

PJN

ISSN 1680-5194

PAKISTAN JOURNAL OF
NUTRITION

ANSI*net*

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Iron Status of Hill Tribe Children and Adolescent Boys: A Cross Sectional Study at a Welfare Center in Chiang Mai, Thailand

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Abstract: Iron Deficiency Anemia (IDA) is one of the major public health problems worldwide. High prevalence of anemia has been found in the developing countries, including Thailand. The purpose of this study was to investigate the iron status of 181 hill-tribe school boys who lived in Wat Donchan Welfare Center (DWC), Chiang Mai, Thailand. A cross-sectional study was conducted in 2 groups of children which were divided by age (7-11 years and >11-15 years). Body weight and height of each child were measured. Anthropometric status was classified using CDC 2000 Growth chart reference by Weight for Age (W/A) and Height for Age (H/A). Hemoglobin (Hb) and serum ferritin (Ft) were analyzed as indicators for iron status. Student's t-test and Pearson correlation coefficient was used to determine the significant differences and the relationship between the 2 groups or parameters at $p \leq 0.05$. Results showed that 32% and 60% of children and adolescents were under weight and stunted, respectively. Mean Hb in young children was 11.9 ± 0.8 g/dL while in older children was 12.5 ± 1.1 g/dL. The prevalence of anemia was 31% using only Hb as an indicator, while the prevalence of IDA was 12% and Iron Deficiency (ID) was 23% when using Hb and Ft as the indicators. Iron deficiency was a major cause of anemia in this group; 25% and 41% of anemic children in younger and older group, respectively. The study found that 8% of children had the thalassemia trait and 93% of them were anemic. It was suggested that anemia in these hill-tribe children might be associated with insufficient iron food sources, poor sanitation, poor hygienic practice and hemoglobinopathies.

Key words: Iron deficiency anemia (IDA), school children, adolescent, Welfare center, serum ferritin, thalassemia

INTRODUCTION

Iron Deficiency Anemia (IDA) has been considered as one of the leading risk factors for disability and death (WHO, 2008). According to the current report from the WHO (2008), approximately 24.8% or 1.62 billion of world population suffered from IDA and among this number, 305 millions were schoolchildren (aged 5-14 years). The major causes of IDA in the risk group were low iron intake and low dietary iron bioavailability. In addition, IDA symptoms are more likely to occur in those who consume rice-based diets and have low intakes of readily available heme iron from animal protein (Zimmermann *et al.*, 2005).

It has been documented that IDA impairs physical growth, mental development, social attention and learning capacity in young children (Grantham-McGregor and Ani, 2001; Sachdev *et al.*, 2005). In addition, IDA increases susceptibility to infections, mainly of the upper respiratory tract (Longfils *et al.*, 2008; de Silva *et al.*, 2003). Children who have IDA are less likely to recover from upper respiratory tract infection than those who are healthy. Moreover, IDA could lead to deficiencies of vitamins and other minerals (Fishman *et al.*, 2000; Ahmed *et al.*, 2005). Results from previous studies

showed that children who live in rural areas and who are from poor families tended to have higher risk of Protein-Energy Malnutrition (PEM) and multiple nutrients deficiencies including IDA than those who resided in urban area. In addition, children in rural areas are more susceptible to bacteria and virus infection due to poor hygiene and sanitation than those in urban area (Van Den Broek, 2003; Unsal *et al.*, 2007; Malkanthi *et al.*, 2010).

Although, iron deficiency is a major cause of anemia, anemia could be caused by genetic hemoglobinopathies (Borges *et al.*, 2001; Muncie and Campbell, 2009; Galanello and Origa, 2010), multiple micronutrient deficiencies (Fishman *et al.*, 2000; Van Den Broek, 2003; Ahmed *et al.*, 2005), chronic inflammatory disorders (Filiopoulos and Vlassopoulos, 2009; Roy, 2010; Guidi and Lechi Santonasta, 2010) and infection (Loukas *et al.*, 2006; Antia *et al.*, 2008; Drakesmith and Prentice, 2008; Haldar and Mohandas, 2009; Roy, 2010). The characteristics of community and race could play a role for the etiology of anemia as well (Adebisi and Strayhorn, 2005; Veghari *et al.*, 2007).

