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THE PREVALENCE OF THE THALASSEMIA TRAIT AMONG THE EAST MALAYSIAN STUDENTS IN THE FACULTY OF HEALTH SCIENCES, UNIVERSITI TEKNOLOGI MARA, UITM SELANGOR

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Abstract: The thalassemia trait is an asymptomatic thalassemia syndrome that increases the risk of developing a more severe condition. This study aimed to determine the prevalence of the thalassemia trait among Sabah and Sarawak students enrolled in the Faculty of Health Science at Universiti Teknologi MARA, UiTM Selangor. The study was carried out over 3 months following approval from the ethics and research committees of Universiti Teknologi MARA, UiTM Shah Alam. A universal sampling method was used where the blood samples were collected from the 40 students, 16 from Sabah and 24 from Sarawak, of various ethnicities. All subjects were asked to fill out consent forms. A volunteer medical laboratory staff member assisted in taking whole blood samples from the subjects' venous blood. The blood was then collected in a 3 mL EDTA (ethylenediaminetetraacetic acid) tube and was tested for thalassemia using a full blood count (FBC) and a full blood picture (FBP), as well as a Mentzer Index (MI) calculation, gel electrophoresis, and an H-inclusion test. In conclusion, the prevalence of the thalassemia trait is 7.5% (3) out of 40 volunteers among students from Sabah and Sarawak in the Faculty of Health Sciences at UiTM Selangor.

Keywords: Thalassemia trait, East Malaysian, Full blood count (FBC).

Introduction

Thalassemia is one of the most common inherited disorders in the world, commonly found in the Mediterranean region, the Indian subcontinent, Southeast Asia and West Africa (Beutler *et al*., 2001). There are two common types of thalassemia which are alpha-thalassemia (α-thalassemia) which is caused by a decreased synthesis of alpha chains and beta-thalassemia (β-thalassemia) which is caused by a decreased synthesis of beta chains. Both α-thalassemia and β-thalassemia are public health concerns in Malaysia (Wee *et al.,* 2005). In a normal person, each haemoglobin molecule in the red blood cells comprises 2 alpha (α) globin chains and 2 beta (β) globin chains. Each α globin chain is encoded by 4 α genes and each of β globin chains is encoded by 2 β genes. However, deletion or mutation of genes decreases the production of globin chains; thus less haemoglobin is produced. Eventually, anaemia is developed due to less production of red blood cells (Sandra & Edward, 1995). Alpha and β-thalassemia can be classified into thalassemia major, intermedia, and minor based on the genetic traits and their clinical anaemia severity (Monica, 2000).

In Malaysia, the Bumiputeras are known as Malaysia's original inhabitants, the Malays and the indigenous peoples of Sabah and Sarawak. In Sarawak, the Iban community remained the largest ethnic group at 723,400, followed by the Chinese (619,900), Malay (607,800), Bidayuh (197,000), other Bumiputera (141,300), Melanau (133,400), and Indian plus others (14,800) (The Borneo Post, 2018). While in Sabah, about 30 per cent of the population of Sabah comprises of Kadazandusun tribe and they are the largest ethnic group in Sabah, making up almost 30% of the population. Bajau is the second largest ethnic group in Sabah. Other indigenous ethnic groups include the Bisaya, Brunei Malay, Bugis, Kedayan, Lotud, Ludayeh, Minokok, Rungus,

and Suluk. The ubiquitous Chinese comprise about 20% of the Sabah population (Discovery Tours Sabah, 2022).

In Malaysia, it was reported that 4.1% of the Malays and Chinese are α thalassemia carriers (Rahimah *et al.,* 2012). Current estimation shows that 6.8% of Malaysians are thalassaemia carriers who might be affected by various degrees of anaemia (Ngim *et al.,* 2015). Alpha-thalassemia 1 (SEA type) is common in Malaysia and 10.6% of antenatal patients show positive with alpha thalassemia 1 (Rosnah *et al.,* 2006). In another study, 7.4% of Malaysians in blood donors showed α thalassemia deletion (My *et al.,* 2012). Regarding different major ethnicities in Malaysia, only small percentages of Indians were thalassemia while high reported thalassemia among Malays and Chinese (Tan *et al.,* 2006). There were different distributions of alpha thalassemias among different ethnic groups available in Malaysia as reported (Ahmad *et al.,* 2013).

