A STUDY OF IRON DEFICIENCY AMONG BLOOD DONORS IN HUSM

by

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CERTIFICATE

This is to certify that the dissertation entitled

"A Study of Iron Deficiency among Blood Donors in HUSM"

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ABSTRAK

Kadar pendermaan darah yang tinggi boleh menyebabkan kekurangan zat besi dalam tubuh penderma darah. Ia berlaku disebabkan kerapnya kehilangan zat besi yang terkandung dalam setiap unit darah yang didermakan. Jika keadaan ini berlarutan ia akan menyebabkan terjadinya penyakit anemia kekurangan zat besi. Objektif kajian ini adalah untuk mengkaji kekerapan kekurangan zat besi di kalangan penderma darah di Hospital Universiti Sains Malaysia (HUSM).

Kajian ini dijalankan di Unit Perubatan Transfusi Darah HUSM, Kubang Kerian serta di beberapa buah daerah yang telah dipilih secara rawak iaitu Tanah Merah, Tumpat, Bachok dan Kota Baru. Seramai 211 orang penderma lelaki telah mengambil bahagian dan mereka dikategorikan ke dalam tiga kumpulan. Kumpulan pertama terdiri daripada penderma kali pertama, mereka adalah kawalan dalam kajian ini (43.6%). Kumpulan kedua adalah penderma darah yang pernah menderma dalam jumlah dua hingga empat kali sahaja (19.4%) manakala kumpulan ketiga terdiri daripada penderma tetap yang mempunyai jumlah pendermaan sebanyak lima kali dan ke atas (37.0%).

Semua penderma telah melepasi ujian kepekatan hemoglobin sebelum menderma. Sebanyak 5 ml isipadu darah di ambil daripada setiap penderma untuk asai 'serum ferritin'. Taksiran ujian ini akan menunjukkan paras kandungan zat besi dalam tubuh penderma. Nilai aras yang ditetapkan sebagai paras rendah dalam kajian ini adalah berdasarkan kepada garis panduan WHO iaitu kurang dari 15 μg/l.

Sebanyak 4% daripada jumlah keseluruhan penderma yang mengambil bahagian telah dikenalpasti mempunyai paras ferritin yang rendah. Dari peratusan ini, 44% adalah penderma kali pertama dan 56% adalah penderma tetap. Jumlah purata 'serum ferritin' didapati sangat rendah bagi kumpulan penderma tetap iaitu sebanyak 62.0 ± 39.78 μg/l, jika dibandingkan dengan purata 'serum ferritin' penderma pertama (90.17 ± 66.63 μg/l) dan penderma dari kumpulan kedua (114.12 ± 66.97 μg/l). Terdapat perkaitan yang signifikan ('fair correlation') di antara frekuensi pendermaan darah dengan aras 'serum ferritin' ($P < 0.001$). Bagaimana pun, tiada sebarang perkaitan yang signifikan di antara kepekatan hemoglobin dengan aras 'serum ferritin' ($P = 0.76$).

Kesimpulannya, penggunaan ujian kepekatan hemoglobin sebagai kriteria tunggal dalam saringan penderma darah perlu dinilai semula. Ujian 'serum ferritin' dicadangkan supaya disertakan dalam kaedah atau protokol penyaringan penderma darah, terutamanya penderma tetap yang menderma lebih daripada lima kali dalam tempoh dua tahun.

ABSTRACT

Regular blood donation can result in iron deficiency among blood donors. This is due to regular iron loss from each donated whole blood unit. If not treated or controlled, the condition might lead to the development of iron deficiency anemia. The objective of this study was to determine the prevalence of iron deficiency among blood donors of Hospital Universiti Sains Malaysia (HUSM).

This was a cross – sectional study, conducted at Transfusion Medicine Unit of HUSM and also other provinces which were randomly selected namely Tanah Merah, Tumpat, Bachok and Kota Baru, Kelantan. A total of 211 eligible male blood donors had taken part and they were categorized into three groups. The first group was made of the first time donors (43.6%) and they were the control group of this study. The second group consisted of donors who had donated from two to four times only (19.4%) and the third group comprised of the regular donors who had donated equal to or more than five times previously (37.0%).