In Thailand, several studies showed that the prevalence of PEM has been decreased, however; micronutrient

deficiency, especially IDA, still remains a serious problem. The National Nutrition survey conducted by the Ministry of Public Health in 2003 (Department of Nutrition, Thailand's Ministry of Public Health (MOPH, 2003) reported that IDA was not only a problem among infants and preschool children, but also among school children and adolescents. It was found that the prevalence of anemia was 46.7%, 25.4% and 15.7% in children aged 6-8 years, 9-12 years and 12-14 years, respectively.

Hill-tribe people, who are living on the highlands in the northern part of Thailand, were in low socioeconomic status and most of them are agricultural (Joseph, 1996; Phumpatrakom *et al.*, 2007). It was estimated that the hill-tribe population accounted for 2% (about 1.2 million) of Thai population in 2002 (Thailand's Ministry of Social Development and Human Security (MSDHS, 2002). The prevalence of PEM, micronutrients deficiency, infection and infestation were relatively high among their people (Panpanich *et al.*, 2000; Saksirisampant *et al.*, 2004; Tienboon and Wangpakapattanawong, 2007). Because of the rapid growth of the hill-tribe population, they tend to migrate to the city and work as employees (Social development center unit 43, MSDHS, 2006). At the same time, many welfare centers (107 registered welfare centers, Thailand's Ministry of Interior (MOI, 2008) have been established to take care of these children, including hill-tribe children. The purpose of this study was to determine the iron status of hill-tribe school children who are living in welfare centers in the cities of northern Thailand.

MATERIALS AND METHODS

The study was conducted at the Donjan Welfare Center (DWC), Chiang Mai province, Thailand during November 2006 and February 2007. The DWC is the non-profit organization, was established in 1984 by the Dean of Donjan Temple. The DWC provided housing, education and basic requirements for children.

Subjects: Subjects were 181 hill-tribe boys, age ranging between 7-15 years, who lived at DWC. The research study was approved by The Mahidol University Institutional Review Board (MU-IRB). Written consent was obtained from children and their parents before the beginning of the study.

Sixty three percent of subjects were Karen (the largest mountain ethnic minority group in Thailand), 16.6, 14.4 and 6.0% are Akha, Meo and others, respectively. All participants came from 5 different provinces of Northern, Thailand; 73% came from Tak, 17.5% came from Chiang Rai, 5%, 4% and 0.5% came from Chiang Mai, Phayao and Mae Hong Son, respectively.

Anthropometry assessment and evaluation: Body weight was measured using digital bathroom scale

model no.900 (Salter housewares limited, Tonbridge, Kent, Made in Japan). Standing height was measured using a wall-mounted stadiometer (Stanley-Mabo, Poissy, France) while children were wearing light clothing and no shoes (Lohman *et al.*, 1991). Measurement of body weight and height was performed in duplicate and the average value was used as data. Dates of birth of children were verified with school registry. Nutrition status of children was assessed using Weight for Age (W/A) and Height for Age (H/A) percentile. W/A and (H/A) were calculated individually. Nutritional status was categorized using the 2000 Centers for Disease Control and Prevention (CDC) Growth chart as the reference. While W/A was applied to identify children who were at risk of underweight (W/A < 5th percentile), H/A was applied to identify children who were at risk of short stature (Height for age (H/A) < 5th percentile) (CDC, 2000).

Biochemical assessment: Five milliliters of fasting blood sample were obtained from participants and were collected into 2 tubes. The first tube contained EDTA as anticoagulant for determination of Hemoglobin (Hb), Hematocrit (Hct), Mean Corpuscular Volume (MCV), white Blood Cell Count (WBC) and Hb typing and for screening test for hemoglobinopathies and thalassemia. For the second tube, an aliquot serum was separated from clotted blood and used to determine serum ferritin and C-Reactive Protein (CRP) levels.

