THE EFFECTIVENESS OF APPLIED MUSCLE TENSION IN REDUCING VASOVAGAL REACTION AMONG YOUNG BLOOD DONORS IN KELANTAN

By

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DISCLAIMER

I hereby certify that this dissertation's work is my own except for the quotations and summaries, which have been duly acknowledged. I declare that I have no financial interest in the instruments or materials used in this study.

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LIST OF ABBREVIATION

AABB	American Association of Blood Banks
ADR	Adverse donor reactions
AMT	Applied muscle tension
BDRI	Blood donation reaction inventory
BTS	Blood transfusion services
BP	Blood pressure
EBV	Estimated blood volume
EMG	Electromyography
HUSM	Hospital Universiti Sains Malaysia
ISBT	International Society of Blood Transfusion
LL	Lower limbs
LOC	Loss of consciousness
NBC	National Blood Centre

SPSS	Statistical Package for Social Sciences
UL	Upper limbs
VVR	Vasovagal reaction

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ABSTRAK

Latar belakang: Reaksi Vasovagal (VVR) merupakan kesan sampingan utama pendermaan darah yang memberikan impak negatif terhadap keselamatan dan pengulangan pendermaan. Tujuan kajian adalah untuk menentukan keberkesanan Aplikasi Penegangan Otot (AMT) dalam mengurangkan kejadian VVR di kalangan penderma darah muda di Kelantan.

Kaedah: Kajian intervensi, perbandingan dan prospektif melibatkan 306 penderma darah muda yang telah dibahagikan kepada kumpulan AMT dan bukan AMT. Penderma telah diperiksa menggunakan inventori reaksi pendermaan darah pada minit ke-30 dan 48 jam selepas pendermaan. Data susulan mengenai sama ada penderma kembali menderma darah selepas enam bulan dikumpulkan.

Keputusan: Peratusan VVR adalah sebanyak 24.8% daripada 306 orang penderma. Kumpulan AMT menunjukkan pengurangan ketara insiden VVR berbanding kumpulan bukan AMT ($p<0.001$). Tiada kenaikan peratusan penderma kembali menderma darah yang ketara bagi kumpulan AMT berbanding kumpulan bukan AMT. Faktor-faktor yang

meningkatkan risiko VVR adalah jantina perempuan (p=0.002), berat badan(p=0.002), anggaran isipadu darah badan (p=0.033) dan tekanan darah sistolik yang rendah (p=0.005).

Kesimpulan: Kajian ini menunjukkan AMT adalah salah satu strategi yang berkesan bagi mengurangkan kejadian VVR di kalangan penderma darah muda di Kelantan. Pelaksanaan AMT disarankan kepada penderma yang mempunyai risiko yang tinggi terjadinya VVR.

***Kata Kunci**: Aplikasi Penegangan Otot (AMT), Reaksi Vasovagal (VVR), Faktor risiko VVR, penderma kembali menderma, inventori reaksi pendermaan darah.*

ABSTRACT

The effectiveness of Applied Muscle Tension in reducing Vasovagal Reaction among young blood donors in Kelantan

Background: The vasovagal reaction (VVR) incidence is the most common adverse donor reaction poses a major risk to the donor's safety and a disincentive for repeat donation. This study's main objective is to determine the efficacy of applied muscle tension (AMT) in reducing the VVR incidence among young blood donors in Kelantan.

Methods: A prospective comparative interventional study was conducted among 306 young blood donors that were equally assigned to AMT and non-AMT groups. Donors were assessed with Blood Donation Reactions Inventory at 30 minutes and 48 hours post blood donation. The subjects will be followed-up on whether they make at least one donation attempt in the next six months.

Results: Out of 306 young blood donors, the incidence of VVR was 24.8%. The AMT group showed a significant reduction in the VVR incidence rate compared to the non-AMT group ($p<0.001$).

There was no significant increment of donor return rate among the AMT group than non – AMT group upon follow-up in six months. In addition, female (p=0.002), low body weight (p=0.002), low estimated blood volume (p=0.033) and low systolic blood pressure (p=0.005), are important predictors of VVR.

Conclusions: This study showed that AMT was an effective strategy to reduce the VVR incidence among young blood donors in Kelantan. Implementation of AMT is recommended to donors with high risk of developing VVR.