All donors had passed the haemoglobin concentration test by HemoCue prior to donation. 5 ml of blood was drawn for the determination of body iron stores evaluated by serum ferritin measurement. The cut – off point of serum ferritin was based of WHO guideline (<15 μg/l).

Among the donors, 4% were iron – deficient. From the prevalence, 44% were the first time donors and 56% were regular donors. Serum ferritin level were found to be significantly lower among the regular donor (62.0 ± 39.78 μg/l) as compared to the first time donors (90.17 ± 66.63 μg/l) and the second group donors (114.12 ± 66.97 μg/l). There was a significant (fair) correlation between frequency of donation and serum ferritin level (P <0.001). However, there was no correlation between haemoglobin concentration and serum ferritin level ($P = 0.76$).

It is concluded that the used of haemoglobin measurement as a single parameter to screen blood donors should be reviewed. It is also suggested that besides haemoglobin estimation, serum ferritin assessment should also be included in the screening protocol for blood donors, especially for the regular donors who had donated for more than five time per two years.

Chapter 1

Introduction

Resolution of 28.72 of the World Health Assembly established the principle that blood donation should be voluntary and non – remunerated (WHO, Blood Transfusion Safety, Geneva, 2002). It means that the voluntary non – remunerated donors donate their own blood freely to unknown recipients but did not receive any payment or personal benefit. Their willingness to donate regularly is essential in maintaining adequate blood supplies since the need of blood in hospitals is highly demanding. The question is, how frequent these kind – hearted donors are allowed to donate their blood?

In year 2000, as reported by Health Ministry of Malaysia in its annual book report, total blood donations at government hospitals throughout Malaysia was 361 898. This number includes 114 967 new volunteer blood donors and 230 590 regular donors. As compared to 1999, total blood donation in year 2000 was increased as much as 8.2%. On the contrary, Transfusion Medicine Unit of Hospital Universiti Sains Malaysia reported that blood donations were decreased by 18.7% (8228) in year 2003 as compared to 2002 which were 10 124 donations.

A possible explanation for high numbers of donors drop out was probably due to decreased hemoglobin concentration and low iron status among the regular blood donors. These lead to the feeling of unwell and prevent them to come to donate blood voluntarily after three months of previous donation. Recent scientific studies have shown that high

frequency of blood donations can lead to anemia (Nadarajan VS and Eow GI, 2002). Most of the cases were iron deficiency anemia. Finch *et al.* (1997) also concluded that blood donation was associated with a decrease in serum ferritin. More frequent donations would result further decreases. If more and more regular volunteer blood donors developed anemia, this will continuously increases the donors drop out. As the result, blood bank can not fulfill the need of blood from patients who are totally depending on blood for their survival.

Since donating blood is a humanitarian act from healthy individual towards the sick, we need to appreciate the generous donors by providing care to their health status. Reducing the risk of developing iron deficiency anemia is one of the appropriate ways.

1.1 Haemoglobin

Red blood cell is produced in bone marrow under the influence of erythropoietin. With the life-span of 120 days, this biconcave disc cell is normally 7 μm in diameter and has no nucleus. The primary function of the red cell is to carry oxygen (O_2) to tissues and to return carbon dioxide (CO_2) from tissues to lung. To accomplish the task, it has special protein called haemoglobin.

Haemoglobin is mainly a combination of haem (an iron – containing porphyrin) and globin. Each red cell contains 640 million of haemoglobin molecules which are synthesized in mitochondria. It has high affinity to combine with oxygen. Each gram of

haemoglobin can carry up to a maximum of 1.36 ml of oxygen. Reduction in haemoglobin concentration will significantly reduce the oxygen supply to the tissues. Normal range of haemoglobin concentration as indicated by World Health Organization (WHO) is given in table 1.0.

