ASSOCIATION BETWEEN ETHNICITY AND THALASSEMIA CARRIER STATUS AMONG SECONDARY SCHOOL STUDENTS INVOLVED IN THALASSEMIA SCHOOL SCREENING PROGRAMME IN PAHANG IN 2016

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2018

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by

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Research project submitted in partial fulfilment of the requirement for the degree of Master of Public Health

MAY 2018

ACKNOWLEDGEMENT

Bismillahirrahmanirrahim

In the name of Allah S.W.T, the Most Gracious, the Most Compassionate and the Most Merciful, whose blessings have eased my journey towards completing this task. This dissertation would not have been possible without His guidance and rahmah.

I wish to express my sincere and deepest gratitude to my supervisor, Associate Professor Dr Azriani Binti Berahim @ Ab. Rahman for her constant encouragement and continuous guidance to carry out and complete my dissertation, who always give a helping hand and motherly advice in guiding me on completing the whole process of my project.

My warmest and sincere thanks also go to Dr Nurly Zahureen Binti Mustapha, Senior Principal Assistant Director of Family Health Unit from Pahang State Health Department for her support and help especially in obtaining data on thalassemia school programme. Not to forget also all the staffs in Family health unit that willingly explained all my inquiries during the data collection process.

I also forever grateful to Dr Najib Majdi bin Yaacob in guiding me and sharing his expertise in data analysis process as well as Dr Suhaily Binti Mohd Hairon in lending a hand in this research project. I would also like to express my gratitude to all other lecturers, staff and my caring and supportive course-mates who shared their

knowledge and help throughout this incredible journey.

Last but not least I want to take this opportunity to express a sense of gratitude and deepest love to my beloved father and mother, my understanding brothers and sisters for their continuous courage, support and prayer; and to the one above all of us, Allah, for answering my prayers and giving me the strength and courage to accomplish the task. Alhamdulillah…

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LIST OF ABBREVIATIONS

CI	Confidence interval
DOSM	Department of Statistic Malaysia
FBC	Full Blood Count
HB	Haemoglobin
HLPC	High Performance Liquid Chromatography
HTAA	Hospital Tengku Ampuan Afzan
HOSHAS	Hospital Sultan Ahmad Shah
IDA	Iron deficiency anaemia
IMR	Institute for Medical Research
JKNP	Jabatan Kesihatan Negeri Pahang
MCH	Mean Cell Haemoglobin
MCV	Mean Cell Volume
MOH	Ministry of Health
MS	Microsoft Software
NMRR	National Medical Research Registry
OR	Odd ratio
RBC	Red blood cell count
RDW	Red cell distribution width

ROC	Receiver operation characteristic
SEA	South East Asia
WHO	World Health Organization

LIST OF SYMBOLS

>	More than
<	Less than
=	Equal to
≥	More than and equal to
≤	Less than and equal to
α	Alpha
β	Beta
δ	Delta
%	Percentage

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ABSTRAK

HUBUNG KAIT DIANTARA ETNIK DAN STATUS PEMBAWA TALASEMIA DI KALANGAN PELAJAR SEKOLAH MENENGAH TERLIBAT DALAM PROGRAM SARINGAN TALASSEMIA SEKOLAH DI PAHANG PADA 2016

Latar Belakang: Talassemia kekal sebagai kebimbangan dalam kesihatan masyarakat di Malaysia walaupun program saringan telah dijalankan semenjak tahun 2004. Pengambilan ujian saringan secara sukararela masih kurang memuaskan kerana kurangnya kesedaran orang ramai tentang kepentingan aktiviti saringan talassemia. Orang ramai tidak akan tampil ke hadapan untuk ujian jika mereka tidak menyedari bahawa mereka terdedah menjadi pembawa talassemia.Penduduk pelbagai etnik seperti di Pahang menimbulkan lebih cabaran untuk mengatasi keaadaan ini.Dalam pengetahuan kami, belum ada lagi kajian analitik dilakukan untuk melihat hubungan antara etnik dan status pembawa talassemia di Malaysia.

Objektif: Tujuan kajian ini dilakukan adalah untuk menggambarkan jenis pembawa thalassemia yang berlainan dan menentukan hubungan antara etnik dan status pembawa thalassemia di kalangan pelajar sekolah menengah di Pahang.

Metodologi: Kajian kes kawalan telah dijalankan daripada bulan Mac 2018 sehingga Mei 2018 melibatkan sepuluh buah daerah di Pahang. Kajian ini dibuat dengan menggunakan data sekunder yang diperoleh dari borang ‘Rekod Saringan Talassemia

Sekolah' ***(KSK/ST/101/2016)*** sebagai sumber data. Kes adalah pelajar yang didiagnos pembawa thalassemia manakala kawalan adalah pelajar yang bukan pembawa thalassemia seperti yang di dokumenkan di dalam pangkalan data. Regresi logistik mudah dan regressi logistic berganda telah digunakan untuk menganalisa hubungan antara etnik dan status pembawa thalassemia.