Keywords: *applied muscle tension, vasovagal reaction, risk factors for VVR, donor return, Blood Donor Reaction Inventory.*

CHAPTER 1

INTRODUCTION

1.1 Overview

This chapter covers introduction on vasovagal reaction, blood donation reaction inventory and applied muscle tension. This chapter will explain further the research justification and research question.

1.2 Background of study

Malaysia is progressing to become an ageing nation by 2035, as 15% of its populations are classified as senior citizens (1). More blood is needed each year to fulfil an ageing population's health needs, especially in oncology treatments and surgical procedures (2). The retention and recruitment of novice donors are crucial because meeting the growing demand for these recruits would contribute up to six times per year. The truth is that most young donors are not donating blood regularly (3).

In younger and novice donors, vasovagal reaction (VVR) is one of the most common adverse reaction during and after donation, has become primary discouragement to both retention and recruitment (4,5). In the National Blood Centre (NBC), the most typical adverse donor reaction (ADR) reported is VVR. NBC has reported 489 cases (0.27%) and 384 cases (0.21%) in the year 2018 and 2019 respectively (6). In the same year, Hospital Universiti Sains Malaysia (HUSM), Kelantan has recorded 40 cases (0.37%) and 43 cases (0.36%) (7). There is a potentiality of under-reporting VVR cases due to limitation in the staff's ability to detect VVR especially for mild symptoms and when there is no complaint from the donor,

thus contributing to a lower VVR rate. To date, the VVR rate in Malaysia is low compared to other countries (8-11).

Several methods used to reduce VVR among blood donors have been demonstrated and Applied Muscle Tension (AMT) is one of the approaches. AMT is a technique that involves tensing muscles in the body. During the donation process, donors need to repeatedly tense their major muscles in their upper limbs (UL) and lower limbs (LL) at five-second intervals with normal breathing pattern. AMT effectively improves patient safety by reducing vasovagal reaction as reported in several studies (5,12,13).

1.3 Literature review

In general, whole blood donation is a safe process and well-tolerated without adverse reactions. Yet, varying severity of adverse reactions can happen (14,15). VVR are the most prevalent adverse donor reactions with reported cases between 1.5% to 8.2% worldwide (8-11). The occurrence of VVR following blood donation has been explained in a considerable amount of literature, where it can be described as a diphasic process (16). The presyncopal phase, is classically portrayed by an increment in both cardiac and peripheral resistance, resulting in slightly to moderately elevated blood pressure (BP), which is considered a normal physiologic response to stress and blood loss. On the other hand, the syncopal phase also known as the syncopal reaction is described by an abrupt decline in peripheral vascular sympathetic activity. Thus, it will lead to peripheral dilatation and resulting in blood pooling and hypotension. A minor role in causing the syncopal reaction occurred by an increment in parasympathetic activity although it often happens (17). Loss of consciousness (LOC) can

occur when brain perfusion is dramatically decreased.; i.e. a systolic BP below 75 mm Hg (18).

VVR evaluation tools were developed to detect and analyse VVR by inspecting and recording reactions by the bleeding staff. One of the tools was developed by the American Association of Blood Banks (AABB) Donor Biovigilance and International Society of Blood Transfusion (ISBT) Committee in which the data can provide valuable information like the site of reaction, the symptoms and sign as well the presence of any injury and duration of reaction that enables the staff at donation site to identify donors who had VVR through observation (19). However, it has a limitation in detecting mild VVR as it might not be obvious or recorded. Self-assessment by the donor or a written interview can give a more definite assessment of donor reaction. Hence, post-donation surveys (20) or post three weeks interview (21) demonstrate a significantly higher occurrence of VVR in the general donor population.

The Blood Donation Reaction Inventory (BDRI) is one of the donor self-assessment tools. It is a persuasive self-survey approach that can pick up more detailed data from the blood donor, normally after the donation period. The donor will grade the severity of VVR symptoms ranging from 0 for no reaction to 5, the most severe reaction symptoms. BDRI has eleven questions and the donor will answer the questions themselves and detect even the slightest of VVR symptoms such as mild headache and giddiness (22). It has been shown to execute better results in evaluating VVR as it allows researchers to obtain more detailed data ranging from a small group of donors to a much larger pool of subjects for analysis which can be of more relevance and significance (23). Summarily, merging both observational and donor surveys can result in a higher sensitivity in detecting even the mildest VVR.

