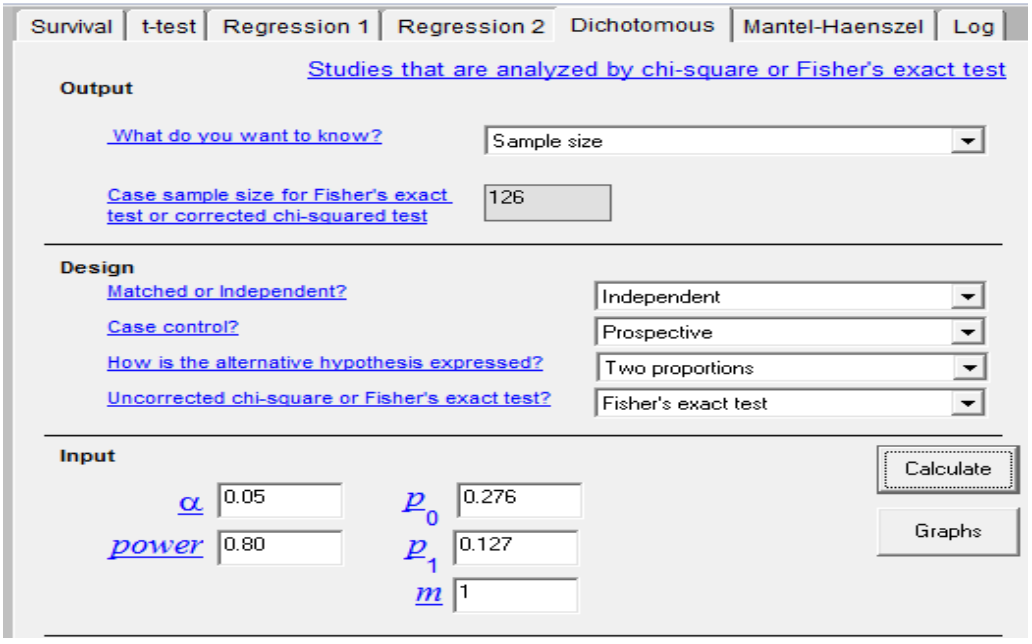
3.2.2 Objective 2

For the second objective, using comparison between two means formula, with the alpha of 0.05, power 0.8, difference between the two mean (δ) : 2.275, standard deviation (σ) : 6.5 and the ratio control to intervention (m): 1, sample size of 129 per group and 258 samples for both groups. To include 10% rate, 142 per group was calculated and total number for both group are 284. It has 0.65 similarities to pilot study ; Pre donation water ingestion attenuates negative reactions to blood donation (Hanson and France, 2004).



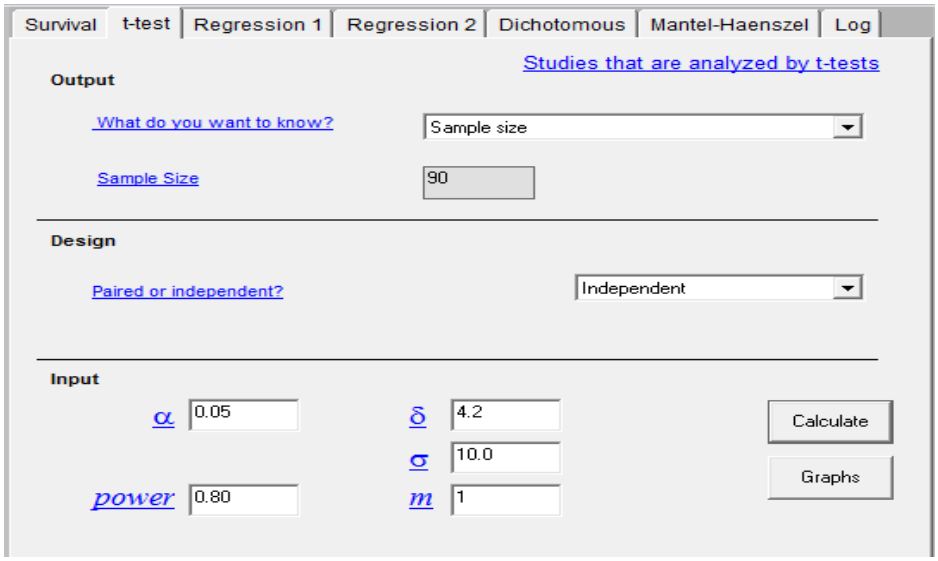
3.2.3 Objective 3

To compare mean of age between the two groups, sample size for third objective was calculated using difference between two means formula, with α: 0.05, power: 0.8, VVR incidence in control population (P0): 0.276, VVR incidence in water ingestion population (P1): 0.127 and ratio of control to intervention group (m): 1, sample size calculated was 126. When including 10% drop out subjects the total subjects for each in each group making the calculated sample size was 278 subjects in both groups (France *et al.*, 2010).



