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Prevalence of iron deficiency anaemia and thalassaemia trait among undergraduate medical students

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Abstract

Background. Anaemia is a global health problem including Malaysia. In adults, anaemia may affect work productivity. Iron deficiency anaemia and thalassaemia are common causes of anaemia in Malaysia. However, there is scarcity of data on national prevalence of iron deficiency anaemia and thalassaemia, especially in young adults. This cross sectional study was performed to determine the prevalence of iron deficiency anaemia and thalassaemia among medical students of Universiti Kebangsaan Malaysia Medical Centre (UKMMC).

Materials and Methods. Blood samples collected in EDTA tubes were analyzed for haemoglobin level and red cell parameters such as MCV, MCH and red cell counts. Samples with abnormal red cell indices were sent for analysis of RBC morphology, iron status, haemoglobin analysis and DNA analysis.

Results. A total of 400 samples were available for this study. Fifty-eight (14.5%) students had hypochromic microcytic red cell indices in which 44 (11%) showed thalassaemia red cell indices while 14 (3.5%) had iron deficiency red cell indices which were finally confirmed by serum iron/TIBC analysis. Amongst those suspected to have thalassaemia, 12 (27.3%) were confirmed as alpha thalassaemia trait ($\alpha\alpha/--^{SEA}$), 11 (25%) as Haemoglobin-E trait, 8 (18.2%) as beta thalassaemia trait and 2 (4.5%) as Haemoglobin Constant Spring ($\alpha\alpha/\alpha^{CS}\alpha$). However, eleven students (25%) with thalassaemia red cell indices could not be confirmed with the common thalassaemia primers available, thus causes have yet to be established.

Conclusion. Our prevalence of thalassaemia was high and thus we opine that better screening methods should be adopted. ***Clin Ter 2012; 163(4):287-291***

Key words: *anaemia, iron deficiency, medical, students, thalassaemia, trait*

Introduction

Anaemia is a common world health problem. It affects developing and developed countries with major consequences for individuals as it affects body function. It also affects social and economic development. Anaemia is more common among pregnant women and young children. The most dramatic health effects of anaemia, which is increased risk of maternal and child mortality due to severe anaemia has been well documented (1). In addition, the negative consequences of anaemia on educational and physical performance particularly work productivity in adults is also of major concern (2).

A study performed in rural villages and estates in Peninsular Malaysia by Tee ES and friends (3) from 1992 – 1995 showed that prevalence of anaemia among female adults were significantly higher (25%) compared to male adults (14%). A study performed by Zulkifli A and colleagues among pregnant women in rural Kelantan showed that the prevalence of anaemia was 47.5% at the first antenatal visit (4). In a study conducted in 1994 among 1,408 adolescent girls in three schools near Kuching, Sarawak in Malaysia, the prevalence of anaemia was found to range between 9.1 and 36.8% (5).

From the World Health Report, 50% of the cases of anemia were reported to be due to iron deficiency (6). In Malaysia, most of the cases of anaemia is also due to iron deficiency. However prevalence of iron deficiency anaemia (IDA) among children and adolescent was variable and it depends on socioeconomic status of the family. In one study among 165 adolescences in the rural area of Sabah, Malaysia, the iron deficiency anaemia (85%) contributed largely to the prevalence of anaemia (7). In another study in children aged between six months to two years amongst different community groups, it was estimated that the prevalence of IDA varied between 15% and 60% (8). A study performed by Al-Mekhlafi and friends showed that 34% of school children in rural peninsular Malaysia (Kelantan) were affected by IDA (9). Among blood donors in Klang Valley, the prevalence of IDA for first time blood donors (7.4%) was lower compared to regular blood donors (17.4%) (10).

Besides IDA, thalassemia is also another public health problem in Malaysia. It is the commonest single gene disorder affecting mainly the Malays and Chinese population. It is

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characterized by abnormal, partial or no production of alpha or beta globin chains which form part of the structure of the haemoglobin in red blood cells. Based on statistics, at least one out of every 20 Malaysians, is a thalassaemia carrier, or approximately 600,000 to one million of the population (11). About 4.5% of the Malays and Chinese-Malaysians are carriers for beta-thalassaemia (12). The Ministry of Health of Malaysia estimated that 120-350 babies are born with thalassemia each year with 46 births being beta-thalassaemia major. The Health Ministry's registry showed that there were 4,385 beta-thalassaemia major patients now undergoing monthly blood transfusion (11). On the other hand, the prevalence of alpha-thalassemia trait based on a study performed among anaemic pregnant women in University Malaya Medical Centre was found to be 15.8% (103/650) (13). Haemoglobin E, which is a beta chain variant, also showed high prevalence rate in Malaysia (7–23%) (14).