Various factors have been determined as predictors for VVR. Age of blood donors is one of the most important predictors of VVR. Donors aged 18 to 24 have a significant risk of VVR (24) while younger donors aged between 17 to 18 have the greatest risk (25). Female gender has a greater risk among first-time donor categories for VVR (10,24).

Donors with low estimated blood volume (EBV) present another risk factor for VVR. A study by Wiltbank et al. (25) revealed that the incidence of VVR is 3.49% in those who had less than 3500 ml EBV, 2.34% and 1.44% in those in the 3500 ml – 4000 ml and 4000 ml – 4750ml groups respectively. Being first-time donors present a crucial risk factor for VVR (25,26). The selected donor should not donate more than four times before because the rate of syncopal occurrences may stabilise after fourth donation (27). A study on the amount of sleep showed that 30% of donors who slept less than six hours experienced VVR while only 13% of those who slept more than six hours experienced it (28).

Fear has been identified to be related to VVR based on a study using BDRI, where experienced donors who did not expressed fear had a lower rate score than those who did expressed fear (29). VVR risk also associated with the location of the donation. Wong et al. (10) proved that donating at a mobile drive has a significantly higher incidence of VVR than donating in centres. Other than that, the risk for LOC is also higher at a mobile drive (30). Hashizume et al. (31) has confirmed that a comfortable environment with suitable and stable room temperature is crucial for preventing VVR.

A considerable amount of research has been published on the impact of VVR on blood transfusion services (BTS). A study performed by Gillet et al. (32) revealed that VVR incidence during the first donation reduces the return rate from 47.4% to 29.0%. Another study by Wong et al. (10) proved that donors who had history of VVR are less likely not to donate again four times more than those who do not have any reaction. Since donation can

be repeated after three months, we consider six months to analyse the donor return rate in this study.

VVR can increase blood discard due to suboptimal volume collected. A study performed in Tanzania reported that whole blood discard rate was 1.76% despite no statement for under volume (33). While in Malaysia, a study showed that the main cause for the disposal of the whole blood unit was underweight that occurred because of the VVR incidence (34).

Besides, VVR may also result in injury. The injury might become more severe if the donor experiences delayed VVR such as LOC while performing critical work or driving. Previous studies showed that nearly 60% of the syncope reaction happens as the donor falls from the bed when leaving. Between 10% to 12% of the reactions develop after the donor exits the donation vicinity (30,35). Syncope is a serious risk as the donor can suddenly fall with about 4% to 9% risk of injuries (30). Despite most of the injuries being minor (35), serious injuries such as closed-head injuries, fractures and lacerations need to be considered (36).

Numerous study has been performed and several methods used to reduce VVR among blood donors demonstrated such as applied muscle tension (5,37), pre-donation water hydration (12), fear reduction of needles among blood donors (38,39), pre-donation salt ingestion (40), social support (41) and distraction technique (42).

AMT is a method generally used as behavioural therapy that involves the repeated tensing of the major muscles of the UL and LL with normal breathing pattern. This repeated tensing can prevent the rapid drop of donor's blood pressure that usually occurs during VVR (43). France et al. (44) demonstrated that muscle tensing evokes physiological adaptations which may minimise the risk of VVR. Ditto et al. (45) proposed that AMT can serve as a buffer towards hypovolaemia as it reduces heart rate changes throughout the blood donation

process. A previous study by Kowalsky et al. (46) found that cerebral oxygenation decreased in the control group and that this decrease was attenuated in blood donors practising AMT. All these conditions may explain that AMT may be effective in part through increased oxygen availability to the brain and further prevent vasovagal symptoms and syncope. Besides, it has been proven that a donor can learn AMT within five minutes (47).