Keputusan: Sebanyak 1875 orang subjek yang memenuhi kriteria inklusi dan eksklusi telah dikaji, di mana 625 adalah kes dan 1250 adalah kawalan. Kajian ini mendedahkan bahawa pembawa talasssemia HbE adalah jenis paling biasa (58.2%), diikuti oleh pembawa talasssemia jenis Alpha (24.2%), pembawa talasssemia jenis Beta (16.8%), pembawa talasssemia jenis HbS (0.64%) dan pembawa talasssemia jenis HbC (0.16%) dikesan di kalangan pelajar-pelajar tersebut. Kajian selanjutnya menunjukkan bahawa status pembawa talassemia adalah berkait rapat dengan etnik Melayu (Adj. OR 3.73, 95% CI; 1.34, 10.34; p= 0.012) dan etnik Orang Asli (Adj. OR 3.94, 95% CI; 1.34, 10.34; p= 0.012) berbanding etnik India.

Rumusan: HbE adalah jenis pembawa talassemia paling biasa dikesan di kalangan pelajar sekolah menengah di Pahang. Etnik Melayu dan Orang Asli adalah lebih berisiko untuk menjadi pembawa talassemia berbanding etnik India. Dengan dapat mengenali kumpulan yang berisiko untuk menjadi pembawa talassemia, pastinya akan membantu kita mengoptimakan program kawalan dan pencegahan untuk mengurangkan jumlah kes talassemia di Malaysia terutamanya di Pahang.

KATA KUNCI: Etnik, pembawa talassemia, pelajar, Pahang

ABSTRACT

ASSOCIATION BETWEEN ETHNICITY AND THALASSEMIA CARRIER STATUS AMONG SECONDARY SCHOOL STUDENTS INVOLVED IN THALASSEMIA SCHOOL SCREENING PROGRAMME IN PAHANG IN 2016

Background: Thalassemia remains a public health concern in Malaysia despite screening programme has been conducted since 2004. Voluntary uptake towards screening test still unsatisfactory as there was lack of public awareness about the importance of screening activities. The public will not come forward for testing if they did not perceive they are susceptible to be a carrier. Multi-ethnicity population as in Pahang poses further challenges to overcome this situation. To our knowledge, there is yet no analytical study done looking at the association between carrier status with ethnicity in Malaysia.

Objective: This study aimed to describe the different types of thalassemia carrier and to determine the association between ethnicity and thalassemia carrier status among secondary school students in Pahang.

Methodology: A case control study was conducted from March 2018 till May 2018 involving ten districts in Pahang. The study used secondary data derived from *'*Rekod Saringan Talassemia Sekolah' ***(KSK/ST/101/2016)*** form as the source of data. Cases were students who were diagnosed thalassemia carrier and controls were students who were not thalassemia carrier as documented in the database. Simple

logistic and multiple logistic regression analysis were used to analyse the association between ethnicity and thalassemia carrier status.

Result: A total of 1875 subjects who fulfil the inclusion and exclusion criteria were studied, of which 625 were cases and 1250 were controls. This study revealed that HbE thalassemia carrier was the commonest type (58.2%), followed by Alpha thalassemia carrier (24.2%), Beta thalassemia carrier (16.8%), HbS thalassemia carrier (0.64%) and HbC thalassemia carrier (0.16%) detected among the students. Further analysis showed that thalassemia carrier status was significantly associated with Malay ethnicity (Adj. OR 3.73, 95% CI; 1.34, 10.34; p= 0.012) and Orang Asli ethnicity (Adj. OR 3.94, 95% CI; 1.34, 10.34; p= 0.012) compared to Indian ethnicity.

Conclusion: HbE thalassemia carrier was the commonest type detected among secondary school students in Pahang. Malay and Orang Asli ethnicity are at higher risk to be a thalassemia carrier compared to Indian ethnicity. Being able to recognize groups at risk to become a thalassemia carrier will certainly help us to optimize the control and prevention programme in order to bring down the number of thalassemia cases in Malaysia especially in Pahang.

KEY WORDS: Ethnicity, thalassemia carrier, students, Pahang

CHAPTER 1

INTRODUCTION

1.1 Study background

The thalassemia syndromes, a type of haemoglobinopathies is the most common inheritable genetic disorder worldwide and still remains a major public health concern globally up to this age. It is an autosomal recessive disorder characterized by the absence or reduction in the synthesis of one or more globin chain of haemoglobin, mainly the alpha or beta chain. A person is called a carrier when he/she has one defective alpha or beta gene (heterozygous state) and usually is asymptomatic (Angastiniotis *et al.,* 2013).

Due to this, many of them are unaware of their carrier status if they do not commit themselves for blood testing for thalassemia screening. If two carriers marry each other, they have 25% chances to produce an offspring with thalassemia disorder. Because of the severity of the anaemia and other serious complications associated with this condition, many of the affected patients do not have long survival without proper and adequate treatment (Weatherall and Clegg, 2008). Modell and Darlison summarized that roughly about 24% of world population is a carrier for haemoglobin gene variant. It is also estimated that there are about 300,000 affected births each

year for haemoglobin disorder among the world population. Approximately 60,000-70,000 among these affected babies is beta thalassemia major. They also stated that haemoglobin disorder cause at least 3.4% of deaths in children aged less than 5 years old worldwide and at the same time agreed that this figure is still very much underestimated (Modell and Darlison, 2008).

In Malaysia, thalassemia maintains to be a main public health problem and the effort to curb and prevent this disorder poses a huge challenge to all the public health practitioners. Malaysia is considered a high prevalence country for thalassemia. It was reported that approximately 4.5% of the Malaysian populations are Beta (β) thalassemia carrier and about 3-40% are Haemoglobin E (HbE) carriers. It is estimated that about 250 thalassemic babies are born in Malaysia annually (George, 2001).