The HbE/β-thalassaemia forms the largest group of thalassaemia patients in Malaysia with 2878 (35.19%) patients, followed by β-thalassaemia major with 2671 (32.66%) patients, HbH disease with 1593 (19.48%) patients, β-thalassaemia intermedia with 738 (9.02%) patients, whereas the remaining 298 (3.64%) patients have other forms of thalassaemia (Hishamshah *et al.,* 2019).

The Malaysian Ministry of Health (2009) has reported that the treatment cost for thalassemia patients is very expensive and therefore management of thalassemia is important in the community in Malaysia. Symptomatic thalassemia major and intermedia requires life long-term medical care and support (Ismail *et al.,* 2006). Population screening for carrier detection has been proposed to reduce its occurrence and is being practised in many countries (Bozkurt, 2007; Samavat & Modell, 2004).

Thalassemia may occur in either parent or both and in both men and women. A previous study conducted in Tanjung Karang, Malaysia, highlighted that thalassemia is one of Malaysia's most common genetic disorders and that alpha thalassemia and beta thalassemia are widespread (Othman & Soon, 1994). Many previous studies on the prevalence of thalassemia were done on the major ethnic groups in Malaysia, including the Malays, Chinese and Indians (Tan *et al.*, 2004; 2006; Wee *et al.,* 2005). A high prevalence was reported among Malays and Chinese, with 4.1% having the α thalassemia trait while 4.5% being β thalassemia carriers (Rahimah et *al.*, 2012; George *et al.,* 2001). However, little research and information on its prevalence in Sabah and Sarawak's multi-ethnic populations has been limited. As a result, an investigation into the prevalence of thalassemia traits among a small population of students from Sabah and Sarawak of various ethnicities at Universiti Teknologi MARA, UiTM Selangor Branch, Puncak Alam Campus, has been conducted.

Materials and Methods

Sample Collection

The study was carried out over 3 months following approval from the ethics and research committees of Universiti Teknologi MARA, UiTM Shah Alam. Since the population is small, the population of this study was randomLy selected among students in the Faculty of Health Sciences from Sabah and Sarawak, Malaysia. A universal sampling method was used to collect blood samples from the 40 students, 16 from Sabah and 24 from Sarawak. All the subjects were asked to fill out the consent forms before their blood samples were collected. Whole blood samples were taken from the venous blood of the subjects with the help of a volunteer medical laboratory staff member. The blood samples were then collected in an ethylenediaminetetraacetic acid (EDTA) tube in a 3 mL volume for the full blood count (FBC) test. The EDTA tubes were inverted a few times to mix the blood thoroughly with the anticoagulant in order to prevent blood clotting *in vitro*.

Full Blood Count (FBC)

FBC test was performed in the haematology laboratory of UiTM Puncak Alam by using a Beckman Coulter LH500 analyser within 4 hours after the blood collection. The Beckman Coulter LH500 is an instrument that evaluates complete blood cell (CBC) counts and white blood cell (WBC) differential counts on whole blood samples in vitro. The analyser can detect the parameters including red blood cell (RBC) concentration, haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), hematocrit (HCT), platelet (Plt) counts and differential counts of white blood cells (WBC).

However, in this study, the red blood cell indices which include RBC concentration, Hb concentration, MCV, MCH, MCHC, HCT, and RDW were analysed. This analyser uses an impedance counting system to detect red blood cells. Pulses measured in the RBC aperture representing cells as 36FL or greater are classified as red blood cells. While for Hb detection, the transmittance of light (525nm wavelength) through the lysed WBC solution in the haemoglobin cuvette is compared to the transmittance of the same light through a reagent blank.

The system converts this ratio to Hb value in g/dl using a calibration factor. MCV: The average volume of individual red blood cells derived from the RBC histogram. RDW: The size variation of the red blood cell population is derived from the RBC histogram, whereas HCT, MCH and MCHC are derived from calculated parameters. HCT is the relative volume of packed red blood cells to whole blood. FBC procedure was performed according to the manual provided by Beckman Company. Once the procedure was completed any blood sample with MCH < 27 pg was subjected to further test; full blood picture (FBP).