AMT involves several techniques such as LL tensing, LL crossing, UL tensing and abdominal tensing (5,37,48). Research conducted to determine which type of AMT was the most effective in reducing VVR showed that lower-body muscle tensing (legs and abdomen) combined with leg crossing is most effective in maintaining BP and cerebral oxygenation (44,46). However, this method has not been universally used in evaluations of the efficacy of AMT. For example, in some AMT trials, donors have been instructed to lift one leg at a time, while in others, donors have been told to tense their body's major muscles (5,37,48). We chose LL and UL tensing as the method in this study as it was the most effective and pleasant technique to learn and apply during mobile donation.

Few studies initially suggested that AMT does not appear to be equally effective for both males and females. In a study by Ditto et al. (45), females who practised AMT had significantly fewer self-reported symptoms and were more likely to complete the donation than females in the control group. Another analysis of group difference was conducted by France et al. (49) and revealed a significant VVR reduction by AMT in a group of women but not men. But, a more recent study by Tomasulo et al. (12) in 1000 donations showed a reduction of 20% of the total (mild, moderate and severe) reaction rate in both young male and female donors (17- 22 years old) after the intervention (AMT). The symptoms of LOC also showed a 22% reduction. Another study by Thijsen et al. (5) showed a significant

decrease of VVRs incidence among both male and female donors who practised AMT (1.6%) incidence compared to the standard donation practice (6.3%).

A study by Ditto et al. (50) revealed that female donors in the AMT group were significantly more likely to return than in the control group after a two-year follow-up period. It was opposed to another study by Ditto et al. (37) that showed no significant donor return rate effects in both groups. Upon further search and literature review, there were limited studies on AMT during blood donation in Malaysia and Asia (51).

1.4 Research Justification

Being the most common ADR, VVR presents a threat to major blood banks worldwide. Research has consistently shown its negative impacts on BTS, such as reducing donor retention and blood collection discarding due to underweight. Staff workload will increase as they need to manage donors' ADR during blood donation. It will also bring a negative perception regarding blood donation to the society. With all those impacts, mitigation steps are imperative.

This study is the first interventional study conducted in Malaysia that focuses on the effectiveness of AMT. The research in Malaysia is different from other countries (where previous studies were done) in terms of the demographic background (ethnicity and body weight), weather and volume of collected blood. The findings will be analysed and if AMT is proven to reduce VVR incidence, it can be proposed as a routine technique during donation process to our BTS in Malaysia since it is cost-effective and requires only minimal effort.

This study will also provide a potential improvement in donor haemovigilance data, particularly on VVR cases. At present, many centres adopt observational methods only in

reporting VVR cases that lead to under-reporting VVR cases. In this study, we will employ observational methods and assessment using the BDRI form. Thus, we can determine a more definite VVR incidence rate among blood donors. The associated risk factors like low body weight, female gender, first-time donors and higher blood volume collection with VVR incidence can also be calculated.

1.5 Research question(s)

1. Is AMT effective in reducing frequency and severity of VVR?
2. Is AMT able to increase donor return rate?
3. Is there any association between donor characteristic (donor's weight, gender and EBV) and donation characteristic (number of donations, total blood volume collected) with the VVR?

CHAPTER 2

OBJECTIVES

2.1 General objective

To study the effectiveness of AMT in reducing VVR 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.

ii. To determine the effectiveness of AMT in reducing frequency and severity of VVR.

iii. To compare the donor return rate for both AMT and non – AMT groups.

iv. To determine the association between donor characteristics (donor's weight, gender and EBV) and donation characteristics (number of donations, total blood volume collected) with vasovagal reaction.

2.3 Alternative hypotheses

H_{A1}: There is significant difference in the frequency and severity of VVR between AMT group and non – AMT group.

H_{A2}: There is significant difference in donor return rate between AMT group and non – AMT group.

H_{A3}: There is a significant association between donor characteristics and donation characteristics with VVR.

2.4 Null hypotheses

H_{01}: There is no significant difference in the frequency and severity of VVR between AMT group and non – AMT groups.

H_{02}: There is no significant difference in donor return rate between AMT group and non – AMT group.

H_{03}: There is no significant association between donor characteristics and donation characteristics with VVR.