In Pahang specifically, a discouraging situation occurs in which the numbers of thalassemia patient being diagnosed keep increasing each year. In 2013 there are about 450 patients being diagnosed with thalassemia compared to 263 patients in the previous year. As for thalassemia carrier, for the year 2012, about 622 thalassemia carriers detected from government health facilities in Pahang from voluntary screening, and it should be stated here that, it is among the lowest detection rate compared to other states in Malaysia (MOH, 2016).

Managing thalassemia is costly not only in term of medical cost shouldered by the government but also has a strong impact both economically and emotionally on the

affected family and as well as the community as a whole (Miskam *et al.*, 2016). The lifetime cost of managing one Beta thalassemia major patient is estimated around RM 1,000,000 (Karnon *et al.*, 1999). Fortunately, due to its simple Mendelian inheritance of autosomal recessive disorder, prevention of thalassemia is mostly possible to achieve. Thalassemia can be prevented effectively if no carrier marries another carrier as in each pregnancy there will be 25% risk to get a child with thalassemia if both parents are carriers of the defective gene of thalassemia (Angastiniotis *et al*., 2013).

Ministry of Health had initiated a nationwide thalassemia school screening programme in early 2016 to complement current thalassemia screening programme in the community. This programme involved voluntary participation from secondary school students from government schools in all states including Pahang (MOH, 2016).

Public acceptance and awareness regarding thalassemia play a vital role in the uptake of voluntary screening test among the community as being practised in our country so far (MOH, 2008). We need a good response for screening test among the public because this will help in making an accurate estimation of thalassemia burden in Malaysia for successful and cost effective prevention policy. Malaysia is a multi-ethnicity country and this undoubtedly poses further substantial challenges in detecting carrier via screening programme. This is because; thalassemia is regionally specific and may differ between ethnicity in each local population in view that it can have its own characteristic spectrum of mutations even in a neighbouring

geographical area (Angastiniotis *et al*., 2013). A study by Ahmad *et al.* (2013) revealed that there is great variability in the prevalence of haemoglobinopathies among different ethnicity in Malaysia. Certain ethnic groups have higher chance to become a carrier (MOH, 2008). Pahang has estimated population of about 1,626,700 as in 2016. The main ethnic composition was Bumiputera 74.9%, Chinese 14.3%, Indian 4.0% and others 0.34% with non-Malaysian citizen 6.4% (DOSM, 2016). According to Evers (2014), Malaysian states shows a great difference in term of ethnic diversity with Pahang was categorized under medium ethnicity density index along with Melaka, Perlis and Kedah state.

1.2 Study justification, rationale and benefit

Despite the Thalassemia Prevention and Control Programme had been conducted since 2004 by MOH, the number of new thalassemias is still not declining and expected to incur more cost for government in the future (MOH, 2009). It is crucial to make the public aware the importance of screening test as they will not come forward for testing if they did not perceive they are susceptible to be a carrier. Furthermore, ethnicity and cultural diversity may influence the perception of risk and health seeking behaviour in a person (Wong *et al.*, 2011; Vasudeva Murthy *et al*., 2015; Karimzaei *et al*., 2015). Thus, multi-ethnicity population as in Pahang poses further challenges in promoting and improving voluntary participation from the public for thalassemia screening test.

The information on common types of thalassemia carrier in a different population helps a country to estimate the real burden of thalassemia in order to develop an

effective prevention and control programme. Specific prevention and control strategies can be developed for population identified at high risk of having the severe type of thalassemia.

Furthermore, there is limited information looking at the association between carrier status with ethnicity in Malaysia especially in an individual state like Pahang which ethnicity density is quite high and has a large number of Orang Asli populations. Most of the currently available studies are descriptive studies mainly focusing on genetic, molecular study and treatment of thalassemia patient (homozygous state). By analysing the association between ethnicity and carrier status, we can identify which ethnic at higher risk to become a carrier. In addition to that, our study also will examine the haematological characteristics of thalassemia carrier in Pahang secondary school students. According to Angastiniotis *et al.* (2013) it is important to establish haematological characteristic of the carrier for each specific population because due to the interaction of various gene in the different population, it may alter the usual haematological manifestation of thalassemia carrier. It is hoped that findings from this research would help health care provider in promoting the importance of voluntary thalassemia screening in the community, especially among the adolescents. It also may be utilized to increase the awareness among public thus improving the uptake of voluntary thalassemia screening. Finally, it may serve as a guide to improve current thalassemia screening programme for better provision in health care service in Malaysia in the future. A cost effective prevention programme is important especially for population screening in a high prevalent country with thalassemia like Malaysia.

1.3 Research Questions

1. What are the common types of thalassemia carrier detected among secondary school students in Pahang?

2. Is there any association between ethnicity and thalassemia carrier status among secondary school students in Pahang?

1.4 Objectives

1.4.1 General Objective

To study the common types of thalassemia carrier among secondary school students in Pahang for the year 2016 and the association of carrier status and ethnicity

1.4.2 Specific Objective

1.4.2.1 To describe the different types of thalassemia carrier detected among secondary school students in Pahang in 2016.

1.4.2.2 To determine the association between ethnicity and thalassemia carrier status among secondary school students in Pahang in 2016.

1.5 Hypothesis

There is a significant association between ethnicity and thalassemia carrier status among secondary school students in Pahang in 2016.