Whole blood was immediately analyzed for complete blood count, i.e., Red Blood Cells (RBC), Hemoglobin (Hb), Hematocrit (Hct), White Blood Cells (WBC) and Platelets (PLT), using the fully automated ABX Pentra 60C+ Analyzer (Horiba ABX, Montpellier, France). In addition, Hb typing was measured by HPLC and by Cellulose acetate electrophoresis, erythrocyte osmotic fragility test (OF test) and Dichlorophenolindolphenol Precipitation (DCIP) test. In addition, serum ferritin was measured using an immunoradiometric assay (Ramco Laboratories, Inc.) and C-Reactive Protein (CRP) levels was measured by Nephelometry (cardioPhase High sensitivity CRP). Children who had CRP more than 10 mg/L were identified as infected cases (Clyne and Olshaker, 1999; Pearson *et al.*, 2003). Excess WBC greater than 11,000 per mm³ was identified as infection (Abramson and Melton, 2000).

Anemia was defined when Hb concentration levels were less than 11.5 g/dL for children aged 6-11 years and less than 12.0 g/dL for those aged more than 11 years to 15 years (WHO, 2001). The cutoff value for serum ferritin of less than 30 µg/L was used for Iron Deficiency (ID) without anemia (in the presence of infection) (Zimmermann and Hurrell, 2007). IDA was defined as serum ferritin less than 30 µg/L and Hb concentration less than 11.5 g/dL for 6-11 years of age or less than 12.0 g/dL for more than 11-15 years of age.

Table 1: Characteristic of study children

Variables	Age range (yrs)		
	7-11	>11-15	All
Number of subjects	n = 34	n = 147	n = 181
Age (yrs)**	10.0±1.0 ^a	13.6±1.2 ^b	12.9±1.8
Weight (kg)**	27.1±4.0 ^a	40.5±8.8 ^b	37.9±9.7
Height (cm)**	126.5±6.5 ^a	144.5±10.2 ^b	141.0±12.0
BMI (kg/m ²)**	16.9±1.3 ^a (n = 34)	19.1±2.0 ^b (n = 143)	18.7±2.1 (n = 177)
Weight for age			
Underweight [†] (n, (%))	10 (29.4)	48 (33.6)	58 (32.8)
Height for age			
Short stature ^{††} (n, (%))	20 (58.8)	88 (61.5)	108 (61.0)
Underweight and Short stature (n, (%))	9 (26.5)	47 (32.9)	56 (31.6)
Normal weight and normal height (n, (%))	13 (38.2)	53 (37.1)	66 (37.3)

**Different superscript letters are significantly difference between groups (p<0.01).

[†]W/A less than the 5th percentile = underweight.

^{††}H/A less than the 5th percentile = short stature

Statistical analyses: Data were analyzed using SPSS (version 13.0; SPSS Inc., Chicago, USA) and are presented as Mean±SD and percent of anemia stratified by age groups. Independent sample t-test was used to determine the significant differences in anthropometric and biochemical parameters between the 2 groups. Pearson correlation coefficient was used to examine the relationship between biochemical and anthropometric parameters of children. Statistically significant differences are indicated by p≤0.05.

RESULTS

All anthropometric and biochemical parameters of all 181 boy children were included in data analysis. Subjects were divided into two groups according age group; 7-11 years and >11-15 years old. The mean age of younger children was 10.0±1.0 years and the older children was 13.6±1.2 years.

The body weight, height, Body Mass Index (BMI) and nutritional status of children are shown in Table 1.

Nutritional status was classified by Weight for Age (W/A) and Height for Age (H/A) from CDC Growth charts standard (CDC, 2000). W/A classification indicated that more than 32.8% of children in both age groups were underweight. Moreover, H/A classification indicated that 61.0% of children in both age groups were stunted. Mean of age, body weight, height and BMI of younger children were significant less than older children age group. The findings showed that the prevalence of stunted children was twice that of the underweight children in both age groups; about 31.6% of children with underweight and short stature. Only 37.3% of children had normal weight and normal height.