Full Blood Picture (FBP)

Full blood pictures were performed on blood samples with MCH < 27 pg. Thin blood films were prepared to observe the blood's full picture or morphology. Before performing a blood film, a slide was labelled with the sample's identification number. A drop of blood was dropped on a glass slide using a hematocrit microtube. Then, the blood was spread using a cover slip on the glass slide at an appropriate angle. The slide was allowed to dry before staining the smear using commercially prepared Leishman's stain by Merck USA. A duplicate smear of each sample was also prepared. First, the blood smears were placed on a staining rack neatly to stain the smear with Leishman's stain. The blood smears were then covered with Leishman's stain and left for 4 minutes. Next, a pH 6.8 buffer solution was added to each smear with the same amount as Leishman's stain. The mixture of the buffer and Leishman's stain was then mixed by blowing using the rubber bulb and leaving the smears for 11 minutes to stain. After that, the blood smears were washed thoroughly with tap water. Lastly, the smears were left to dry. The morphology of the red blood cells was then observed using a light microscope.

Haemoglobin (Hb) Gel Electrophoresis

Haemoglobin electrophoresis was performed using the alkaline gel electrophoresis kit HE 10 by Hellabio. This kit contained commercially prepared agarose gels, hemolysing solution, concentrated pH 8.6 electrophoresis buffer and concentrated Amido Black staining solution. This haemoglobin alkaline gel electrophoresis kit can identify different types of haemoglobin molecules where HbA, HbF, HbS (HbG, HbD, Lepore), Hb A2 (HbC, HbE and HbO) that appear in separated bands thus any haemoglobin abnormalities can be diagnosed. Before performing the haemoglobin electrophoresis, a dilution of pH 8.6 buffer was prepared. Twenty millilitres of concentrated pH 8.6 buffer was diluted with 980 mL of distilled water to make

1 litre of pH 8.6 buffer. Next, to prepare a protein staining solution, 20 mL of concentrated Amido Black solution was diluted with 120 mL 10% acetic acid solution to make a 140 mL dilution of 0.4% Amido Black solution. The electrophoresis chamber and power supply were set up to perform the haemoglobin electrophoresis. The hemolysates were prepared and the electrophoresis chamber was filled with an adequate volume of electrophoresis buffer depending on the chamber volume. The agarose gel was then discovered and put in a horizontal position. The gel was blotted on the sample application zone with a gel blotter strip.

Next, the sample template was placed on the application zone and rubbed using a forefinger to get in contact with the gel surface, thus avoiding the presence of air bubbles. 5 µL of each hemolysate was applied across the slit of the sample template. Hemocontrol AFSA2 by Helena Biosciences was used for haemoglobin control. The hemolysate was let absorbed for 50 to 60 seconds. The excess hemolysates were then blotted with a gel blotter strip. Both the sample template and gel blotter strip were gently removed and discarded. After that, the gel was placed into the chamber with the samples on the cathodic side (-). The chamber was connected to the power supply and ran for 20 minutes at 200 V. Once the electrophoresis was done, the gel was dried in a 65 ˚C drying oven and stained for 5 minutes with a protein staining solution. The gel was then destained for 5 minutes in 3 destaining 10% acetic acid solution baths. The gel was then dried again in the drying oven. The result of the electrophoresis was then visually evaluated.

Supravital Staining

Simultaneous with hb electrophoresis, supravital staining was also performed on the blood samples. Commercialised brilliant cresyl blue solution (BCB) by Merck, USA was used. BCB can best stain Hb H as small pale blue granules on red blood cells. Red blood cells containing these Hb H inclusions are also called golf balls as they appear under the microscope. To perform BCB staining, 200 µL of each blood was pipetted in each test tube. Then one drop of BCB was added to the tube. The blood was mixed thoroughly with the stain and incubated at 37 ˚C for over 2 hours. Once ready, a thin blood smear of BCB supravital staining was prepared. A duplicate of each sample was also prepared. The smears were allowed to dry before coverslipping them. The blood smears were then observed under a light microscope for H inclusions.