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CHAPTER 2

LITERATURE REVIEW

2.1 The classification of thalassemia and carrier status

Thalassemia and structural haemoglobin variant (abnormal haemoglobin) are the two main group of hemoglobinopathies and are genetically passed down through autosomal recessive inheritance (Kohne, 2011; Fuchareon and Winichagoon, 2011) meaning that only in a homozygous state where two copies of abnormal gene present, the disease will develop. Thalassemia syndromes are a heterogeneous group of haemoglobin disorder characterized by either reduced synthesis or complete absence of synthesis of globin chain. It can be divided into two main groups based on which globin chain affected the Alpha (α) or Beta (β) thalassemia (Weatherall and Clegg, 2008).

Another way to classify thalassemia is by phenotypic classification based on severity (clinical classification) of its clinical manifestation. It can be divided into the *major* forms in which there is the presence of severe anaemia and commonly the affected people need a regular blood transfusion to prolong their survival or *minor* forms which usually are symptomless. Thalassemia minor also frequently referred as a

carrier state or traits. Carrier can only be identified haematologically via blood testing. As carrier of thalassemia is usually asymptomatic, most of them look healthy without obvious clinical symptoms. Due to this, many of them are unaware of their carrier status if they do not commit themselves for blood testing for thalassemia screening (Weatherall and Clegg, 2008; Angastiniotis *et al.*, 2013). However, some carrier of thalassemia may exhibit sign like mild pallor that resembles iron deficiency anaemia (IDA). Because of this almost similar presentation between thalassemia carrier and IDA patient, many carriers are often wrongly treated with unnecessary iron supplement for IDA (Ainoon and Cheong 1994).

Another familiar term used in clinical practice is thalassemia *intermedia* which encompass a wide spectrum of the clinical presentation describing patient with more severe features than asymptomatic carrier but milder than transfusion dependant major type (Chern *et al*., 2006). Thalassemia and haemoglobin variant genotype can interact with each other and produce complex haematological phenotypes that are often difficult to interpret especially in a population with a high frequency of different forms of these genetic conditions (Angastiniotis *et al.*, 2013; Weatherall and Clegg, 2008).

Due to its nature of autosomal recessive in inheritance, if two carriers with thalassemia get married and reproduce, based on probability, 25% of their children will be affected and can have a severe type of thalassemia, 50%, will becoming a carrier of thalassemia and 25% will be with normal genotype. Figure 2.1 below shows the possible combination if both parents are a carrier and the risk of the

affected foetus (Angastiniotis *et al.*, 2013).