Age / gender	**Normal Hb range (g/dl)**	**Anemic if Hb range less than: (g/dl)***
Birth (full term)	13.5 – 18.5	13.5 (Hct 34.5)
Children: 2 – 6 months	9.5 – 13.5	9.5 (Hct 28.5)
Children: 6 months – 6 years	11.0 – 14.0	11.0 (Hct 33.0)
Children 6 – 12 years	11.5 – 15.5	11.5 (Hct 34.5)
Adult males	13.0 – 17.0	13.0 (Hct 39.0)
Adult females: non – pregnant	12.0 – 15.0	12.0 (Hct 36.0)
Adult females: pregnant		
First trimester: 0 – 12 weeks	11.0 – 14.0	11.0 (Hct 33.0)
Second trimester: 13 – 28 weeks	10.5 – 14.0	10.5 (Hct 31.5)
Third trimester: 29 weeks – terms	11.0 – 14.0	11.0 (Hct 33.0)

Table 1.0: WHO guidelines for haemoglobin concentration based on age and gender. *These values simply define anemia. They are often used as thresholds for investigation and treatment, but are not indications for transfusion.

Low hemoglobin level might indicate the presence of anemia. It can be further classified as mild (Hemoglobin (Hb) greater than 10.0 g/dl), moderate (Hb 7.0 – 10.0 g/dl) and severe (Hb below 7.0 g/dl) anemia. However, it is relatively simple to diagnose iron deficiency anemia based on their haemoglobin concentration only. Further detailed assessment is required for determination of anemia. As suggested by Cook *et al.*, 1992a, in screening for iron deficiency and iron deficiency anemia, four laboratory measures have been identified as suitable for single use or in combination which includes:

Single measures	Combination measures
Haemoglobin	Serum ferritin and haemoglobin
Serum ferritin (SF)	Erythrocyte protoporphyrin and haemoglobin
Erythrocyte protoporphyrin	Serum receptor and haemoglobin
Serum transferrin receptor	Serum ferritin and serum transferin

In a scientific study, the iron status of 203 women was examined. Iron status tests included examination of stainable bone-marrow iron, a radioactive iron absorption test, serum ferritin, hemoglobin, MCH, MCV and transferrin saturation. Women were classified according to the presence or absence of stainable bone marrow iron. 75% women with no stainable bone marrow iron had serum ferritin less than or equal to 15 µg/l. The diagnostic efficiency to correctly classify subjects as iron replete and iron deficient was superior for serum ferritin measurement as compared to the other tests examined. A serum ferritin level less than 16 µg/l resulted in a specificity of 98% and a sensitivity of 75%.

As suggested by Cook *et al* (1984)., combination of haemoglobin concentration and serum ferritin measurement provides higher specificity and sensitivity. If both are normal, iron deficiency is excluded. If both are low, iron deficiency anemia is the diagnosis. If serum ferritin is low but haemoglobin is normal, then the individual may have depleted iron stores or mild iron deficient erythropoiesis. This study thus combined serum ferritin and haemoglobin concentration as iron deficiency parameters.

1.2 Iron

Haemoglobin and myoglobin are iron – containing proteins. Each molecule of haemoglobin has four irons that fundamentally assist in the binding of haemoglobin with oxygen. Lacking of iron may reduce the oxygen – carrying capacity of blood. Thus, tissues do not have enough oxygen supply and this becomes the main reason of tiredness and fatigue in iron – deficient individual. Besides oxygen transportation, iron also plays essential roles in enzymatic actions, protein metabolism, energy production, maintaining the body immune system, etc. Normal adult body contains approximately 3.5 – 4.5 g of mineral iron. Approximately 60% of this iron is present in blood and the remainder is stored as ferritin in the liver, spleen, bone marrow and muscles (figure 1.0).

