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*Original Research Article*

# The Prevalence of Thalassaemia Trait among Medical Laboratory Technology Students of UiTM Puncak Alam, Malaysia

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## ABSTRACT

**Objective:** The aim of this study is to detect level of hemoglobin and identify subject with thalassaemia carrier among Medical Laboratory Technology (MLT) students of UiTM Puncak Alam.
**Methodology:** This study was conducted at Department of Medical Laboratory Technology, Faculty of Health Sciences, UiTM Puncak Alam, from March 2013 to July 2013. One hundred and sixty two (162) blood samples were collected from volunteered students who agreed to participate. After ethical approval, blood samples were collected from 162 MLT volunteered students in the five mL of EDTA tubes and the volunteer need to fulfill consent form before blood sample collected. Thalassaemia detected by performing four compulsory tests started with full blood count (FBC), secondly is full blood picture (FBP), third step is serum ferritin lastly hemoglobin electrophoresis to identify hemoglobin pattern. The prevalence, percentages, means, interquartile range, P-value calculated by Science Social Software (SPSS) version 18.0 by using Mann-Whitney u test.
**Results:**The prevalence of thalassaemia trait among MLT students is 4.32% comparing to iron deficiency anemia (IDA) which is 5.56% and normal MLT students which is 90.12%. There is significant association between level of hemoglobin and thalassaemia as p-value <0.05. Level of hemoglobin is the main parameter for detection of thalassaemia together with mean cell volume (MCV) and mean corpuscular hemoglobin (MCH).
**Conclusion:** This study has managed to identify carrier of thalassaemia among MLT students of UiTM Puncak Alam.

***Keywords:*** Thalassaemia, Full Blood Count and Hemoglobin

## INTRODUCTION

Thalassaemia is an inherited red blood cell disorder which is caused by abnormal gene lead to unable to produce normal and functioning hemoglobin in the red blood cell. Thalassaemia is one of hereditary disorders of commonest single-gene disorders.[1] S. Jameelaet al., 2011 states that thalassaemia known as an autosomal recessive disorder and usually the thalassaemic syndromes from an individual are inherited from both parents, father and mother whom are carriers of thalassaemia.[2] According to Shivashankara A.R, Jailkhani

R and Kini A (2008), the World Health organization WHO reported that there are about 5% of the whole world populations are positive as carriers of hemoglobinopathies and about 370000 newborn every year was affected severely with either homozygotes or compound heterozygotes of thalassaemia.[3] Other than that, Pratima, Shraddha, Akhilesh, Wahid and Sunita (2013) reported that 60000 new cases of β-thalassaemia were estimated every year worldwide for about 80 to 90 million people of world population.[4] Thalassaemia also identified as inherited hemoglobinopathy and the two common types of thalassaemia which are alpha-thalassaemia (α-thalassaemia) and beta-thalassaemia (β-thalassaemia).[5]

A.Victor, Paul and John, 2006 in his book highlighted that thalassaemic circumstance was firstly identified by Thomas B. Cooley a Detroit physicians in 1925. Thomas clarified that there are some infants that become anemic and suffered enlargement of spleen or splenomegaly over their first year of life. A.Victor Hoffbrand also added that the word 'thalassaemia' originally come from Greek θαλασσα, meaning 'the sea' that created by Whipple and Brandford in 1936, due to most of their patients came from Mediterranean region.[6] Besides that, Aziz, Abolghasem and Reza (2012) also highlighted that thalassaemia which is worldwide disease more commonly in Meditteranean region, Middle East, Asian subcontinent, and southeastern Asia, also as well as southwestern Europe and central Africa.[7] Previous study conducted in Tanjung Karang, Malaysia in 1994 highlighted that thalassaemia is one of the most common genetic disorder seen in Malaysia and alpha thalassaemia and beta thalassaemia are prevalent.[8] Based on this statement, recently there are much attention has been paid to the screening program, hemoglobin DNA analysis, genotype study, perception study and others focused on thalassaemia over around the world. This study also performed since there is lack of such study among university students in Malaysia. So, the aim of this study is to detect level of hemoglobin and identify subject with thalassaemia carrier among Medical Laboratory Technology (MLT) students of UiTM Puncak Alam.