3.2.4 Objective 4

To compare the difference between mean BDRI from each group**, t**he fourth sample size was calculated using two means formula. α was set at 0.05, power of the study: 0.80, the difference between mean (δ) : 4.2, standard deviation within group (σ) : ±10 and ratio to interventional group (m) : 1, the sample size calculated was 90 in each group making 180 samples for both groups. The addition of 10% dropout will make the total sample size 198 (France *et al.*, 2010).



3.2.5 Final sample size

The highest sample size was calculated using the second specific objective, to determine the association of donor's weight, gender, estimated blood volume, number of donation and total volume collected with severity of VVR. Therefore the sample size used in this study was 284 subjects, 142 subjects per group.

3.3 Inclusion and Exclusion Criteria

3.3.1 Inclusion Criteria

i. Malaysian Citizen, aged from 17 to 23 year old (Newman, 2014) and eligible for blood donation through blood donation criteria according to the donor selection criteria by the National Blood Centre Guidelines 4TH Edition 2016 from January 2017 to December 2018. This age group was known to have highest VVR incidence

ii. Donors who provide informed study consent.

iii. Donors less than 18 years old with both informed study consent and parental consent.

iv. Donors who successfully drink 500 ml of mineral water 20 minutes before phlebotomy starts in hydration group.

3.3.2 Exclusion criteria

i. Donors who do not provide informed study consent.

ii. Donors who do not understand Bahasa Malaysia, English or understand instructions.

iii. Donors who do not drink successfully 500 ml of water pre-donation in hydration group.

iv. Donors who drink any amount of water in non-hydration group 20 minutes before phlebotomy starts.

v. Donors who wait more than 20 minutes after drinking 500 ml of water in hydration group before phlebotomy starts.

vi. Donors who wait more than 20 minutes after health screening by Medical Officer in non- hydration group before phlebotomy starts.

vii. Donors who are 23 years old and above.

3.4 Study Subjects

A total of 284 eligible and consented donors age from 17 to 23 years old have been included from various blood mobile.

3.5 Data Collection Method

Eligible and consented donors were divided into two groups, hydration (intervention) and non hydration (control) group using simple random sampling. This was done by each subject drawing a piece of paper in a sack, written with either hydration or non hydration which was equal in number, 142 per group. Donors were not divided according to gender, age, donation status or ethnic group. In hydration group, donors need to drink successfully 500ml of mineral water 20 minutes before they were bled and the phlebotomist were there to ensure that the study participants finished their drinks. In non hydration group, there will be no water given and donors were not allowed to drink any

water within 20 minutes period starting from when they were assigned the group till the bleeding process started.

Blood volume collected from a donor was not more than recommended 13%-15% of total blood volume. Based on this rule, male weighing 50kg and above and female 55kg and above were allowed to donate 450ml in a triple bag collection. Those who were not eligible for 450ml collection, 350ml of a double bag will be collected. Estimated blood volume was calculated using Nadler's Formula for volume collection which shown as below.

For Males: 0.3669 X Height in M^3 + 0.03219 X Weight in Kg + 0.6041

For Females = 0.3561 X Height in M^3 + 0.03308 X Weight in Kg + 0.1833

Thirty minutes after the donation process has completed, subjects were requested to answer the BDRI - 4 items questions which was in English. Subject's demographic data including risk factors for VVR development which include donor's gender, age, weight, estimated blood volume, number of donations, blood volume collected, sleeping hours, systolic blood pressure and ethnic group were retrieved from the donors blood donation form. Estimated blood volume (EBV) of each donor was calculated using Nadler's formula. Duration of VVR, syncopal attack occurrence and duration, and injuries were recorded from donor interview and observation by donation staff. After 48 hours post donation, they were contacted again by phone call and was asked regarding the BDRI - 4

items questions and score to assess the BDRI score at 48 hours delayed VVR. At the end of the study, each donor will receive honorarium of RM10 for their participation. All data were entered into the study proforma as in Appendix 1.

3.6 Statistical Analysis

Statistical analysis was performed using SPSS version 24.0 for window software (SPSS, Chicago Illinois, USA) to present the descriptive, statistical and multivariate analysis. Numbers, percentages, mean with standard deviation and median with interquartile range were used in the descriptive analysis. To study the comparison between categorical variables, Chi square was used. For numerical data analysis, Student t-test was used for independent parametric data while the Mann Whitney U test was used for independent non parametric data. Pearson correlation was used to study correlation between the two BDRI-4 Items score. To study the association of multiple factors influencing the VVR incidence, multiple logistic regressions was used.

3.6.1 Objective 1

The percentage was used to describe the incidence of VVR in both groups and chi square was used to compare both groups statistically.

3.6.2 Objective 2

To compare between hydration group and non hydration group with the incidence, severity and type of VVR, Chi Square test and Fisher Exact test was used.