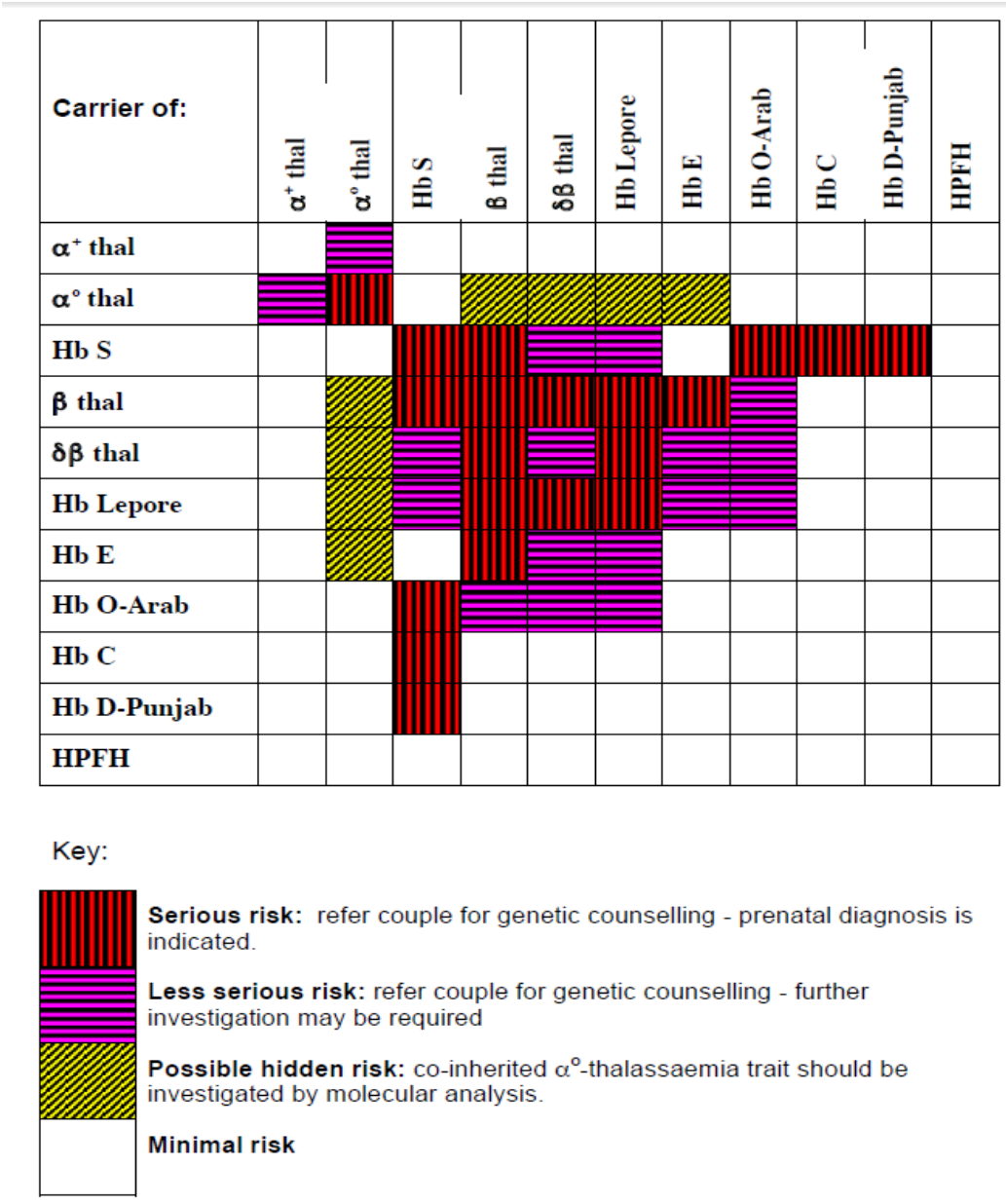


Figure 2.1: Risk of foetus with possible parental carrier state combinations

2.2 The types of thalassemia

Thalassemia is endemic in certain regions around the world mainly in Mediterranean, Middle East, Indian continent and South East Asia (SEA).Gene frequencies for the different thalassemia types are distributed differently across these regions (Weatherall and Clegg, 2008). There are hundreds of thalassemia variants recognised around the world (Welch, 2015) and each at risk population has its own spectrum of common mutations. As stated previously, thalassemia syndrome is a complex disorder with a wide range of clinical severity due to numerous ways the thalassemia and variant genotypes can interact with each other. So those that are clinically important include Beta thalassemia, the compound Beta and HbE thalassemia and some forms of non-deletional Alpha thalassemia which can cause severe anaemia and most of the time required lifelong blood transfusion (Angastiniotis *et al.,* 2013). Globally, HbE carriers are the most encountered hemoglobinopathies and the incidence can approach as high as 60% of most of Southeast Asia population (Vichinsky, 2005; Fucharoen and Winichagoon, 2011; George, 2013). Alpha and Beta thalassemia also are prevalent in SEA. The gene frequency can range between 2.6 to 30% in a certain region of SEA (Fucharoen and Winichagoon, 2011; Ahmad *et al.,* 2013; Yamsri *et al.,* 2015).

The knowledge of frequency distribution and common types of thalassemia carrier in a different population helps a country to estimate the real burden of thalassemia. For example, even there was a high prevalence of Hb Constant Spring in minority group

in Central Vietnam, it will not cause public health problem for the community as severe thalassemia syndrome was less likely to occur because alpha and beta thalassemia were not found in their community (Nguyen *et al.,* (2017).

In Malaysia, Alpha thalassemia, Beta thalassemia, Hemoglobin E (HbE), δβ-thalassemia and Hb Constant Spring are prevalent (Ainoon and Cheong, 1994). In peninsular states of Malaysia, HbE β-thalassemia is more common in northern states bordering Thailand (MOH, 2009). Meanwhile in Sabah, especially among the Kadazan-Dusuns, the predominant mutation is the β mutation/n-Filipino deletion type A (Tan et *al.,* 2010). A study was done by Jameela *et al. (*2011) in Ampang involving 310 school students age of 15-16 shows the carrier rate for all thalassemia was 9.3% and the commonly identified carrier types are α-Thalassemia (34.5%), β-thalassemia (31%) and HbE thalassemia (24.1%). Whereas Meng *et al.* (2014) revealed among 499 of attendees from health clinics and a government hospital in Perlis, the overall carrier rate for hemoglobinopathies is 42.7% with common thalassemia trait detected are HbE trait (19.3%) and β-thalassemia trait (14.5%). Meanwhile, Ezalia *et al*. (2014) found that 4.9% of carrier rate among the 738 of healthy blood donors when conducted the study in Klang between July-September 2010 and she describes that the most common type of carrier noted to be HbE (2.6%), β-thalassemia (0.7%) and Hb Constant Spring (0.3%).

A Talib *et al*. (2011) conducted a study in Kuantan, Pahang among 72 Medical and science students in International Islamic University Malaysia (IIUM) revealed that 17 (23.6%) students were diagnosed as carriers with predominance of α-Thal1SEA type

(12.5%), HbE trait (8.3%) and β-thalassemia (5.5%).

As for thalassemia school screening programme conducted in Pahang among the secondary school students for the year 2016, the thalassemia types that were analysed by the designated laboratory are Alpha carrier, Beta carrier, HbE carrier, HbS carrier, HbQ Thailand carrier, HbC carrier, HbD carrier, HbG carrier, HbJ carrier, HbJ Meerut carrier, HbJ Bangkok carrier, Hb Lepore carrier Hb Malay carrier, Hb New York carrier, HbO Indonesia carrier and HbO Arab carrier (MOH, 2016).

2.3 Thalassemia carrier and ethnicity

Malaysia is a unique multi-ethnicity, multi-lingual and multi-religion country that is changing rapidly for the past few decades. Its population has doubled in 30 years with populations of nearly 32 million in 2016. It is considered high upper-middle income country in South East Asia with its population comprises majority of Malay (50.1%) , Chinese (23.4%), indigenous people of Sabah and Sarawak (11%), Indian (7%) and other minority group (8.5%) making up for the rest of population (DOSM, 2016 and Lewison *et al.,* 2016). The Malays and Orang Asli are considered the original inhabitants of peninsular Malaysia. The Malaysian Chinese were mainly originated from the southeastern China provinces of Zhejiang, Fujian, Guangdong, Hainan and, Guanxi. Whereas Malaysian Indians are a group largely discovered to be descended from those who migrated from southern India. Thalassemia is more prominent among the Malays and Chinese, whereas the Indians form only a small percentage of those with thalassemia (Tan *et al.,* 2006 and Nagaraj *et al.,* 2015).