Mentzer Index (MI) Calculation

Mentzer Index was also done to screen for subjects suspected of thalassemia. Mentzer Index differentiates between microcytic anaemia due to iron deficiency anaemia (IDA) or thalassemia. Mentzer Index is based on the calculation of MCV/RBC. Recently, a study has correctly diagnosed 86.85% of beta-thalassemia trait cases using Mentzer Index (Niazi *et al.,* 2010). Another study by Jiminez (1995) proved that the Mentzer Index has 86.9% sensitivity and 80.1% specificity. MI of the FBC result was analysed where <13 and has low MCV and normal RBC level suggestive of thalassemia.

Statistical Analysis

Data was collected and analysed using the Statistical Package for the Social Sciences (SPSS) version 21 by PASW Statistics. The percentage of students with thalassemia was calculated and FBC parameters were evaluated using Mann-Whitney test with p-value < 0.05 to be considered statistically significant.

Results and Discussion

Socio-Demographic Characteristics of the Subjects

Sabah accounted for 40% (16) of the 40 subjects, whereas Sarawak accounted for 60% (24). In Sabah, the majority of the subjects were Bajau, with Dusun, Kadazan, Murut, Malay, Bugis, Suluk, Banjar, and Irranum close behind. On the other hand, Sarawak's subjects were mostly Malay, with one subject each for the ethnic groups of Iban, Kedayan, Bisaya, Melanau, and

Lunbawang. In all, 17 (42.5%) participants were Malay, while 23 (57.5%) were non-Malay, also known as indigenous people in Sarawak and Sabah. The participants ranged in age from 20 to 27 years old. Only 5 (12.5%) of the participants were male, while the remaining 35 (87.5%) were female as shown in Table I.

Full Blood Count (FBC)

Table 2 provides a summary of the results of the haematological parameters of the studies. Ten out of 40 (25% with 95% CI, 15% to 40%) people had anaemia based on their haemoglobin level (hb). However, only 8 (20%) subjects with MCH <27pg underwent FBP testing, Hb gel electrophoresis and H-inclusion for further detection of thalassemia.

Full Blood Picture (FBP)

Eight out of forty subjects with the FBC result of MCH <27 pg were further examined for full blood picture (FBP) examination. Three samples with MCH<27 pg showed hypochromic red blood cells, whereas another sample with both low MCV and MCH showed hypochromic microcytic red blood cells. Other 4 subjects with MCV<80 fL, MCH<27 pg and high RDW showed anisopoikilocytosis red blood cells, including red blood cell fragments, teardrop cells, pencil cells, and ovalocytes. Out of this, 3 samples among the later 4 subjects with target cells seen, which may indicate or suggest thalassemia, such as in Figures 1, 2, and 3.

Haemoglobin (Hb) gel electrophoresis

The result of gel electrophoresis was interpreted visually for the qualitative measurement. However, no positive result for beta thalassemia trait was observed in all samples. All samples did not show HbA2 band formation or any other haemoglobin besides the smearing of the hb bands of AFSA2 Hemo control in Figure 4 indicates that the deterioration of hb control occurs thus, the result of the hb alkaline gel electrophoresis is invalid.

H-inclusion test

The microscopic examination of the samples for H-inclusion showed no positive results. None of them showed the presence of golf ball-like cells.

Table 1: Socio-demographic characteristics of the subjects

Variables	Frequency (Total n = 40)	Percentages, %
State		
Sabah	16	40
Sarawak	24	60
Race		
Malay	17	42.5
Non-malay	23	57.5
Gender		
Male	5	12.5
Female	35	87.5
Age		
20 – 23	21	52.5
24 – 27	19	47.5

Table 2: Summary of the FBC results of the subjects

FBC Parameters	Frequency (Total n=40)	Percentage (%)
Hb level (g/dl)		
< 12	10	25
> or equal 12	30	75
RBC (10^{12}/L)		
< 4	2	5
> or equal 4	38	95
MCV (fL)		
< 80	5	12.5
> or equal 80	35	87.5
MCH (pg)		
< 27	8	20
>27	32	80
MCHC (g/dL)		
< 32	10	25
> or equal 32	30	75
RDW (%)		
< 15	35	87.5
> or equal 15	5	12.5
HCT (%)		
< 40	18	45
> or equal 40	22	55