Hemoglobin (Hb), Hematocrit (Hct), Mean Corpuscular Volumes (MCV), White Blood Cells Count (WBC), serum Ferritin (Ft) and Hb typing values of children stratified by age group are presented in Table 2. The average values of Hb and Hct in children aged 7-11 years were significantly lower than those aged more than 11 years

Table 2: Hematological and biochemical variables of the children (mean±SD) or n (%)

Variables	Age range (yrs)	
	7-11	>11-15
Number of subjects	34	147
Hemoglobin (g/dL)*	11.9±0.8 ^a	12.5±1.1 ^b
Hematocrit (%)*	37.0±4.9 ^a	40.0±3.0 ^b
MCV (fL)	79.1±5.2	78.9±6.2
WBC (10 ³ /mm ³)*	9.0±2.8 ^a	7.1±1.9 ^a
Serum ferritin (µg/L) [‡]	48.1±33.2 (n = 32)	45.8±61.2 (n = 142)
Hb typing for Thalassemia (n, (%))		
Normal	33 (97.1)	133 (90.5)
β thalassemia trait	1 (2.9)	11 (7.5)
α thalassemia trait	-	3 (2.0)
CRP		
≤10 mg/L	32 (96.9)	142 (96.6)
>10 mg/L	1 (3.1) (n = 33)	5 (3.4) (n = 147)
WBC**		
≤11 10 ³ per mm ³	26 (76.5)	141 (95.9)
>11 10 ³ per mm ³	8 (23.5)	6 (4.1)
Anemia status		
Normal [†]	24 (70.6)	101 (68.7)
Anemia ^{††}	10 (29.4)	46 (31.3)
Iron status^{†***}		
Iron Deficiency Anemia (IDA) [†]	2 (6.3)	19 (13.2)
Iron Deficiency (ID) ^{††}	9 (28.1) (n = 32)	29 (21.5) (n = 144)

*Different superscript letters denoted significant mean difference in Hb, Hct, MCV and WBC between 2 groups by independent sample paired t-test (p<0.01).

**Significant association between WBC and age group by Chi-square test at p-value = 0.000

***Significant association Iron status and age group by Chi-square test at p-value = 0.053

[‡]Excluded infected children (who had CRP>10 mg/L)

[†]IDA = Hb <11.5 g/dL for aged 7-11 yrs or Hb <12 g/dL for aged >11 yrs and serum ferritin <30 µg/L

^{††}ID = Hb normal and serum Ferritin <30 µg/L

(p<0.01). However, the result showed the average value of WBC in younger children were significantly higher than those older children (p<0.01). Mean Hb in children

Table 3: Iron status and anthropometric status of children, n (%)

Iron status	Anthropometric status		p-value	Anthropometric status		p-value
	Normal height	Short stature		Normal weight	Under weight	
All subjects [‡]	65	107	0.157	113	58	0.543
IDA [†]	7 (10.8)	14 (13.1)		12 (10.6)	09 (15.5)	
ID ^{††}	18 (27.7)	22 (20.6)		25 (22.1)	12 (20.7)	

[‡]Excluded infected children (who had CRP>10 mg/L).

[†]IDA = Hb <11.5 g/dL for aged 7-11 yrs or Hb <12 g/dL for aged >11 yrs and serum ferritin <30 µg/L.

^{††}ID = Hb normal and serum Ferritin <30 µg/L

aged between 7-11 years was 11.9±0.8 g/dL while in those aged more than 11 years was 12.5±1.1 g/dL. Mean of MCV of younger and older children were 79.1±5.2 and 78.9±6.2 fL, respectively. Although mean of serum Ferritin (Ft) in younger children was a little bit higher than those older children, it was not significantly different from that of older children.

The screening test for thalassemia indicated that 2.9% in younger children and 7.5% in older children had the β-thalassemia trait and 0% in younger children and 2% in older children had the α-thalassemia trait. While positive test of C-Reactive Protein (CRP) test (CRP> 10 mg/L) showed that 3.1% of younger children and 3.4% of older children were infected, excess White Blood Cell Count (WBC) in blood (>11,000 per mm³) was found in 23.5 and 4.1% of younger and older children, respectively.