CHAPTER 3

METHODOLOGY

3.1 Study background

Hospital Universiti Sains Malaysia (HUSM) being the second largest blood collection centre in Kelantan after Hospital Raja Perempuan Zainab, Kota Bharu. Blood collection was done at the static blood donation site located in Transfusion Medicine Unit (TMU), HUSM and blood donation mobiles conducted mainly in Kota Bharu, Pasir Mas, Jeli, Bachok and Machang districts. The blood donation mobiles usually were held in higher institutions, government and private offices, schools, and malls. The total blood collection in 2018 was 10869 units. Yet, there was no requirement in applying AMT during donation. Therefore, this study aimed to determine the VVR incidence rate and study the AMT's effectiveness among young blood donors at higher risk of developing VVR.

3.2 Study design

This study was a prospective comparative interventional study among young blood donors who donated blood in Kelantan, conducted by Transfusion Medicine Unit, Hospital Universiti Sains Malaysia.

3.3 Study area

This study was done during blood drives conducted by Transfusion Medicine Unit, Hospital Universiti Sains Malaysia in Kelantan area. Fifteen mobile blood drives with the target of more than 50 donors were selected by random selection in each monthly planned

mobile. All mobiles selected were from the higher educational institutions. The institutions include Kolej Komuniti Kok Lanas (2 mobile blood drives), Kolej Matrikulasi Pasir Puteh (2 mobile blood drives), Kolej Poly-Tech Mara Kota Bharu (2 mobile blood drives), Kolej Politeknik Kota Bharu (2 mobile blood drives), Universiti Malaysia Kelantan (2 mobile blood drives), Universiti Teknologi Mara Kota Bharu, Universiti Teknologi Mara Machang, Universiti Malaya Nilam Puri, Kolej Vokasional Pengkalan Chepa and Kolej Kemahiran Mara Kota Bharu.

3.4 Study duration

The study was conducted from 1st January 2019 until 31st December 2020 and the subjects were recruited from 1st June 2019 until 31st March 2020.

3.5 Study population

- Target population : Population in Kelantan
- Source population : Eligible blood donors in HUSM, Kota Bharu and mobile donation conducted by HUSM.
- Study population : Young blood donors age 18 to 23 years old
- Sampling unit : Eligible Young blood donors age 18 to 23 years old in HUSM, Kota Bharu and mobile donation conducted by HUSM

3.6 Inclusion criteria

Non - AMT group (Control)

i. Malaysian Citizen aged from 18 to 23 years old and eligible for blood donation.
ii. Donors who provide informed study consent.
iii. Donors who had donated blood less than four times in their lives.
iv. Donors in HUSM or Mobile donation conducted by HUSM.

AMT group (Intervention)

i. Malaysian Citizen aged from 18 to 23 years old and eligible for blood donation.
ii. Donors who provide informed study consent.
iii. Donors who had donated blood less than four times in their lives.
iv. Donors in HUSM or Mobile donation conducted by HUSM.
v. Donors who able to practise AMT during blood donation.

3.7 Exclusion criteria

Non - AMT group (Control)

i. Donors who do not understand Bahasa Malaysia or English and understand instructions.
ii. Donors who have an underlying medical illness (e.g. cardiovascular disease, bronchial asthma or vision and hearing impairment).
iii. Donors who take medications that might influence cardiovascular activity.

AMT group (Intervention)

i. Donors who do not understand Bahasa Malaysia or English and understand instructions.
ii. Donors who have an underlying medical illness (e.g. cardiovascular disease, bronchial asthma or vision and hearing impairment).
iii. Donors who take medications that might influence cardiovascular activity.

3.8 Sample size calculation

The sample size calculation was done in accordance with objectives.

Objective 1

To determine the incidence, severity and type of vasovagal reaction (VVR) among young blood donors.

Single proportions:

$n = (z/\Delta)^2 p (1-p)$

n = sample size

z= z statistic for a level of confidence = 1.96 (95% confidence interval)

p= expected prevalence or proportion (in proportion of one; if 100%, p=1)

Δ = precision (in proportion of one; if 1%, $\Delta = 0.01$)

Where,

n=calculated sample size

z= 1.96 for 95% confidence interval

p= 0.08, according to Tomasulo et al. (12)

Δ=0.05

n= 113 + 10% drop out

n= 124

Objective 2

To determine the effectiveness of AMT in reducing frequency and severity of VVR.