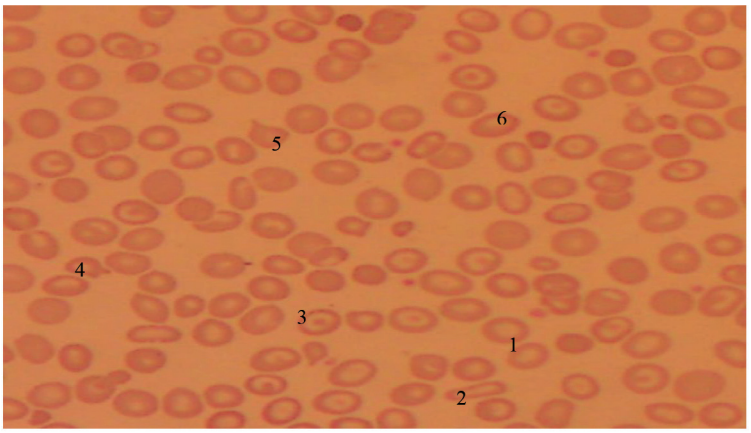

Figure 1: FBP result of sample no. 1 with characteristics of thalassemia trait. Hypochromic microcytic RBC (1), pencil cell (2), target cell (3), keratocyte (4), teardrop cell (5), ovalocyte (6)

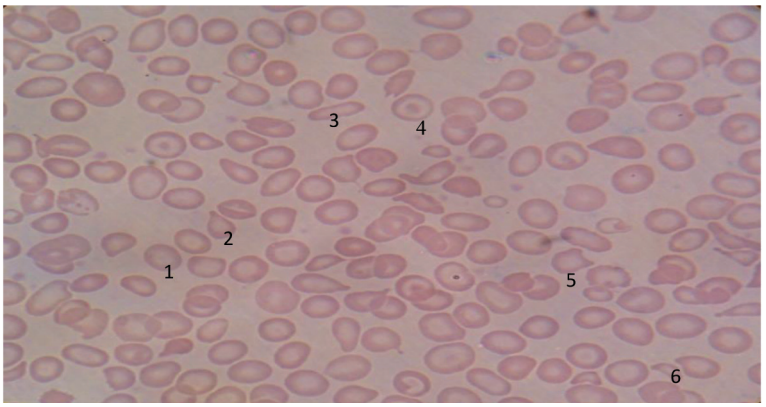

Figure 2: FBP result of sample no. 2 with characteristics of thalassemia trait. Hypochromic microcytic RBC (1), teardrop cell (2), pencil cell (3), target cell (4), keratocyte (5), fragment

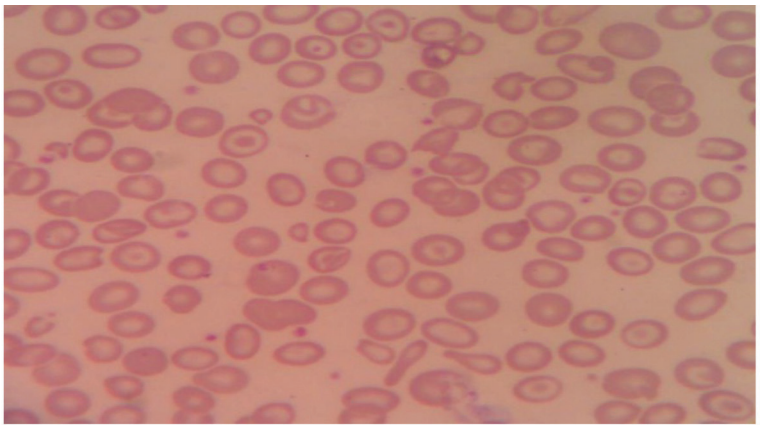

Figure 3: FBP result of sample no. 3 with characteristics of thalassemia trait. Hypochromic microcytic RBC (1), target cell (2), ovalocyte (3), pencil cell (4), teardrop (5)