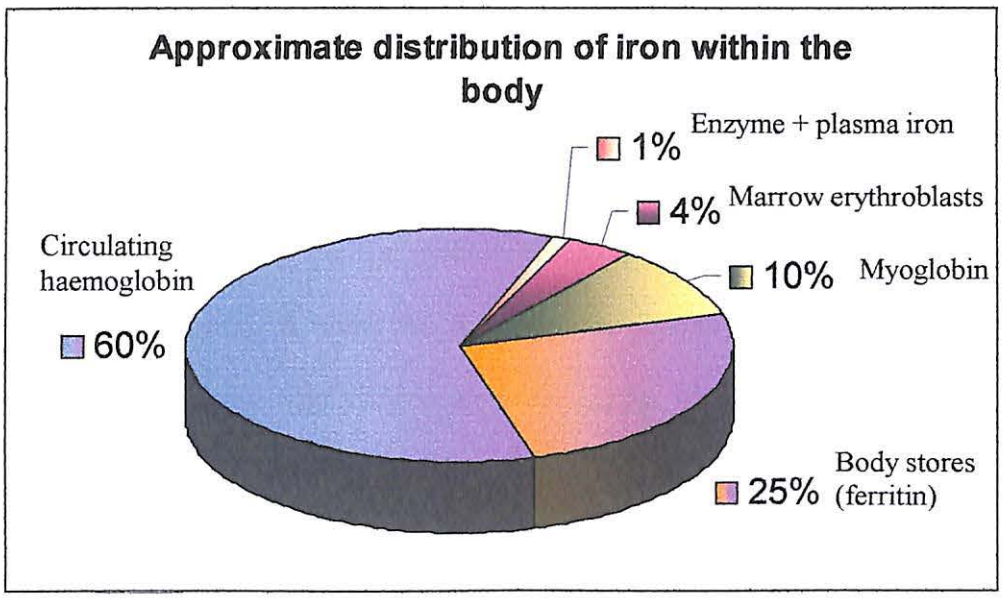

Figure 1.0: Iron distribution within the body (C J Pallister, 1999)

Iron is transported by transferin and absorbed in intestines by certain mechanisms. Interestingly human body does not have specific pathway for iron excretion. Thus, iron is never 'excreted' but instead, it is 'lost'. Bleeding such as gastrointestinal bleeding and menstruation or blood loss during accident will cause iron lost. Donating blood also resulted in iron lost (Ahmed Badar *et al.*, 1996). A volume of 450 ml of blood will cost about 200 – 225 mg of iron lost. A normal healthy donor will require at least three months to replenish their iron stores (Cook JD *et al.*, 1984). However, donors with high prevalence of donations, their iron stores are in constant pressure. Without appropriate treatment, these iron – deficient donors might suffer from anemia since iron deficiency induces microcytic, hypochromic anemia.

1.2.1 Serum Ferritin

Ferritin exists as a spherical structure with a diameter of 5 nm and hydrated ferric (Fe^{3+}) phosphate as its core. Ferritin, with molecular weight of 450 000 D, serves as the principal storage of iron. A small fraction of body ferritin circulates in the serum is known as serum ferritin. The concentration of serum ferritin reflects the amount of storage iron and that 1 µg/l per liter of this compound is roughly equivalent to 10 g of iron stores. Normal value for male is 40 – 340 µg/l while the value is slightly lower for female which is 14 – 150 µg/l.

Serum ferritin shows a high correlation to the storage iron in the reticuloendothelial cells of the bone marrow. Therefore it is a reliable test to watch over the body iron stores of blood donors (Henneberg KB, 1980). Frewin *et al.*, (2003) also suggested that ferritin measurement is a reliable parameter to distinguish iron deficiency anemia from other types of anemia. This is due to low serum ferritin level in iron – deficient patients. In order to screen iron deficiency, the standard serum ferritin level applied in this study is based on WHO recommended cut off point which is below 15µg/l.

1.3 Iron Deficiency Anemia

Iron deficiency anemia is one type of microcytic hypochromic anemia. The word anemia is composed of two Greek roots that together mean "without blood" (Ed-Uthman, 1998). Signs and symptoms of anemia include weakness, fatigue, palpitation, headache,

difficulty in swallowing, loss of appetite, nausea, constipation, diarrhea, stomatitis and others.

In Malaysia, anemia has been extensively documented since 1950's and most of anemia was due to iron deficiency (reported by WHO). The epidemiological study of anemia in Malaysia as provided by a survey data stated that anemia has major effects in pregnant women, infants, young children and the elderly (Tee ES, 1998).

Generally, anemia is defined as reduction in the haemoglobin concentration and hematocrit values of the blood. Normal haemoglobin concentration as outlined by WHO at sea level are 13.0 – 17.0 g/dl for men and 12.0 – 15.0 g/dl for women. The values differ from an individual to another according to age, sex and altitude. Any values lower than the normal range is suspected to experience anemia.