Although IDA is an important public health concern in our country, surprisingly there is lack of national prevalence data especially in young adults and males. Most survey data are related to preschool-age and school-age children, pregnant women, and non-pregnant women of reproductive age. Similarly in the case of thalassaemia, there is lack of prevalence data on each subtypes of thalassaemia.

Keeping in view the above facts, the present study was performed to observe the prevalence of iron deficiency anaemia and thalassaemia trait in a group of medical students in Universiti Kebangsaan Malaysia (UKM). In general, university students hail from different socio-economic background. Analysis on UKM medical students may give an overall picture of the anaemic status as well as causes of anaemia among young adults in the Malaysian population.

Materials and Methods

This cross sectional study was carried out in Kampus Cawangan, Universiti Kebangsaan Malaysia, Kuala Lumpur and Universiti Kebangsaan Malaysia Medical Centre (UKMMC). The study was conducted under the Special Study Module (SSM) programme for undergraduate students. The subjects were medical students from Universiti Kebangsaan Malaysia from Year 1 to Year 5. A total of 400 medical students volunteered to be involved in the study. Ethical approval was obtained from Institutional Ethics committee. The students were interviewed regarding their dietary and medical status, family background and questions related to risk factors of iron deficiency anaemia and thalassaemia. Peripheral blood samples were taken after written consent was obtained. All blood samples which were collected in EDTA tubes were analyzed in the Haematology Unit, Department of Diagnostic Laboratory Services, UKMMC.

The haemoglobin (Hb) concentrations and red cell indices were determined using an automated blood cell counter (Coulter LH 750®) and the morphology assessment were performed by qualified medical officers. Anaemic samples were defined using WHO definition of anaemia (15). All samples that showed low mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were subjected to serum iron and TIBC, and haemoglobin analysis. Serum iron and TIBC were determined colorimetrically using Guanidine/FerroZine® method on the COBAS INTEGRA 800 analyser®. As for Hb analysis, the quantitation of Hb A_2, Hb E, Hb A and Hb F were performed using automated high performanced liquid chromatography (HPLC) system, beta thalassaemia short (BTS) program by Bio-Rad VARIANT®.

Samples that showed normal Hb analysis but had mildly low or normal Hb with normal or raised RCC were subjected to DNA analysis. DNA was extracted from peripheral blood leucocytes using QIAamp DNA Blood Mini Kit (GENE ALL®). Multiplex-PCR amplification was performed using 300ng of genomic DNA and 10pmol each of primers LIS-F, LIS-R, SEA-F, SEA-R, 4.2-F, 4.2-R, α2/3.7-F, α2-R and 3.7/20.5-R for detection of α thalassaemia gene deletion (16). The PCR was carried out on the 9700 PCR Thermal Cycler (Applied Biosystem, USA®). The amplified products were electrophoresed in agarose gel with Ethidium Bromide. DNA bands were visualized under ultra-violet light illumination.

Real-Time PCR was performed for the detection of HbCS gene using specific primers and probes for the point mutation. The probes were dual labeled TaqMan probes with both a fluorophore and a quencher dye (Biosearch Technologies®), to detect amplification of specific DNA targets. The real-time PCR was carried out using a RotorGene 6000 (Corbett Research, Aus®). Confirmation for the point mutation was done by sequencing method (ABI Genetic Analyzer 3130®).

Results

A total of 400 medical students were studied. Their ages ranged from 19 to 24 years old. The male to female ratio was 3:7 (118 male, 282 female). There were more Malays (70.8%) as compared to Chinese (25.5%) and Indians (13.8%).

The prevalence of anaemia among medical students was 7.3% (29/400). The prevalence of anaemia in male and female students were 0.84% (1/118) and 9.9% (28/282), respectively. Prevalence of anaemia in Malay, Chinese and Indian races were 6.6% (18/283), 6.9% (7/102) and 26% (4/15) respectively.