Single Mean:

$n = (z^*\sigma / \Delta)^2$

n = sample size

z = z statistic for a level of confidence = 1.96 (95% confidence interval)

σ = expected mean

Δ = precision

Where,

n=calculated sample size

z= 1.96 for 95% confidence interval

σ= 14.3, according to France et al. (13).

Δ= 2.65

n= 112 + 10% drop out

n= 123 x 2 groups = 246

Objective 3

To compare the donor return rate for both AMT and non – AMT groups.

Two proportions:

$n=[[p1(1-p1) + p2(1-p2)]/ (p1-p2)^2](z\alpha + z\beta)^2$

n=sample size

p1=proportion of the associated factor in high risk group

p2=proportion of the associated factor in low risk group

$z\alpha = 1.96$ for $\alpha = 0.05$ (two tailed) or 2.58 for $\alpha = 0.01$ (two tailed)

$z\beta = 0.84$ for 80% power or 1.28 for 90% power

Where,

n=calculated sample size

p1=0.55, p2 = 0.71, according to Ditto et al. (50)

$z\alpha = 1.96$ for $\alpha = 0.05$ (two tailed)

$z\beta = 0.84$ for 80% power

n= 139 + 10% drop out

n= 153 x 2 groups= 306

Objective 4

To determine the association between donor characteristic (donor's weight, gender and EBV) and donation characteristic (number of donation and total blood volume collected) with the VVR.

Two proportions:

$n=[[p1(1-p1) + p2(1-p2)]/ (p1-p2)^2](z\alpha + z\beta)^2$

n=sample size

p1=proportion of the associated factor in high risk group

p2=proportion of the associated factor in low risk group

$z\alpha = 1.96$ for $\alpha = 0.05$ (two tailed) or 2.58 for $\alpha = 0.01$ (two tailed)

$z\beta = 0.84$ for 80% power or 1.28 for 90% power

Where,

n=calculated sample size

p1= 0.12, p2= 0.02, according to Wiltbank et al. (25)

$z\alpha = 1.96$ for $\alpha = 0.05$ (two tailed), $z\beta = 0.84$ for 80% power

n= 98 + 10% drop out

n= 108 x 2 groups= 216

The highest number of samples is 306 (153 x 2) according to specific objective 3. Thus, this is the total number of samples for this study which is 153 per group (AMT and non-AMT).

3.9 Sampling method and subject recruitment

A total of 306 subjects were selected from blood donors who were eligible for blood donation. Simple random sampling was used as the sampling method in this study. The selection was based on every second subject. The sample was selected at number 2, 4, 6, and so on. All the selected subjects were checked against the inclusion and exclusion criteria. As a result, a total of 34 samples were excluded. Additional 34 subjects were selected to replace excluded subject. Finally, total subjects selected were 340. The selected eligible subjects were separated into AMT and non-AMT groups. The division was randomly assigned by balloting a paper from a box. The papers in the box were pre-write with AMT or non-AMT. Each group had 153 participants.

3.10 Research tool

Blood Donation Registration Form (Appendix B)

All blood donors will complete the Blood Donor Registration Form and before donation, they will be counselled by the blood bank medical personnel. It ensures excluding persons with high-risk behaviours exposed to infectious diseases. They will also give their consent for blood samples to be taken for testing of viral markers and put down their signature sign in the presence of the interviewer or the interviewer should verify the signature if the donor had already signed earlier (52). Most of the data regarding donor and donation details will be extracted from this form.

Blood Donation Reaction Inventory (Appendix C)

The Blood Donation Reaction Inventory consists of eleven subjective physiologic reactions associated with blood donation on a 0 to 5 scale, with total scores ranging from 0 to 55. The eleven items include 1) faintness, 2) dizziness, 3) weakness, 4) facial flush, 5) visual disturbance, 6) difficulty hearing, 7) lightheadedness, 8) rapid or pounding heartbeat, 9) sweating, 10) rapid or difficult breathing, and 11) nausea or upset stomach. The total scores have a high internal consistency level and have significantly corresponded to phlebotomist classification of donor reactions (5,12,22).

Two-minutes Video

A short video describing the AMT technique will be played on a notebook (5,12). For reducing distraction by other activity, the subject will listen to an audio track with headphones.