Haemoglobin concentration alone is not a reliable parameter in diagnosing anemia. The cornerstone of the laboratory identification of iron deficiency anemia is a low haemoglobin and serum ferritin concentration although a normal serum ferritin does exclude iron deficiency anemia (Cook JD, 1974). Other parameters such as bone marrow iron, red cell indices (MCV, MCH and MCHC) and iron assessment tests (SF, TIBC, and sTfR) will further assist in determining the underlying causes of anemia. Blood film is also helpful in classifying the types of anemia.

1.3.1 Pathogenesis of Iron Deficiency Anemia

Iron deficiency anemia is a nutritional anemia that occurs worldwide especially in both developed and developing countries. Blood donors with high frequency of donations are prone to develop iron deficiency anemia. This is because approximately 225 mg of iron is lost during a single donation (Ahmed Badar *et al*, 1996). The pathogenesis of anemia is simply classified into three successive stages.

In earlier stage, iron stores are lowered but the patient has no clinical effects. In the next stage, iron is depleted. Laboratory indices show that iron stores are exhausted while haemoglobin concentration is within the normal range. In the final stage, the patient develops iron deficiency anemia. Biochemical hallmarks of iron deficiency are; no iron is left in the marrow and haemoglobin production falls. The progression of iron deficiency anemia happens insidiously and thus, it is difficult to detect.

1.4 Blood Donation

Transfusion Medicine Unit of HUSM has practice a standard guideline for the procedure of blood donation. All blood donors will be registered prior to donation. Their haemoglobin concentration will be checked at the time of registration by using portable Hemocue. Donor with low haemoglobin concentration is temporarily deferred while donors with normal haemoglobin value are eligible to donate. A maximum of 450 ml of blood is drawn in a single donation.

1.4.1 Criteria for Whole Blood Donors

Blood donors must have certain standard criteria to donate their blood. This criteria requirement is made in order to make sure that the donors will not develop any serious complication during post – donation. The criteria that should be met are:

i. Age between 18 – 55 years old
ii. Weight more than 45 kg.
iii. Haemoglobin concentration within normal range (refer to WHO cut off level).
iv. Last donation must be three months before.
v. Have enough sleep for at least 5 hours in last night.
vi. Healthy enough (feeling well and can perform normal activities).
vii. Pulse rate within 50 – 100 beats/min.
viii. Blood pressure can be up to 140 / 90 mmHg (maximum)
ix. Not pregnant (for female donors)

1.4.2 Exclusion Criteria for Blood Donors

There are donors who are deprived from donating blood. They are:

i. Donor with confirmed HIV positive, or may be HIV positive.
ii. A carrier for Hepatitis B or Hepatitis C virus
iii. Previously had become a drug addict
iv. Haemophiliacs.
v. Have ever injected, or been injected, even only once, with illegal drugs

including body building drugs.

vi. A man who has had sex with other men since 1977 (homosexual)

vii. A man who has had sex with other men and women (bisexual)

viii. A man who is sexually active with prostitutes.

ix. A man who has multiple sexual partners.

x. A prostitute.

xi. Anyone with Crueutzfeldt – Jakob disease (CJD) or signs and symptom f CJD or who has an immediate family member with CJD.

1.4.3 Pre – donation Care

Prior to donation, the eligible donors are advised to eat something. Normally, meals are provided by Transfusion Medicine Unit of HUSM. The donors should be explained clearly with the procedure of donation and if possible, calm the donors.

1.4.4 Post – donation Instructions

After donation, the donors will be asked to rest on the donation chair for at least 10 minutes before getting up. They are also encouraged to eat and drink something as refreshment before leaving. They are required to tell a staff member if they feel something wrong or any unwanted reaction happens such as headache, nervousness, flushing or perspiration. If there is other complication that occurs within 3 to 4 days, they are asked to contact the department with information of the illness.