The haemoglobin level of anaemic medical students were between 9.2–11.9g/dL while non anaemic medical students were between 12–15.6 g/dL. Out of 29 students who had low haemoglobin level, 51.7%(15/29) had red cell indices suggestive of thalassaemia (low MCV, MCH with normal or raised RCC) while 48.3% (14/29) showed red cell indices suggestive of iron deficiency (low MCV, MCH and RCC), which were confirmed by iron studies. None of the students with IDA tested positive for thalassaemia.

All the iron deficient students were female, with a prevalence of 5% (14/282) among female medical students. The prevalence of iron deficiency anaemia among medical students was 3.5% (14/400).

Out of 58 students who had low MCV and MCH, 75.9% (44/58) had red cell indices of thalassaemia while 24.1% (14/58) showed red cell indices suggestive of iron deficiency anaemia. The prevalence of hypochromic microcytic red cells among medical students was 14.5% (58/400).

Table 1. Distribution of thalassaemia types and mean of red cell indices in different types of thalasaemia.

Types of thalasaemia	n = 44	Mean Hb (g/dl)	Mean MCV (fl)	Mean MCH (pg)	Mean RCC ($x10^{12}$/L)
α-thalassaemia trait ($\alpha\alpha$ / --^{SEA})	12 (27.3%)	12.5	69.3	22.2	5.6
Hemoglobin-E trait (HbE)	11 (25%)	12.5	73.3	24.0	5.2
β-thalassaemia trait	8 (18.2%)	11.7	69.4	22.3	5.1
Hb Constant spring ($\alpha\alpha$ /$\alpha^{CS}\alpha$)	2 (4.5%)	12.6	76.4	25.1	5.0
Thalassaemic indices with causes not established/known	11 (25%)	13.2	72.9	23.8	5.4

Alpha thalassaemia trait ($\alpha\alpha$ / --^{SEA}) accounted for 27.3% (12/44), Beta thalassaemia trait 18.2% (8/44), Hemoglobin-E trait 25% (11/44) and Hb Constant spring ($\alpha\alpha/\alpha^{CS}\alpha$) 4.5% (2/44). For 11 students, (25%) although their red cell indices was suggestive of thalasaemia but hemoglobin analysis, iron study and DNA analysis performed were normal (Table 1). The mean value of Hb, MCV, MCH and MCHC in different types of thalassaemic students were shown in Table I.

Majority of alpha thalassaemia trait ($\alpha\alpha$ / --^{SEA}) students were Chinese, while all HB E trait and Hb Constant Spring ($\alpha\alpha/\alpha^{CS}\alpha$) were Malay students (Table 2). Thalassaemia was not found in any Indian students in this study.

The red cell indices among affected students, the mean Hb level and RCC were much lower in IDA compared to thalassamia. However, there were no differences in the mean for MCV or MCH (Table 3)

Discussion

Subjects in our study population were mainly young adults aged between 19 – 24 years with male to female ratio of 3:7, which hailed from different socioeconomic groups. The overall prevalence of anaemia and iron deficiency anaemia (IDA) in this study population was 7.3% (29/400) and 3.5% (14/400), respectively. In comparison to another study done among adolescent in Tuaran, Sabah, Malaysia (in age group 12 – 20 years), the prevalence of anaemia and IDA were 20% (33/165) and 17% (28/165) respectively (7). This indicates that IDA was not a major problem among medical students. All the affected students with IDA were females. A survey conducted among these anaemic medical students showed that most of them had no history suggestive of bleeding disorder or inadequate nutrition; however it might due to inadequate iron supplement to replace the iron loss from their monthly menses. This was different when compared to the study done in Sabah, Malaysia where the high prevalence of IDA was mainly attributed to nutritional factor.

The worldwide prevalence of anaemia among adolescence has been reported to be 15% (27% in under developed countries and 3% in developed countries) (6). This showed that the prevalence of anaemia in medical students of Malaysia was much lower compared to worldwide prevalence.

In one research carried out in Turkey among adolescent students in high school, the prevalence of anaemia was found to be 6.8% (23/338), the prevalence of IDA was 5.9% (20/338) while the prevalence of thalassaemia was 1.1% (4/338) (17). This indicated that anaemia due to IDA was a major concern in Turkey. While in our study the prevalence of IDA was much lower even when compared to another study done in this country by Loh and Khor which was 10.3% (18). This might due to different target group analyzed in their studies and all the subjects being females.