Pahang has estimated population of about 1,626,700 as in 2016. The main ethnic composition was Bumiputera 74.9%, Chinese 14.3%, Indian 4.0% and others 0.34% with non-Malaysian citizen 6.4% (DOSM, 2016). Pahang has the largest group of Orang Asli in Malaysia approximately 74,035 populations (JKNP, 2016). According to Evers (2014), Malaysian states shows a great difference in term of ethnic diversity with Pahang was categorized under medium ethnicity density index along with Melaka, Perlis and Kedah state.

Nguyen *et al.* (2017) reported based on his studies done previously regarding ethnicity and thalassemia in Vietnam that there was a very high prevalence of thalassemia among ethnic minorities namely Co-tu and Ta-oi compared to Khin ethnicity, which was the Vietnamese majority group. Meanwhile, in northern Thailand, Kulaphisit *et al.* (2017) revealed that after analysing 688 blood samples from thirteen different Thai ethnic groups, they found a higher percentage of Yuan and Paluang ethnic group carried alpha thalassemia genotype compared to other ethnic groups.

To our knowledge, there is no analytical study looking at the association between ethnicity and thalassemia carrier status in Malaysia. Several descriptive studies done in Malaysia related to thalassemia and ethnicity shows that among the three major ethnics in Malaysia, Malays are more likely to be diagnosed as a thalassemia carrier compared to Chinese and Indian. One of the study findings conducted in Kuala Lumpur by Wee *et al.* (2005) among 650 Antenatal women in University Malaya Medical Centre (UMMC); Malays show predominance to become a carrier with 65%

of the cases. Another study by Rosnah *et al.* (2012) in Kelantan involving 400 first time blood donors also identified Malays are most common to be a carrier for α-thalassemia with 3.7 deletion type. Surprisingly she also reported that no α-thalassemia deletion type detected in any Indian subjects. Similar with this finding was a study conducted by Ainoon *et al.* (2012) for 400 medical students from University Kebangsaan Malaysia Medical Centre (UKMMC) in which also found out that no thalassemia carrier detected in Indian students. Meanwhile, Pauzy *et al.* (2018) reported that in his study done in Sabah, Kadazandusun ethnic had the highest incidence of thalassemia carrier compared to other ethnic groups in that state.

2.4 Thalassemia carrier and other associated factors

A few haematological factors such as Mean Cell Volume (MCV), Red Blood Cell Count (RBC), Red Cell Distribution Width (RDW) and Haemoglobin (HB) were reported to be associated with the carrier status. MCV is usually reported in femtoliters (fL) and in most adult populations the normal ranges are between 80-100 fL. In most thalassemic patient, MCV was discovered to be low than normal ranges (Clarke and Higgins, 2000). However, in certain populations, a carrier of thalassemia variant may have the value of MCV more than >80fL (Chan *et al.*, 2001). RBC frequently expressed in the count of 10^{12}/L and most of the time found to be increased in thalassemia more than normal range of 4.50-6.50 10^{12}/L. According to a study done by Rathod *et al.* (2007), RBC value was significantly difference between beta thalassemia trait group and non-thalassemia trait group studied in Ahmedabad, India. RDW is a measure of the degree of variation in red cell size and commonly

reported as a percentage (%). In thalassemia, RDW value usually within the normal range 11-15% as compared to increase RDW more than normal in iron deficiency anaemia. Haemoglobin concentration (HB) is measured in gram per deciliter (g/dL) and usually, the normal ranges reported based on gender. HB concentration typically decreased in thalassemia patient but in carrier or trait condition, the HB concentration only mildly affected, thus giving the picture of normal HB level in the carrier (Clarke and Higgins, 2000; Rathod *et al.,* 2007).

As for the association of gender with thalassemia carrier status, Acemoglu *et al.* (2007) who conducted a study in Turkey, Erzurum conclude that from 1610 couples screened during premarital screening in 2005, no significant association between gender with carrier status was established. Babiker *et al.* (1999) also found similar finding when he studied 1020 school children age 6-15 in Jordan. Another study was done involving 466 healthy participants in Sindhi community in Nagpur City India by Mulchandani *et al.* (2008) also made the same revelation that there was no difference found in the overall prevalence of beta thalassemia trait between both genders.

According to Weatherall and Clegg, (2008) haematological picture of thalassemia may vary at a different stage of life. Furthermore, Berman *et al.*(1980) also reported there was striking age-related changes occur in haemoglobin and may alter hematologic manifestation of carrier depending on at what age haematological screening was done to detect carrier especially during childhood and young adulthood stage.

In the past few decades, migration between countries all over the world has become more prominent and this remarkably affects the gene frequency and clinical picture of thalassemia worldwide (Angastiniotis *et al.,* 2013). Migration has led to population mixes and the possibility of interracial marriage. According to Vichinsky (2005) due to rapid migration from thalassemia prevalent country especially from Middle Eastern, Indian and Asian region in the last decade, the haemoglobin (Hb) E–β-thalassemia and HbH disease which previously were rare to be found, becoming more common in North America and Europe especially among immigrants, making it a more significant health problem in the region.

