PREVALENCE OF ANEMIA, IRON DEFICIENCY, THALASSEMIA AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY AMONG HILL-TRIBE SCHOOL CHILDREN IN OMKOI DISTRICT, CHIANG MAI PROVINCE, THAILAND

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Abstract. The prevalaence of anemia, iron deficiency, thalassemia and glucose--6-phosphate dehydrogenase (G-6-PD) deficiency were examined among 265 hill-tribe school children, 8-14 years of age, from Omkoi District, Chiang Mai Province, Thailand. Anemia was observed in 20 school children, of whom 3 had iron deficiency anemia. The prevalence of G-6-PD deficiency and β-thalassemia trait [codon 17 (A>T), IVSI-nt1 (G>T) and codons 71/72 (+A) mutations] was 4% and 8%, respectively. There was one Hb E trait, and no α-thalassemia-1 SEA or Thai type deletion. Furthermore, anemia was found to be associated with β-thalassemia trait in 11 children. These data can be useful for providing appropriate prevention and control of anemia in this region of Thailand.

Keywords: anemia, G-6-PD, hill-tribe school children, iron deficiency, thalassemia, Omkoi District, Thailand

INTRODUCTION

Anemia is a common public health problem affecting people of all ages with a global anemia prevalence of 32.8% in 2010 (Kassebaum *et al*, 2014). The highest anemia prevalence was in the post-neonatal period and followed by children aged 1 to 4 years. The etiology of anemia is multifactorial, including nutrition (*viz*, deficiencies in iron, folic acid and micronutrien), genetic hemoglobin disorders (*viz*. thalassemia, hemoglobinopathy, and glucose -6-phosphate dehydrogenase (G-6-PD) deficiency) and infectious disease (viz. parasitic infection) (Balarajan *et al*, 2011). Globally, iron deficiency is the major cause of anemia, estimated to account for appoximately 50% of all anemias.

In the northeast Thai school children population, the prevalence of anemia is 31%, with only a small propotion due to iron deficeincy (Thurlow *et al*, 2005). Genetic hemoglobin disorders are widespread in Thailand, with a high prevalence of heterozygous α-thalassemia (10%-30%), β-thalassemia (3%-9%) and Hb E

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(10%-53%) (Fucharoen and Winichagoon, 2010). These common genetic disorders are the major causes of anemia in the Thai population, inculding school children, aldolescents and pregnant women (Thurlow *et al*, 2005; Sanchaisuriya *et al*, 2006; Pansuwan *et al*, 2011). However, another study showed a high prevalence of iron deficiency anemia (12%) among hill-tribe school children living in a welfare center, Chiang Mai, Thailand (Porniammongkol *et al*, 2011). Proper diagnosed and treatment of anemia according to its underlying causes are important to reduce mortality and morbidity in order to improve the quality of life as well as providing an appropriate prevention and control measures.

The purpose of this study was to determine the prevalence of anemia due to iron deficiency, thalassemia and G-6-PD deficiency among a hill-tribe school children population in Omkoi District, Chiang Mai Province, Thailand.

MATERIALS AND METHODS

Subjects

This study was conducted in August 2013. The participating Karen hill-tribe school children were from Baan Yang Poa School, Omkoi District, Chiang Mai Province, Thailand. Omkoi District is located about 179 km southwest of Chiang Mai city. .A total of 265 school children, 8-14 years old, were recruited after receiving informed consent for participation from the director of the school. Informed written consents were obtained from their parents or legal guardians. This study was approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University (number 053E/56-7). Children who were found to have anemia were followed up for treatment and genetic counseling at Omkoi Hospital.

Blood samples

Ten ml of whole blood samples were collected and delivered to the Clinical Service Center, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai for hematological and biochemical analysis. Hematological parameters were measured using an automated blood counter (NIHON KOHDEN MEK-8222K, Tokyo, Japan). Anemia is defined when hemoglobin (Hb) concentration is < 11.5 g/dl for children aged 5-11.9 years and < 12.0 g/dl for those 12-14.9 years old (WHO, 2008).