## MATERIALS AND METHODS

This study was performed at Department of Medical Laboratory Technology, Faculty of Health Sciences, UiTM Puncak Alam from March 2013 until July 2013. This study was approved by Research Ethics Committee, Faculty of Health Sciences, UiTM Puncak Alam. One hundred and sixty-two (162) volunteered Medical Laboratory Technology (MLT) students involved in this study. Consent forms for this study were sent out before the blood sample taken and no refusals to participate. Average age of all volunteers' participants can be divided specifically to two genders which are 31 male and 131 female students with average age twenty to twenty-five years old. Sample size for this study calculated by using Krejcie and Morgan method.

All blood samples were collected in ethylenediaminetetraacetic acid (EDTA) blood tubes by the qualified phlebotomist who is staff nurses from Health Centre of UiTM Puncak Alam and Department of Nursing UiTM Puncak Alam. The EDTA blood tube from the blood collection procedure will then quickly put in the Beckman Coulter LH500 analyzer in hematology laboratory of Department of Medical Laboratory Technology UiTM Puncak Alam for full blood count procedure. Several hematological parameters were check especially gold standard for thalassaemia study that highlighted by Sylvie Langlois*et al.,* 2008 consisted in full

blood count (FBC) by diagnosing mean cell volume (MCV) and mean corpuscular hemoglobin (MCH)[8]. Thus, all samples with characteristics hypochromasia (MCH <25pg) and microcytosis (MCV<80fl) set as the cut-off value in order to identify thalassaemia trait subject and separated. Besides that, mean corpuscular hemoglobin concentration (MCHC), hemoglobin count and red cell distribution width (RDW) also diagnosed properly as main indicator of thalassaemia.

Abnormal FBC then proceed with Full Blood Picture (FBP) will look for abnormal findings and characteristics of thalassaemia such as presence of hyperchromic and microcytic cell, poikilocytosis, dacrocytes or teardrop cell, codocyte or target cell and others. Meanwhile, serum ferritin test used to differentiate iron deficiency (IDA) and thalassaemia trait subject which perform at Faculty of Medicine, UiTM Sungai Buloh.

Then, Hemoglobin electrophoresis will be used to identify types of thalassaemia based on types of hemoglobin result obtained. The kit used is hemoglobin electrophoresis test kit by Hellabio company or known as Hellabio's alkaline agarose gel suitable for detection of different types of hemoglobin molecules such as hemoglobin A, F, S, G, D, $A_2$, C, E and O and determine hemoglobin abnormalities.

Data was analyzed by using Statistical Program for Science Social Software (SPSS) version 18.0. The data analysis had done by using Mann-Whitney u test with 95% confidence interval for comparison between normal group and thalassaemia hematological parameters for example MCV, MCH, MCHC, RDW and also hemoglobin level. P-value less than 0.05 were set and considered as statistically significant.

## RESULTS

The volunteered students consisted of 132 female (82%) and 30 male (18%) students. All the blood samples then analyzed based on hematological parameters in order to detect thalassaemia especially mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), and more importantly hemoglobin levels by full blood count (FBC) done by Beckman Coulture LH500 analyzer. The blood samples with abnormal result that is having lower MCV, MCH, MCHC, RDW and also hemoglobin level was identified and separated for further test. For this reason, 16 out of 162 student's blood samples proceeded with further test which is full blood picture (FBP) and serum ferritin in order to differentiate thalassaemia and iron deficiency anemia (IDA). There are 7 blood samples out of 16 abnormal student' blood samples then separated for last important test for this study which is hemoglobin electrophoresis meanwhile 9 out of 16 abnormal student's blood samples excluded for the reason of positive for IDA.

***Hematological parameters results:*** The results of hematological parameters summarized as in Table 1. Based on all the result hematological parameters in FBC, one male students and fifteen (15) female students were suspected for having thalassaemia and proceed with FBP and serum ferritin test.