In terms of geographical distribution and locality, a study by Hickman *et al.*, (1999) suggested that population who reside in inner city area had a higher prevalence of carrier in England county districts due to clustering of most of the ethnic minorities there. Another study conducted in western India by Colah *et al.,* (2010) reported a significant difference in five districts in both Maharashtra and Gujarat states respectively. According to Whetherall *et al*., (2010) distribution of haemoglobin disorder may differ significantly even in short geographical distances. However, one of the study's findings by Acemoglu *et al.,* (2008) in Turkey shows that even participants who were born in provinces other than Erzurum had a higher frequency of beta thalassemia carrier, but the difference was not statistically significant between provinces.

Another epidemiologic factor that can influence the occurrence of thalassemia carrier is consanguinity. Shawky *et al.,* (2013) reasoned that due to gene variants are trapped

within extended families with consanguineous marriage, their descendants may be at high genetic risk. Studies were done by Hafeez *et al.*, (2007) and Ahmed *et al.*, (2002) in Pakistan found that highest frequency of thalassemia carrier was found in the family that practise consanguineous marriage. However, from a study done by Acemoglu *et al.*, (2008) in Erzurum, Turkey there was no statistically significant association between consanguineous marriage and having thalassemia trait detected.

There are many different types of mutations that can alter gene action and influence thalassemia carrier status. Various types of mutations were already identified with significant association with thalassemia gene expression (Weatherall and Clegg, 2008).

2.5 Thalassemia screening programme

It is crucial to identify the carrier of thalassemia in order to assess the reproductive risk of a couple of having a severely affected child or not. Furthermore, it is important for the couple at risk to be adequately informed regarding all available options that they can have if faced with the dilemma in the future. Thalassemia screening program to detect carrier which includes public awareness and education followed with genetic counselling and coupled with prenatal diagnosis when necessary is found to be an effective way for the prevention of birth of thalassemia baby in many countries (Verma and Puri, 2015). For example, a marked decrease in thalassemia incidence is seen after 20 years following school screening in Montreal due to higher uptake (~99%) of prenatal diagnosis among identified carrier couples (Mitchell *et al*., 1996). Another study in France revealed that 86% of thalassemia carrier detected during school screening in Marseille still remember their carrier status and prompt them to request testing of their partner despite time lapse of 15 years between screening and pregnancy (Lena-Russo *et al*., 2002).

For time being, thalassemia is not only considered as a major health concern in Malaysia but also a socioeconomic problem that affects not only the patients themselves but both their families and community as well. A cross sectional study published by Ismail *et al.* (2006) evaluating the quality of life among affected thalassemia patient in Kuala Lumpur area revealed that by having the disease, it give negative impact on perceived physical, emotional, social and school function irrespective of age, gender, ethnicity and household income of the patient. The

quality of life of mothers of the affected child also greatly reduced due to the consequences of high stress and anxiety level in handling their child (Miskam *et al.*, 2016).

The aim of thalassemia screening in Malaysia is not only to identify carrier and assess the future reproductive risk of carrier couple but ultimately, if possible, to avoid marriage between carrier couple through informed choice and subsequently reducing the possibility of having an affected child with thalassemia. Public acceptance and awareness regarding thalassemia thus play a vital role in the uptake of voluntary screening test among the community as being practised in our country so far (MOH, 2008). Since 2004, thalassemia screening in Malaysia offered mainly under voluntary basis to the couple during premarital screening and antenatal women during first pregnancy as well as cascade screening in which the close relatives of the patient will be screened (MOH, 2008).