In our study, the prevalence of thalassaemia was higher, i.e., 11% (44/400) compared to Turkey, 1.1% (4/338) and Saudi Arabia, 3.4% (307/8918) (19), and all their thalassaemias were β-thalassemia trait. In our study, there were various forms of thalassaemia found in our medical students (β-thalassemia, α-thalassemia trait 2 gene deletion, HbE and Hb Constant Spring). Hence, we should raise our awareness towards thalassemia as a public health problem in the Malaysian population.

The results of this present study varies from past research report. Previous study by Rosline H and colleagues (20) reported that the frequencies of thalassaemia carriers among donors in Hospital Universiti Sains Malaysia were β-thalassemia trait 4/13, Hb E trait 1/13, and Hb E haemoglobinopathy 8/13 (Hb E β-thalassemia 3, Hb E α-thalassemia 5) which was not observed in this study. This might due to variability in the method used and different target groups. Most of the participants involved in their study were Malays.

Table 2. Types of thalassaemia according to race.

Types of thalassaemia	Malay	Chinese
Alpha thalassaemia trait ($\alpha\alpha$ / --^{SEA})	2	10
Hemoglobin-E trait (HbE)	11	
Beta thalassaemia trait	5	3
Hb Constant spring ($\alpha\alpha$ /$\alpha^{CS}\alpha$)	2	
Thalassaemic indices with causes not established/known	11	

Table 3. Mean of Hb, MCV, MCH and red cells count of affected students.

	Mean Hb	Mean MCV	Mean MCH	Mean RCC
Iron deficiency anaemia	11.0	72.8	23.7	4.6
Thalassaemia	12.5	71.5	23.2	5.3

As thalassaemia is among the commonest inherited blood diseases worldwide, knowing the prevalence is very helpful in determining those who are eligible for thalassaemia screening and counselling. In two studies done between 1990 and 1996 in the state of California (21), approximately 1 in 114000 infants had beta thalassaemia major with the prevalence rates being the highest among Asian Indians (about one in 4,000), Southeast Asians (about one in 10,000), and Middle Easterners (about one in 7,000). Meanwhile E/beta thalassaemia was represented approximately one in 110,000 births, all of which occurred in families of Southeast Asian ancestry. From our study, the prevalence of beta thalassaemia trait was found to be 8 out of 400 respondents, in which the percentage was much higher compared to the prevalence of beta thalassemia in Malaysia that was estimated to be 2.1 in 1000 of live births. On the other hand, the prevalence of alpha thalassemia trait based on a study done among pregnant women in University Malaya Medical Centre was found to be 15.8% (103/650)(13) among those with anaemia, which is much less compared to our study 24.1% (14/58) (including Hb Constant Spring) among medical students with red cells abnormalities.

There was difficulty in identifying the causes of anemia in 25% (11/44) of medical students with abnormal red cell indices. In all eleven cases, serum iron level were normal and DNA analysis performed showed negative results. These could be due to test limitations in our laboratory because of unavaibility of primers for certain alpha thalassaemia deletions and mutations.

Based on our result, there is a high prevalence of thalassaemia among medical students in UKMMC, which reflects a serious public health concern. The government policy should lay emphasis on prevention and control of thalassaemia in order to reduce the burden on the national health expenditure. It is important to provide effective education and screening materials to decrease the prevalence of thalassaemia. In Malaysia, screening for thalassaemia should be done at secondary school level. This is the most effective way to prevent thalassaemia. Every hospital in this country should at least have the facilities for thalassaemia screening and medical personnels such as doctors, who play an important role to interprete the screening tests and select individuals that need further testing for confirmation of the diagnosis of thalassaemia. The government should also be aware that all reference centres for thalassaemia such as UKMMC need up-to-date services for thalassaemia diagnosis. The government should provide adequate funding for research development to improve methods in detecting gene abnormalities in thalassaemia.

Implications. Early intervention can also reduce the prevalence of thalassaemia. Genetic counselling clinic aimed at carrier couples could significantly reduce the risk for future offspring to have thalassaemia major. At the public level, effective education and information dissemination using electronic or mass media is essential.

In conclusion, this study showed that prevalence of thalassaemia among the young age group in Malaysia was very high compared to the prevalence of IDA. We must put more efforts to increase the level of self-awareness about thalassaemia inheritance among the public. Efforts are also needed to promote screening for thalassaemia amongst school children, university students, pre-marital couple or perinatal patients. We also opine that the diagnosed cases of thalassaemia should be properly counselled.

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