***Full blood picture (FBP) results:*** Based on FBP results, 16 subjects with abnormal results in FBC have abnormal characteristics in their blood smear especially hypochromic microcytic cell, teardrop cell or dacrocytes and others such in Figure 1.

***Serum ferritin results:*** All 16 anemic subjects identified based on hematological parameters. Number of students having lower serum ferritin <12 is 9 female students (60%) and no male students meanwhile students with higher serum ferritin >12 is 1

male student (100%) and 6 female students (40%) as summarized in Table 2. For this reason, seven suspected students proceed for having hemoglobin electrophoresis analysis.

**Table 1 Summary of result of hematological parameters in FBC of MLT students.**

| Hemoglobin level (g/dL) | Male | Female | Total |
|---|---|---|---|
| >12 | 29(96.7) | 117(88.64) | 146(90.12) |
| <12 | 1(3.3) | 15(11.36) | 16(9.88) |
| MCV level (fl) | Male | Female | Total |
| >80 | 29(96.7) | 115(87.12) | 144(88.89) |
| <80 | 1(3.3) | 17(12.88) | 18(11.11) |
| MCH(pg) | Male | Female | Total |
| >27 | 27(90) | 105(79.55) | 132(81.48) |
| <27 | 3(30) | 27(20.45) | 30(18.52) |
| MCHC (%) | Male | Female | Total |
| >31.5 | 29(96.7) | 110(83.33) | 139(85.8) |
| <31.5 | 1(3.3) | 22(16.67) | 23(14.2) |
| RBC ($10^{12}$/L) | Male | Female | Total |
| >4.0 | 30(100) | 129(97.73) | 159(98.15) |
| <4.0 | - | 3(2.27) | 3(1.85) |
| RDW (%) | Male | Female | Total |
| >15 | 1(3.3) | 15(11.36) | 16(9.88) |
| <15 | 29(96.7) | 117(88.64) | 146(90.12) |

**Table 2 Summary of result of serum ferritin of MLT students.**

| Serum ferritin (µmol/L) | Male | Female | Total |
|---|---|---|---|
| >12 | 1(100) | 6(40) | 7(43.75) |
| <12 | | 9(60) | 9(56.25) |
| Thalassaemia | Male | Female | Total |
| | 1(100) | 6(100) | 7(100) |

***Comparison between normal and thalassaemia students in investigated hematological parameters in FBC and serum ferritin based on median, inter-quartile range (IQR) and Z statistic[a] and P value.***

In all, FBC hematological parameters toether with serum ferritin test results divided subject for this study to two groups which is normal group and thalassaemia group. Table 3 and Table 4 show the summary of FBC hematological parameters and serum ferritin result clearly. Table 3 describes clearly about median and interquartile range of FBC hematological parameters. The median for hemoglobin level of normal group is 13.1 g/dL (1.40) and median for thalassaemia group is 11.0 g/dL (1.60). The median for MCV for normal group is 90.3 fl (5.13) meanwhile median for thalassaemia group is 70.4 fl (1.60). Then, MCHC median for normal group is 32.1% (0.72) meanwhile for thalassaemia group is 31.0% (1.10). For RDW median in normal group is 12.7% (1.10) and thalassaemia group is 15.6% (0.90). Lastly, the median of MCH for normal group which is 29.1 pg (1.90) meanwhile median of MCH for thalassaemia group is 21.5 pg (1.50). On the other hand, Table 4 described about median and interquartile range of serum ferritin results by dividing to two groups which is IDA group and thalassaemia group. The result shows that out of 16 students, nine of them have lower serum ferritin group and seven of them having normal serum ferritin level and suspected as thalassaemia. The median of serum ferritin for IDA group is 8.1 umol/L (3.72) meanwhile median of serum ferritin for thalassaemia group is 76.7 umol/L (163.7). For this reason, 7 suspected students proceed for having hemoglobin electrophoresis analysis.