Electromyography (EMG)

A machine monitors the electrical signal via electrodes placed on the skin surface. The electrode will identify underlying electrical activity by illustrating the waveform on the computer monitor (53). Average EMG is the average electrical activity detected based on the muscle tensing during the entire donation process.

3.11 Data collection method

All potential blood donors were interviewed by the medical officer in charge. The medical officer was available throughout the mobile donation. If they were eligible for recruitment, they completed a standard pre-donation questionnaire that contains demographic information for about five minutes. The reading of pulse rate and BP obtained using a portable B-D digital monitor. Subject was then selected using simple random sampling and separated into AMT dan non – AMT groups. Both groups were in a separate area to reduce contamination bias.

The AMT group watched an instructional video on the AMT method and applied it during blood donation procedures. The subject watched a two-minute video broadcasted on a notebook computer. The video started with a narrator discussing that most VVR reactions were minor and affects only a small number of donors to avoid the unfavourable perception that may discourage prospective donors. AMT will be present as a possible means to enhance the donation experience. Donors were guided by the principal investigator or other research team members to tense their major muscles in their UL and LL at five-seconds intervals while maintaining normal breathing pattern. The priority was focus on repeated tensing, as opposed to tensing and relaxing the muscles in contrast to the progressive muscular relaxation technique. The principal investigator or other research team members will then assess the donor's technique. Once the intended technique was correct, the donors proceeded through the blood donation process while executing the AMT technique until the bleeding completed.

During their blood donation process, electromyography (EMG) probes will be attached to both forearm (ventral side) and both calf (posterior side). On the other hand, the non – AMT group proceeded to the normal blood donation process. The blood donation

process took about 7 – 10 minutes. For all adverse events during the donation process, the principal investigator or other research team members and medical officer in charge attended the donor.

Shortly after the donation process was completed, another reading of blood pressure and pulse rate was recorded. All subjects from both control and intervention groups then completed the BDRI form for about three minutes. Then, they were provided with a light meal in the refreshment area. All the procedures took about 15 – 20 minutes.

We followed up the subjects after 48 hours post donation by a phone call to assess delayed VVR using similar BDRI form. The whole blood donation process's estimated time took about 30 to 50 minutes, and additional 48 hours post donation in total for follow-up purposes. After participating in this study, the subject will be followed-up on whether they make at least one donation attempt in the next six months and data was obtained by phone call. The donor's phone number was taken from the Blood Donation Registration Form.

To date, AMT is not a standard procedure during the donation process. Thus, according to current practice, the control group is considered safe. There is no additional risk in applying the muscle tension technique during blood donation procedure except the known side effects of blood donation, including the development of haematoma, VVR, nerve injury and others.

3.12 Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 26.0 for window software (SPSS, Chicago Illinois, USA), and checked for missing data and outliers (none was found). Assumptions of the statistical procedures used were confirmed. Descriptive statistics were calculated for all study variables. Descriptive data were expressed in numbers, percentages and means with standard deviations. Then, the chi-square test was used to study the association between categorical variables. A $p < 0.05$ value is considered statistically significant. For numerical data analysis, Independent *t*-test was used. Multiple logistic regressions were used to identify the association of multiple factors influencing the incidence of VVR.

3.13 List of variables

Dependent variables

- Vasovagal reactions
- Non – vasovagal reactions

Independent variables

- Age
- Applied Muscle Tension
- Donor return
- Ethnic group
- Gender
- Number of donations
- Pulse rate
- Systolic blood pressure
- Total blood volume
- Weight

3.14 Variables definition

Applied muscle tension:

A technique involves tensing muscles in the body. Donors will tense the major muscle groups in their UL and LL at five-second intervals while breathing steadily (45).

Donor return:

The donor makes at least one donation attempt within the next six months after the last donation attempt. A donor can donate again after the eight weeks of their successive donation (52).

Estimated blood volume:

The total amount of fluid circulating within the arteries, capillaries, veins, venules, and chambers of the heart at any time. The equation for estimating blood volume given the gender, height (H), and weight (W) of the patient following the Nadler's formula (54).

Repeat donor:

A donor who donates at least two times and above (26).