Serum iron determination

Serum iron parameters, including serum iron (SI), total iron binding capacity (TIBC) and serum ferritin, were measured using an automated chemistry analyzer (Dimension® EXL™ 200 Integrated Chemistry System; SIEMENS, Munich, Germany). Transferrin iron saturation was calculated using the formula: (SI/TIBC) x 100. Iron deficiency (ID) is defined when ferritin level is ≤ 20 g/dl, while iron deficiency erythropoiesis (IDE) is defined when SI is < 110 μg/dl, transferrin iron saturation < 15%, ferritin level < 20 ng/ml or TIBC > 350 μg/dl. Iron deficiency anemia (IDA) is considered present if the child has serum iron < 50 μg/dl, transferrin iron saturation < 10%, ferritin level ≤ 10 ng/ml or TIBC > 400 μg/dl.

Thalassemia and hemoglobinopathy diagnosis

Detection of β-thalassemia and hemoglobinopathy including Hb E were performed using high performance liquid chromatography (HPLC) (VARIANT™ β-thalassemia Short Program; Bio-Rad Laboratories, Hercules, CA). Sample is designated as β-thalassemia trait, Hb E trait, β-thalassemia/Hb E or homozygous

Table 1
Prevalence of anemia, iron deficiency anemia, thalassemia and glucose-6-phosphate dehydrogenase (G-6-PD) deficiency among hill-tribe school children.

Feature	Age range (years)		
	8-11 (n = 130) n (%)	12-14 (n = 135) n (%)	Total (n = 265) n (%)
Non-anemia	119 (91.5)	126 (93)	245 (92)
Anemia	11 (8.5)	9 (7)	20 (8)
Iron deficiency anemia	1 (1)	2 (1.5)	3 (1)
β-thalassemia trait	9 (3)	12 (4.5)	21 (8)
Hb E trait	1 (0.5)	0 (0)	1 (0.4)
G-6-PD deficiency	5 (4)	5 (4)	10 (4)

Hb E when level of Hb A_2/E is 4%-9.9%, 10%-29.9%, 30%-60% (with Hb F ≥15%) and > 65%, respectively (Pornprasert *et al*, 2010). Genomic DNA was extracted from whole blood sample using NucleoSpin® kit (Macherey-Nagel, Duren, Germany) according to manufacturers' instructions, and the molecular diagnosis of α-thalassemia-1 Southeast Asian (SEA) and Thai type deletions was performed using SYBR Green 1 quantitative real-time PCR equipped with high resolution melting (HRM) analysis as previously described (Pornprasert *et al*, 2008). In addition, identification of codons 71/72 (+A), codons 41/42 (-TCTT), codon 17 (A>T) and IVSI-nt1 (G>T) β-thalassemia mutations was performed using multiplex amplification refractory mutation system (MARMS)-PCR (Pornprasert *et al*, 2012).

Detection of G-6-PD deficiency

G-6-PD deficiency was analyzed using a fluorescent spot test as described by Beutler *et al* (1979) with minor modifications. In brief, 10 μl aliquot of blood sample was added to 200 μl of G-6-PD screening reagent (Sigma-Aldrich, St Louis, MO) which was prepared immediately prior to use. The solution was incubated at 37°C in the dark for 10 minutes. An aliquot of the solution was spotted onto a Whatman filter paper, air dried and examined under UV light. Control normal and G-6-PD deficient blood samples were included in each assay.

RESULTS

Since the anemia is defined when Hb concentration is < 11.5 g/dl for children aged 5-11.9 years and < 12.0 g/dl for those 12-14.9 years old (WHO, 2008); subjects were divided into two groups: 8-11 and 12-14 years old. Of the 265 children, anemia was detected in 11 (8.5%) of 130 in 8-11 years old children and in 9 (7%) of 135 in the 12-14 years old group (Table 1). Prevalence of iron deficiency anemia was 1% (1/130) and 1.5% (2/135) in the younger and older age groups, respectively. Moreover, the ID or IDE was detected in 1% and 8% in the younger and older age groups, respectively. Prevalence of G-6-PD deficiency was 4% and of carriers of thalassemia or hemoglobinopathy

Table 2
Features associated with anemia among hill-tribe school children.