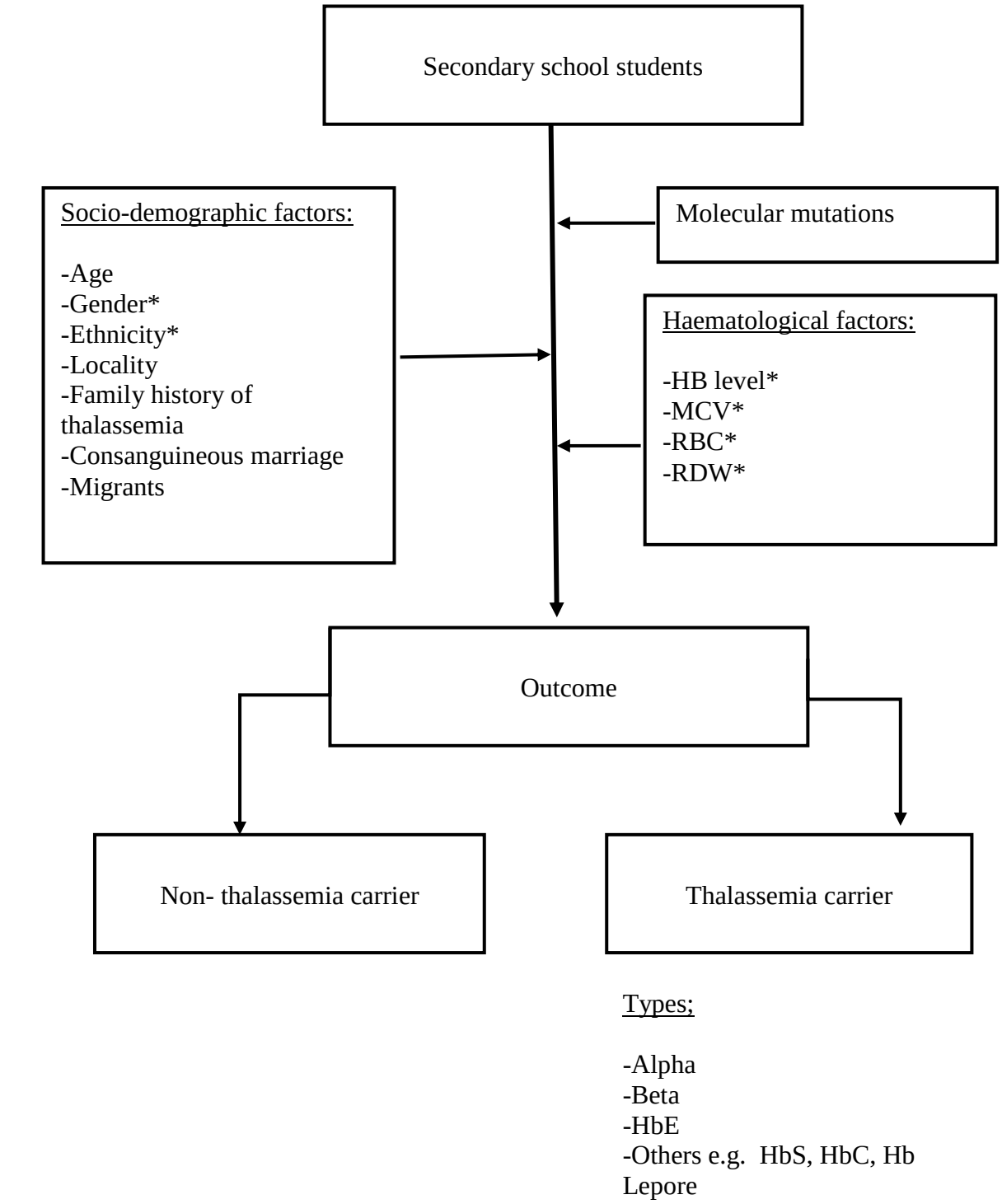
In early 2016, Ministry of Health had initiated nationwide thalassemia school screening programme targeting Form Four students in most of government secondary schools including Pahang state. This programme encompasses educational session regarding thalassemia followed by voluntary participation of students in the screening test with written consent from parents and finally, confidential post-test genetic counselling for the identified carrier will be conducted. This counselling session was given by the in-charged medical health officer in nearest health clinics. The data from this programme then were collected from each health clinic involved and given to respective state health departments for compilation (MOH, 2016).

In regards to screening methodology, Full blood count (FBC) was used as first line for thalassemia screening. FBC was generated by an automated blood counter produce many parameters of red blood cell indices. Among this, the red blood cell count (RBC), mean cell volume (MCV), mean cell haemoglobin (MCH), haemoglobin (HB) concentration and Red cell distribution width (RDW) were considered relevant for haemoglobinopathies screening (Clarke and Higgins, 2000). For national thalassemia school screening programme, cut off value of MCH <27pg and accompanied with normal range for HB for gender, warrant for further investigation of confirmation test of a carrier. For male gender, HB reading of 13 – 18 g/dL is considered normal and for non-pregnant female, the normal value is between 12-18 g/dL. All blood for FBC was analysed within 24 hours after blood taking. All health clinics involved in providing the services were equipped with trained medical laboratory technicians and regularly well maintained and calibrated automated haematology analyser to a standardized level of practice (MOH, 2016).

In Pahang, for confirmation of beta thalassemia carrier and HbE carrier, automated High Performance Liquid Chromatography (HLPC) and Gel Electrophoresis are made available in hospital laboratories namely Hospital Tengku Ampuan Afzan (HTAA), Kuantan and Hospital Sultan Ahmad Shah (HOSHAS), Temerloh. Further confirmation that needs molecular diagnosis especially for alpha thalassemia and other variants of thalassemia will be done either at Institute for Medical Research (IMR) or Hospital Kuala Lumpur laboratory (MOH, 2016). Detailed on each confirmation test for every type of thalassemia carrier is out of scope for this study and thus not discussed in here.

2.6 Conceptual Framework

Figure 2.2 shows conceptual framework of identified factors influencing the determination of thalassemia carrier status. Among the factors are socio-demographic factors, molecular and haematological factors. Those mark with (***)** are variables that were included in this study. They were gender, ethnicity, MCV, HB, RBC, and RDW. Ethnicity was the variable of interest and the others act as confounders. Other associated factors discussed prior were not included in this study as data were not available.



*variables included in the study

Figure 2.2: Conceptual framework of the study

CHAPTER 3

METHODOLOGY

3.1 Study Design

A case control study was conducted.

3.2 Study Duration

This study was conducted from March 2018 untill May 2018

3.3 Study Area

This study was conducted in Pahang and involved all the districts in this state. Pahang is the third largest states in Malaysia after Sarawak and Sabah and located in the east–cost region of Peninsular Malaysia. It is bordered to the north by Kelantan, to the west by Perak, Selangor, Negeri Sembilan, to the south by Johor and to the east by Terengganu and the South China Sea. It ranks as the largest state in Peninsular Malaysia which covers 35,840 km2 (about 10.9% of Malaysia coverage) with a total population of 1.63 million in 2016 (DOSM, 2016).

There are a total of eleven (11) districts in Pahang altogether; Bentong, Bera, Cameron Highlands, Jerantut, Kuantan, Lipis, Maran, Pekan, Raub, Rompin and Temerloh. Kuantan is the capital city of Pahang with the highest density of residence