***Result of hemoglobin electrophoresis analysis:*** Based on serum ferritin results obtained, seven abnormal samples were selected for further test of hemoglobin electrophoresis. Hemoglobin electrophoresis identified movement of bands according to different types of hemoglobin. Based on Hellabio hemoglobin electrophoresis kit procedure, the hemoglobin band will be produced after electrophoresis on alkaline gel runned at 200 volt according to hemoglobin types where hemoglobin as one types of proteins will move from cathode to anode. Hemoglobin pattern or hemoglobin band results obtained and viewed by densitometer as shown in Figure 2. Figure 2 shows the pattern of the hemoglobin bands of all thalassaemia subject, unfortunately there are no positive and negative control provided by this kit, however all the subjects confirmed for

thalassaemia trait as they have been diagnosed as thalassaemia trait before with presence of history of family or parents whom are positive carrier of thalassaemia.

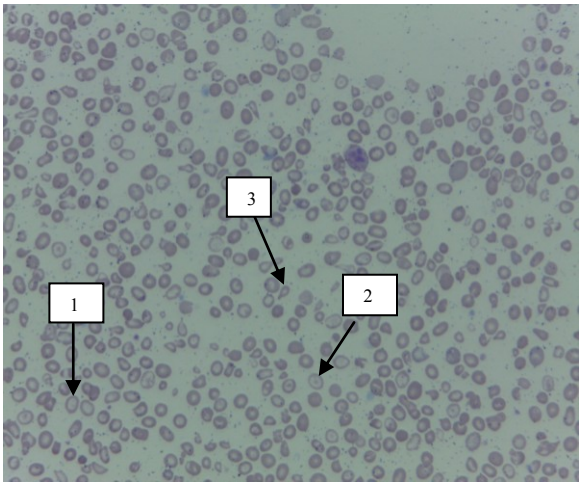

Figure 1. Shows result of FBP with characteristics of thalassaemia trait subjects (1) hypochromicmicroctic cell, (2) target cell (codocyte) and (3) teardrop cell (dacrocyte).

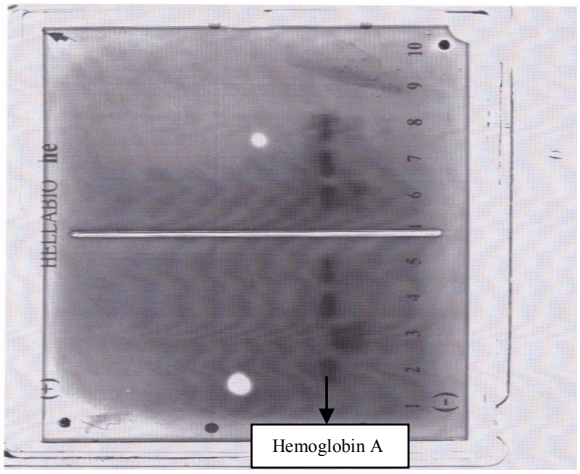

Figure 2. Result of hemoglobin electrophoresis for thalassaemia patients thalassaemia patients on alkaline gel of Hellabio hemoglobin electrophoresis kit by densitometer.

**Table 3 Comparison between normal group (n=146) and thalassaemia (n=7) in the investigated FBC hematological parameters as median, interquartile range (IQR), Z statistics[a] and P-value.**

| | Normal (n=146) Median | IQR | Thalassaemia (n=7) Median | IQR | Z statistics | P-value |
|---|---|---|---|---|---|---|
| RBC ($10^{12}$/l) | 4.63 | 0.49 | 5.02 | 0.59 | -2.09 | 0.036 |
| Hb (g/dl) | 13.10 | 1.40 | 11.0 | 1.60 | -4.47 | 0.000 |
| Hct (%) | 41.20 | 4.33 | 37.5 | 4.10 | -3.98 | 0.000 |
| MCV (fl) | 90.3 | 5.13 | 70.4 | 1.60 | -4.44 | 0.000 |
| MCHC(%) | 32.1 | 0.72 | 31.0 | 1.10 | -3.92 | 0.000 |
| RDW (%) | 12.7 | 1.10 | 15.6 | 0.90 | -3.83 | 0.000 |
| MCH (pg) | 29.1 | 1.90 | 21.5 | 1.50 | -4.41 | 0.000 |

*P-value <0.05: Mann-Whitney test
(RBC) = Red blood cell count,; (Hb) = hemoglobin level, (Hct) = hematocrit; (MCV) = mean cell volume; (MCHC) = mean corpuscular hemoglobin concentration; (RDW) = red cell distribution width; and (MCH) = mean corpuscular hemoglobin.