Feature	Age range (years)		
	8-11 (n = 11) n (%)	12-14 (n = 9) n (%)	Total (n = 20) n (%)
Iron deficiency anemia	1 (9)	2 (22)	3 (15)
β-Thalassemia trait	7 (64)	4 (44)	11 (55)
Others	3 (27)	3 (33)	6 (30)

was 9%. Twenty-one children are β-thalassemia heterozygotes [7 (33%), 6 (29%) and 4 (19%) with codon 17 (A>T), IVSI-nt1 (G>T) and codons 71/72 (+A) mutations, respectively, and 4 (19%) were unidentified]. Furthermore, one child had Hb E trait. Among the 20 children with anemia, iron deficiency accounted for 3 (15%) of the cases, β-thalassemia trait for 11 (55%) and the causes of anemia in the remaining 6 (30%) children remained undefined.

DISCUSSION

Anemia is a global plublic health problem and occurs at all stages of life, especially school children, with the worldwide prevalence of 24.5% (WHO, 2008). A previous study of hill-tribe school children in a welfare center, Chiang Mai Province, Thailand reported the prevalence of anemia and iron deficiency anemia of 30.9% and 12%, respectively (Porniammongkol *et al*, 2011). In this study, there was a lower prevalence of anemia (8%) and iron deficiency anemia (1%) among hill-tribe school children from Omkoi District in the same province. This school provides lunch for all students, thereby providing adequate nutrition, presumably including iron, a deficiency of which is the primary cause of anemia in Thai children (Winichagoon, 2013).

The prevalence of β-thalassemia trait (8%) among the hill-tribe school children from Omkoi District, Chiang Mai Province was within the range reported for the Thai poppulation (3-9%) (Fucharoen and Winichagoon, 2010). The extremely low prevalence of Hb E (1/530 alleles) in this studied population would account for the absence of β-thalassemia/Hb E.

The prevalence of G-6-PD deficiency (4%) among this group of hill-tribe school children is slightly lower than that (6.0%-15.8%) of the Southeast Asian population (Tanphaichitr *et al*, 1994; Iwai *et al*, 2001; Louicharoen and Nuchprayoon, 2005). This may be attributed to the different ethnic origin and/or the small sample size of this study.

A previous study have shown that the majority of anemias in northeastern Thai school children aged 10 to 11 years is not due to iron deficiency but all were carriers of either thalassemia or hemoglobinopathy, particularly β-thalassemia and homozygous Hb E (Panomai *et al*, 2010). In the current study, anemia among hill-tribe school children was poorly attributed to iron deficiency but more associated with β-thalassemia trait. However, it should be noted that only some types of thalassemia

traits may result in anemia, althought all types of thalassemia affect hemoglobin production to some extent (Fucharoen and Winichagoon, 2011). However, in the present study, there were 6 school children whose anemic status could not be defined by the techniques employed. The cause of anemia could be infection with intestinal parasites, such as hookworms and/or *Ascaris*. Approximately 50% of hill-tribe children aged 1-6 years from Mae Chaem District, Chiang Mai Province are infected with intestinal parasites; and *Ascaris lumbricoides* is the most common parasite (Tienboon and Wangpakapattanawong, 2007). Moreover, 3 of 6 undefined anemic children were female aged 10-13 years and blood loss from their menstruation during the study period could be a cause of the anemia.

In summary, anemia and G-6-PD deficiency among hill-tribe school children from Omkoi District, Chiang Mai Province, northern Thailand were detected at a lower prevalence as compared to the previous studies (Tanphaichitr *et al*, 1994; Iwai *et al*, 2001; Louicharoen and Nuchprayoon, 2005; Thurlow *et al*, 2005); and a small number of anemias were attributed to iron deficiency. β-Thalassemia trait was found (8%) and an extremely low prevalence of Hb E in this study population but heterozygous α-thalassemia-1 SEA and Thai type deletions were not detected. These data can be used for genetic counseling, prevention and control of anemia in this region of Thailand.

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