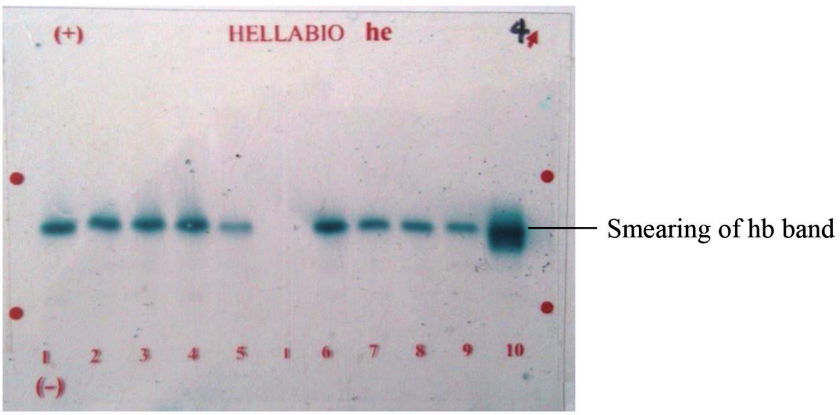

Figure 4: Result of Hb alkaline gel electrophoresis. AFSA2 Control in lane 10, samples in lanes 1 to 9

Mentzer Index (MI) Calculation

Table 3 compares suggestive thalassemia and normal subjects based on MI in investigated haematological parameters based on median, interquartile range (IQR), Z statistics and p-value. It shows that the haematological parameters (HGB, RBC, MCV, MCH, MCHC, RDW and HCT) have significant differences in medians between thalassemia and normal subjects ($p < 0.05$). The median for the haemoglobin level of the normal group is 13.2 g/L while the median for the thalassemia group is 10.4 g/dl. The median for the MCV level of the normal group is 88.5 fl meanwhile for the thalassemia group is 61.0 fl. Besides, 4.53 x 1012/L and 5.71 x 1012/L, respectively, for medians of RBC level of normal and thalassemia group. MCH medians with 29 pg and 18.2 pg of normal and thalassemia groups, respectively, 32.5 g/dl for the normal group and 30.2 g/dl for the thalassemia group for the MCHC level. In addition, 12.6% and 49.5% for both RDW and HCT median for the normal group, whereas 19.6% and 35.1% for both RDW and HCT median levels for the thalassemia group.

Table 4 shows the percentage of subjects suggestive of thalassemia based on the state and race of the subjects. The study shows 12.5% of Sabahan students have thalassemia trait compared to Sarawakian students, with a lower percentage that is 4.2%. According to race, 5.9% of Malay students from Sabah and Sarawak and 8.7% of non-Malay or the indigenous student population have thalassemia traits.

Despite the Hb electrophoresis and H-inclusions test results, MI calculation of the MCV and RBC level among the subjects showed that 3 (7.5%) subjects were suggested to have thalassemia trait, as indicated in Table 5.

Table 3: Comparison between suggestive thalassemia and suggestive normal subjects based on Mentzer Index, MI in investigated haematological parameters based on median, interquartile range (IQR), Z statistics and p-value

	MI Suggestive of Thalassemia (n = 3) Median (IQR)	**MI Suggestive of Non-thalassemia (n = 37) Median (IQR)**	**Z statistics**	**P-value**
HGB (g/dl)	10.4	13.2 (1.2)	2.86	0.004
RBC (1012/L)	5.71	4.53 (0.58)	2.85	0.004
MCV (fL)	61.0	88.5 (4.8)	2.85	0.004

Table 4: Percentage of subjects with thalassemia trait based on state and race

Variables	**Thalassemia (MI < 13)**	**Normal (MI >13)**
State		
Sabah	2 (12.5)	14 (87.5)
Sarawak	1 (4.2)	23 (95.8)
[n = 40]		
Race		
Malay	1 (5.9)	16 (94.1)
Non-malay	2 (8.7)	21 (91.3)
[n = 40]		

Table 5: Prevalence of thalassemia trait in the East Malaysian students in UiTM Puncak Alam

	Suggestive of Thalassemia	Normal
East Malaysian students (Sabah and Sarawak) n = 40	3 (7.5%)	35 (92.5%)