**Table 4 Comparison between IDA group (n=9) and thalassaemia group (n=7) in the investigated serum ferritin test as median, interquartile range (IQR), Z statistics[a] and P-value.**

| | IDA (n=9) Median | IQR | THALASSAEMIA (n=7) Median | IQR | Z statistics | P-value |
|---|---|---|---|---|---|---|
| SF (µmol/l) | 8.1 | 3.72 | 76.7 | 163.7 | -3.33 | 0.001 |

*P-value <0.05: mann Whitney test
(SF) = serum ferritin; (IDA) = iron deficiency anemia.

## DISCUSSION

The prevalence of thalassaemia among MLT students overall is 4.32 consist of male students is 0.62% meanwhile female students is 3.70%. Previous study done by S Jameela*et al.* (2011) reported that the

carrier rate for thalassaemia in Malaysia approximately about 7% among secondary school students in Ampang, Malaysia in 2011.[2] In India, out of 6463 individuals, 145 or (2.24%) individuals were having thalassaemia respectively reported by Bhaskar P. Urade (2012).[10] According to Aziz Batebi, AbolghasemPourreza and Reza Esmailian (2012), 444 out of 11900 individuals among couples intending to marry in Iran had beta thalassaemia minor.[6] Besides that, Elizabeth George *et al.* (2011) stated that in Malaysia about 3.5% to 4.5% of populations are carriers of thalassaemia.[11] Other than that, G. Elizabeth and T J Mary Ann (2011) also stated that there were 4768 transfusion dependent thalassaemia major patient in Malaysia as of May 2010 (Data from national Thalassaemia Registry).[12] In Greece, thalassaemia traits have cumulative frequency approximately 15% in general population majority children with microcytic anemia. In Egypt, beta-thalassaemia also known as most common chronic hemolytic anemia where over one to five million newborns in Egypt expected to be affected by this disease. [1] About 15 million people worldwide have clinically apparent thalasseic disorders and this shows that thalassaemias are most common genetic disorders in human among all ethnicities and almost all country among the world.[13]

Regarding this study, all the participants which are volunteered MLT students, all of them are Malay ethnicity with age range between twenty 20 to 26 years old only. After fresh blood sample drawn the blood sample then tested with full blood count (FBC) test. Based on full blood count result, the result obtained was 16 out of 162 MLT students having anemic with lower hemoglobin level compare to 146 students which has normal results. The anemic subject also choosen by FBC result of hypochromasia (MCH <27pg) and microcytosis (MCV<80fl) as the cut-off value. The blood sample analyzed by using standard laboratory procedures by considering red cell indices with cut-off value MCV<80fl by Y.C. Wee et al. (2008). Sylvie Langlois et al. (2008) reports that combination of finding of normal MCV (>80fl) together with normal MCH (>27pg) would rule out most cases of thalassaemia.

Level of hemoglobin is important in order to detect anemia. According to G Elizabeth and T J Mary Ann (2011), imbalance of globin chains especially absence or reduced globin chains in beta thalassaemia is the main cause of anemia in thalassaemia patient.[12] Thus, based on Table 1 there were 29 male students (96.7%) and 117females subjects (88.64%) having hemoglobin level in normal range (>12g/dL) meanwhile only one male students (3.3%) and 15 female students (11.36%) having lower than range of hemoglobin level (<12g/dL). Overall, in this study there were 16 students or anemic subjects (9.88%). Afterwards, full blood picture (FBP) test done in order to examine morphology on peripheral blood film of 16 anemic student samples. Figure 1 shows example of hypochromic and microcytic red blood cells (RBC) of thalassaemia subject with abnormal morphology in example presence of target cells or codocyte and teardrop cells or dacrocytes which usually found in thalassaemia patient.