1.4.5 Screening test for Donated Blood

Before the blood is transfused into patients, it is tested for blood grouping, rhesus and other unexpected antibodies that may react with recipient's antigen. This is made purposely to minimize and to avoid any serious complication such as Hemolytic Transfusion Reactions. Microbiology tests are also done for evidence of any infections such as:

i. Hepatitis B surface antigen (HBsAg)
ii. Hepatitis C virus antibody (anti – HCV)
iii. HIV – 1 and HIV – 2 antibody
iv. Serologic test for syphilis
v. Malarial test

Chapter 2

Literature Review

A study of anemia and iron status among blood donors in a blood transfusion unit in Malaysia reported that iron deficiency as observed by low ferritin levels was seen in 7.4% of all first time donors as compared to 17.4% in regular donors. They concluded that a high prevalence of iron deficiency is present among regular male donors and all female donors (Nadarajan VS and Eow GI, 2002).

Ton SH and Lopez CG (1980), in their findings of Malaysian donors according to races, stated that serum ferritin levels among Malays donors ranged from 16 – 160 mg/ml, in Chinese donors is ranged from 36 – 500 mg/ml and in the Indian donors it ranged from 5 – 270 mg /ml.

A cross-sectional study in Singapore conducted by Singh K *et a.l*,(1998) also reported that the commonest cause of anemia is due to iron deficiency, which the occurrence was 81.3%. Ting WC and Lim HC (1984) also assessed the iron status in blood donors of Singapore. They conclude that reduction in iron stores was related to the frequency rather than the total number of donation.

In Thailand, the iron status of Thai blood donors was also assessed in year 2000. Depleted iron stores were found in 8.7% of first time female donors, 21.21% in regular male donors and 32.65% in regular female donors. They also stated that the frequency of donations per year was more predictive of decreased iron stores than the number of total donations (Tardtong P *et al*, 2000).

Another study conducted by Alvarez –Ossorio *et al* (1994) was about ferritin level. They evaluated the need to use ferritin in addition to hemoglobin concentration to screen iron deficiency in blood donors. Their results showed that ferritin decreases after 10 donations and with the increase of donation frequency. In 26% of regular donors, ferritin levels were < 15 mg/L and 12% of them were anemic due to low hemoglobin. They concluded that regular ferritin measurement is a useful indicator for iron depletion in blood donors. Their data suggested the usefulness of ferritin screening in first – time donors and regular donors with low hemoglobin levels within normal range.

Haemoglobin concentration and iron status of Danish males comprising of blood donors and non – blood donors had been assessed. They found that haemoglobin levels were identical in donors and non – donors. Donors had lower serum ferritin mean (95 µg/l) as compared to non – donors (136 µg/l). Serum ferritin levels below 15 µg/l (considered as depleted iron stores) were seen in 3.3% of donors and 0.4% of non – donors. Small iron stores indicated by serum ferritin values within 15 – 30 µg/l were seen in 9.8% of donors and 1.4% of non – donors. They concluded that blood donation had a marked influence on iron status in the adult male donor population. They also suggested

optimal donation standard should include monitoring of iron status through measurement of serum ferritin and haemoglobin (Milman M, 1991).

By February 1996, Milman N again studied iron stores among Danish population. His findings were blood donation had a marked influence on the serum ferritin level in the adult population. Among Danes, 20-60 years of age, 27% of men, 15% of premenopausal and 10% of postmenopausal women were blood donors. In all three groups, the prevalence of depleted iron depots was higher in donors than in non-donors. Among premenopausal female donors, 31.7% had depleted iron reserves and 3.3% iron deficiency anemia (Milman N, 1996).

A paper by Finch *et al*, regarding iron stores of blood donors was published in 1997. They believed that blood donation was associated with a decrease in serum ferritin. One unit per year, equivalent to an increased requirement of 0.65 mg/day, halved the serum ferritin level in the male. More frequent donations were associated with further decreases. From the data obtained it would appear that male donors, while depleting their iron stores, were able to donate 2-3 units per year without an appreciable incidence of iron deficiency. Women could donate only about half that amount, and more frequent donations were associated with a high incidence of iron deficiency and donor dropout.

A study evaluated 217 regular blood donors, with the aim of measuring their iron stores and recording the influence of donation frequency. In the population studied, 10% of men donors and 15% of women donors showed reduced iron stores of the

erythropoietic marrow (erythrocyte ferritin less than the normal range) and 64% of the population showed a latent deficiency in iron (serum ferritin values less than the reference values) (Guillemin C *et a.l*, 1992).