According to the results, three out of forty samples were classified as thalassemia, with low MCV, low MCH, normal to low MCHC, low Hb, normal to high RBC, high RDW, and low to normal HCT levels. The haematological parameters (HGB, RBC, MCV, MCH, MCHC, RDW and HCT) have significant values ($p<0.05$) in predicting the thalassemia trait in the subjects. Their FBP results also revealed the abnormal presence of red blood cells with abundant target cells and other abnormalities such as pencil cells, teardrop cells and ovalocytes apart from hypochromic microcytic red blood cells. According to one of the subjects suggestive of thalassemia trait, she has a family history of thalassemia trait where it strengthens that this subject might have thalassemia trait. However, both hb gel electrophoresis and H-inclusions test revealed no positive beta or alpha thalassemia trait results. The positive control blood was not available during the day of the examination. This could be one of the factors of failure in detecting the H-inclusion. There is also a high rate of anaemia with 20% of the subjects having Hb < 12 g/dl. Based on MI, this study revealed that the prevalence of thalassemia among subjects from Sabah and Sarawak is 7.5% which is quite similar to the prevalence in blood donors with multiple ethnicities which is 7.4% (My *et al.,* 2012). MI is based on the calculation of MCV/RBC. The prevalence of thalassemia trait was contributed by the two Bajau students from Sabah and one Malay student from Sarawak which is also consistent with the prevalence of thalassemia trait found in secondary school students in Ampang, Malaysia (Jameela *et al.,* 2011). It shows the contribution of the indigenous population to the prevalence of thalassemia traits among the subjects observed in this study. There are 12.5% of Sabahan students having thalassemia compared to Sarawakian students with a lower percentage of 4.2%. According to race, 5.9% of Malay students and 8.7% of non-Malay or the indigenous student population of Sabah and Sarawak in the samples have thalassemia traits. This study demonstrates that the prevalence of thalassemia trait is demographically and racially different. Previous studies also reported different distributions of alpha thalassemias among different ethnic groups available in Malaysia (Ahmad *et al.,* 2013). Other unpublished data by the Malaysian Thalassemia Registry (2009) reported that Sarawak has the lowest prevalence rate of thalassemia with 4.95 per 100,000 among other states, while Sabah was the second highest state has a higher prevalence rate of thalassemia after Perlis with 38.83 per 100,000.

A study among Malay students at International Islamic University Malaysia, Pahang shows a higher percentage (23.6%) of Malay students has thalassemia trait. (Norlelawati *et al.,* 2011). It was reported that 4.1% of Malaysians are alpha thalassemia carriers, while approximately 4.5% of Malaysian are carriers of β- thalassemia (Rahimah *et al.,* 2012). Since thalassemia is present among students, there is a significant need for education about thalassemia among university students. Furthermore, because most subjects are in the premarital age group, this is the ideal age to educate them. In most conditions, a person will not know that he or she is a carrier of thalassemia until he has gone through a screening program. This is because most carriers are asymptomatic (Fucharoen *et al.,* 1991). In addition, treating thalassemia is very expensive since this can burden the healthcare system and the patient's families (Angastiniotis *et al.,* 1986). Every year there are affected births estimated at 2.1 per 1000. It is also estimated that 5,600 patients with β- thalassemia in Malaysia depend on blood transfusion (George, 2001). Recently, The

Malaysian Thalassemia Registry data recorded that the majority of the thalassemia patients were reported in the state of Sabah (22.72%); the largest age group affected was 5.0–24.9 years old (64.45%); the largest ethnic group involved was Malay (63.95%); and the major diagnosis was haemoglobin E/β-thalassemia (34.37%), (Hishamshah *et al.,* 2020). This study could not represent the entire Sabahan and Sarawakian population due to the small sample size of students from Sabah and Sarawak at the Faculty of Health Sciences at UiTM Selangor.

Conclusion

In conclusion, among students from Sabah and Sarawak in the Faculty of Health Sciences at UiTM Selangor, the prevalence of the thalassemia trait is 7.5% (3) out of 40 volunteers. An education campaign and thalassemia screening programme should be launched to improve health services for indigenous and premarital age groups.

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