Other than that, serum ferritin test also performed in order to differentiate Iron Deficiency Anemia (IDA) subjects and thalassaemia subjects where the result shown that only nine subjects are confirmed having IDA meanwhile the other 7 are suspected for having thalassaemia as explained in Table 4. Serum ferritin test performed in order to diagnose iron storage. Serum ferritin test also known as the most accurate diagnosis for IDA. In beta thalassaemia major and intermedia, this test

result may be either increased or normal in presence of iron chelation therapy explained by G Elizabeth and T J Mary Ann (2011).[12] Other than that, according to Nadeem, Khalid, Muhammad and Samina (2004) iron overload is one of life limiting complication usually found in thalassaemia patients as in case of beta-thalassaemia major patient that require repeated blood transfusions, ineffective erythropoiesis together with increased gastrointestinal iron absorption known as main cause of iron overload.[14] In addition, regular blood transfusion is estimated increased about 0.32 to0.64 mg/kg/day iron together with increased amount of gut iron absorption especially in blood transfusion dependent patients.[15] Because of that reason, serum ferritin result useful in order to identify thalassaemia subjects for this study. Refer to Table 4, the result of serum ferritin shows that 7 suspected thalassaemia MLT students (n=7) with normal and slightly increased serum ferritin with median 76.7µmol/L compare to IDA with median 8.1µmol/L overall.

Based on serum ferritin results obtained, further test performed is manual hemoglobin electrophoresis. Hemoglobin electrophoresis will allows the proportion of each type of hemoglobin presence by referring migration or hemoglobin pattern or bands to be determined. Figure 2 shows the hemoglobin electrophoresis results by using densitometer. The results shows the movement of hemoglobin bands but there is limitations of study due to unprovided positive and negative control and lack of reference materials in this kit for further interpretation and identification for confirmation of thalassaemia patients. As a final point, this study revealed that the prevalence of thalassaemia is 4.32% among MLT students of UiTM Puncak Alam identified as thalassaemia patient. Eliezer A. Rachmilewitz and Patricia J. Giardina (2011) stated that thalassaemia syndrome divided according to globin chains affected either alpha or beta globin chains and thalassaemia pathology can be identified such as decreased hemoglobin production or decreased hemoglobin level, decreased RBC survival or hemolysis and presence of inclusion bodies. Based on previous study, S Jameela et al. (2011) stated that screening of thalassaemia among secondary students in Ampang shows thalassaemia carrier common among Malays and Chinese.[2] This is consistent with this study where all the subjects or participants in this study are Malays.

## CONCLUSIONS

In conclusion, the prevalence of thalassaemia trait among MLT students of UiTM Puncak Alam is 4.32%. The prevalence of male MLT students having thalassaemia is 0.62% meanwhile prevalence of female MLT students having thalassaemia is 3.70%. As a final point, in this study all of seven (7) out of one hundred and sixty two (162) subjects are having thalassaemia. This study revealed the importance of blood screening in order to determine identify subject with thalassaemia trait especially by examining level of hemoglobin together with other hematological parameters in FBC and FBP blood smear examination in order to identify morphology of RBC. Thus, it is important to embark on a thalassaemia screening and education program in order to identify carrier or thalassaemia patient to provide counseling and as prevention steps about this burden disease as thalassaemia which worldwide known as inherited disorder. Other than that, screening program of thalassaemia should be widespread together with educational programs in order to reduce amount of thalassaemia patient especially in Malaysia. For further study, it is recommended to detect hemoglobin pattern by using automated hemoglobin

electrophoresis analyzer or high performance liquid chromatography (HPLC) together with DNA analysis for more specific results of thalassaemia types and mutation.Based on overall procedure for this study, there is several limitation of study. First of all, this study is limited to one small group thus not represent the whole population of UiTM Puncak Alam and whole Malaysia.

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