300 blood donors from Santa Casa Hemocenter of Sao Paulo were studied. The frequency of iron deficiency was higher in multi-time blood donors than in first-time blood donors, for male blood donors (7.6% versus 0.0%, $P < 0.05$) and female ones (41.5% versus 18.5%, $P < 0.05$). The frequency of iron deficiency found was higher among the male blood donors with three or more donations per year ($P < 0.05$) and among the female blood donors with two or more donations per year ($P < 0.05$) (Cancado RD *et a.l*, 2001).

A study done by Szymczyk-Nuzka M, 2003, concluded that they found a high incidence of iron depletion in regular whole blood donors with normal blood count. They suggested to regularly checking the iron metabolism status in those donors in order to prevent them from keeping on giving blood until the restoration of iron balance.

A study done in Norway found that there was no coincidence between low haemoglobin and low serum ferritin when haemoglobin was within normal range. They suggested to include measurement of serum ferritin as a better method of identifying individuals at risk for iron depletion than measuring haemoglobin. (Halvorsen R *et al*, 1990).

A collaborative study of Putra University of Malaysia – Institute of Medical Research (UPM –IMR) has assessed the prevalence of anemia in 69 villages and 7 estates in 9 states in Peninsular Malaysia. In their findings, prevalence of anemia among children below 7 years old was 24%, 21.9% for aged 7-12.9, 17.8% for aged 13-17.9, 21.0% for aged 18-59.9 and 22.7% for those aged 60 above. The female adults has the higher prevalence (25%) compared to male adults (14%) (Tee ES *et al.*, 1998).

Mayang Sari *et al* in their study 'Estimating the prevalence of Anemia: a comparison of three methods' concluded that the prevalence of anemia was overestimated by the indirect assessment method. They suggested that the method of choice in estimating the hemoglobin concentration would be Hemocue method, especially to conduct a study over comparatively large areas. This is followed by the gold standard method, direct cyanmethemoglobin where field conditions and local resources allowed it (Mayang Sari *et al*, 2001).

Chapter 3

Objective

The aims and interest of this study are:

i. To determine the prevalence of iron deficiency among eligible blood donors.

ii. To correlate between frequency of donation with haemoglobin concentration and serum ferritin level.

Chapter 4

Materials and Methods

4.1 Study Area

This study was conducted in Transfusion Medicine Unit of HUSM which located in Kubang Kerian, Kelantan. Donors who took part in blood bank mobile donations were also included. During this study, a few districts from four provinces of Kelantan were randomly selected which were Tanah Merah, Tumpat, Bachok and Kota Baru.

4.2 Sampling Design and Sample Size

This study was a cross – sectional study. All eligible blood donors were given informed consents and structured questionnaires. The purpose of providing questionnaire was to obtain baseline information regarding their age, sex, race, total number of donations, frequency of donations, and factors associated with iron status.

The eligible donors were classified into three groups. Grouping was based on their total number of donations. The first group was made of first time donors. This means that they have just registered as volunteer blood donors during the time this study was conducted. Second group comprised of donors who had previously donated only for two to four times. The third group consists of regular blood donors. Definition of regular

blood donors as applied in this study was donors who have total donations more than or equal to five times. All target respondents aged between 18 to 55 years old.

Sample size was calculated using *PS* software (Dupont and Plummer, 1997). Based on pilot study, it was estimated that the minimum requirement of sample size for this study was 220 blood donors.

4.3 Inclusion Criteria

i. All eligible blood donors, both first time donors and regular donors who agreed to participate in this study.

ii. Donors who were not receiving any medication and antibiotics.

4.4 Exclusion Criteria

i. Donors with haemoglobin concentration below than normal range

ii. Donors who refused to participate in this study.

4.5 Data Collection

The field work was conducted from November 2004 until January 2005. Written informed consents and answered questionnaires were obtained after explaining the aims of this study to the donors. Besides the questionnaires, data was also collected by retrieving their records of donation in Transfusion Medicine Unit of HUSM. All data gained was analyzed by using SPSS software.

The capillary blood was drawn by finger pricking method. For estimation of serum ferritin level, 5 ml of blood sample was taken from the donor's blood beg into labeled plain vacutainer, a product of Becton Dickinson (tube without anticoagulant). The vacutainer was then transported into laboratory for serum ferritin assay.

4.6 Physiological Basis of Laboratory tests used in this study

As quantitative measurements in this study, serum ferritin assay and haemoglobin concentration assessment were done. The former test was carried out in laboratory by AxSYM automated analyzer (Abbot Diagnostic). The latter test was directly done in the field by using HemoCue B – haemoglobin photometer.

4.6.1 Haemoglobin concentration measured by HemoCue

In order to measure haemoglobin concentration in this study, we used portable battery – operated HemoCue since it is light, small, easy and safe to use. The donor's fingertip was cleaned by isopropyl alcohol 70% swab (Becton Dickinson). Then, the finger was punctured by Carelet Lancet (autoclix apparatus). The first drop of blood was discarded. The next drop of blood with approximate volume of 10 μl was then collected into unbreakable HemoCue plastic microcuvette. The microcuvette was then inserted into the photometer for haemoglobin concentration measurement. Within 60 seconds, the result can be directly read and recorded.

Reagents used in HemoCue photometer are sodium desoxycholate, sodium nitrite, sodium azide and sodium fluorescein. The sodium desoxycholate hemolyzes the erythrocytes, liberating haemoglobin. The sodium nitrite converts the haemoglobin into methaemoglobin. This then reacts with the sodium azide methaemoglobin. The sodium fluorescein is used for quality control during the manufacturing process. Measurements are made at two wavelengths, 570 and 880 nm, to compensate for turbidity in the sample. Measurement range is from zero to 256 g/L.

4.6.2 Serum Ferritin Assay

4.6.2.1 Reagents

Principle of AxSYM Ferritin is based on Microparticle Enzyme Immunoassay (MEIA) technology. AxSYM Ferritin reagent pack (7A58 – 20) consists of:

i. 1 bottle (6.9 mL) Anti – Ferritin (Mouse, Monoclonal) Coated Microparticles in TRIS buffer with protein stabilizers. Preservative: Sodium Azide (Reagent Bottle 1).

ii. 1 bottle (4.3 mL) Anti – Ferritin (Rabbit): Alkaline Phospatase Conjugate in TRIS buffer with protein stabilizers. Minimum concentration: 0.1 µg/mL. Preservative: Sodium Azide. (Reagent Bottle 2)

iii. 1 bottle (7.5 mL) Specimen Diluent. TRIS buffer containing surfactant and protein stabilizers. Preservative: Sodium Azide (Reagent Bottle 3).

iv. 1 bottle (50.2 mL) TRIS buffer with 0.3 M Sodium Chloride. Preservatives: Sodium Azide and anti – microbial agents (Reagent bottle 4).

4.6.2.2 Sampling Center:

The AxSYM Ferritin Reagents and sample are pipetted in the following sequence:

i. Sample and all AxSYM Ferritin reagents required for one test are pipetted by the Sampling Probe into various wells of a Reaction Vessel (RV).

ii. Sample is pipetted into one well of the RV.

iii. Anti – Ferritin Coated Microparticles, Anti – Ferritin Alkaline Phosphatase Conjugate, Specimen Diluent and TRIS Buffer are pipetted into another well of the RV.

iv. The RV is immediately transferred into the Processing Center. Further pipetting is done in the Processing Center with the Processing Probe.

4.6.2.3 Processing Center

i. An aliquot of the Specimen Diluent, Conjugate, Microparticles and TRIS Buffer mixture is pipetted and mixed with the sample.

ii. The ferritin, enzyme – labeled antibody and microparticles bond forming an antibody – antigen – antibody complex.

iii. An aliquot of the reaction mixture containing the antibody – antigen – antibody complex bound to the microparticles is transferred to the matrix cell. The microparticles bind irreversibly to the glass fiber matrix.

iv. The matrix cell is washed to remove unbound materials.

v. The substrate, 4 – Methylumbelliferyl Phosphate, is added to the matrix cell and the fluorescent product is measured by the MEIA optical assembly.