Among 181 children, there were no severely anemic children (Hb<8 g/dl). However, when using only Hb as an indicator, the prevalence of anemia was 29.4% in younger children and 31.3% in older children. In addition, the prevalence of IDA identification was 6.3% in younger children and 13.2% in older children, ID was 28.1% in younger children and 21.5% in older children when using Hb and Ft as the indicators. Older children (aged more than 11 years) were anemic from IDA more than twice as much as younger children (aged 8-11 yrs) (Table 2).

The result in Table 3 showed a little bit higher percent of anemic children and IDA with short stature (13.1%) than normal height (10.9%). The same phenomenon, a little bit higher percent of IDA children and was also found in under weight children (15.5%) than normal weight children (10.8%). On the other hand, the number of ID children with normal height (27.7%) or normal weight (22.1%) was higher than in ID children with short stature (20.6%) or under weight (20.7%). However, no correlation between iron status and anthropometric status (classified by H/A and W/A) was found.

The results of the present study demonstrated that some children were anemic, which is not caused by ID. Although there was no correlation between group of Hb typing and blood parameters, means of Hb, Hct, MCV in thalassemia trait children were significantly lower than those in normal children. When comparing the percent of anemic children between normal and those with thalassemia, we found 93.3% of children with

Table 4: Hematological and biochemical variables of the children separated by Hb typing (mean±SD) or n (%)

Variables	Hb typing	
	Normal	Thalassemia trait
Number of subjects	n = 166	n = 15
Age (months)	154.8±22.2	158.7±21.6
Hemoglobin (g/dL)**	12.5±1.0 ^a	11.1±0.6 ^b
Hematocrit (%)**	39.7±3.3 ^a	37.4±2.2 ^b
MCV (fL)**	80.3±3.7 ^a	63.4±5.5 ^b
WBC (10 ³ /mm ³)	7.5±2.2	7.6±2.1
Serum ferritin (µg/L)*	41.6±26.1 ^a (n = 159)	95.3±172.2 ^b (n = 15)
Anemia status***		
Anemia	42 (25.3)	14 (93.3)
Normal	124 (74.7)	1 (6.7)

*Different superscript letters are significant difference between groups (p<0.05).

**Different superscript letters are significant difference between groups (p<0.01).

***Significant association between Anemia status and Hy typing by Chi-square test at p-value = 0.000

thalassemia trait were anemia, while 25.3% of normal Hb children were anemic (p = 0.000) (Table 4).

DISCUSSION

Evaluation of nutritional status using W/A and H/A indicated that high rate of stunting (61%) and under weight (32.8%) was found among these hill-tribe children. High numbers of short stature or stunted children using H/A classification indicated that children had long term malnutrition or chronic malnutrition (WHO, 2001).

When using only hemoglobin as an indicator for IDA, the prevalence of anemia among hill-tribe children aged between 7-11 years was similar to the 2003 national report from the Ministry of Public Health. However, the prevalence of anemic hill-tribe children aged more than 11 years was twice higher than that of the national report (MOPH, 2003). For IDA classification using Hb and Ft as the indicators, nearly half (38%) of anemic children were IDA. The number of ID was also twice higher than that of IDA. In addition, the study demonstrated that 65% of children in the DWC had MCV less than 82 fL and the common morphology type of anemia was hypochromic microcytic anemia, which is the condition that is associated with ID. The high prevalence of IDA and ID in this population might be caused by low dietary iron

intake. Their foods mainly consisted of rice and small amounts of eggs or meat, which are considered as non-heme iron foods. Therefore, iron content from their foods was not sufficient to meet their iron intake and growth requirements (Tatala *et al.*, 1998; Meng *et al.*, 2005; Zimmermann *et al.*, 2005; Singh *et al.*, 2006; Zimmermann and Hurrell, 2007; Coulibaly *et al.*, 2011). However, macrocytosis of the morphology type which is related to vitamin B12 and folate deficiency (Cooper and Rosenblatt, 1995; Ward, 1979; Savage *et al.*, 2000) was not found in these children. In addition, the present study found that around 30% of anemic participants have the thalassemia trait which is characterized by microcytic hypochromic red cells from an abnormal globin chain. Thus it could be assumed that genetic hemoglobinopathies might be another cause of anemia in this population (Koerper *et al.*, 1976; Gwendolyn and Higgins, 2000; Demir *et al.*, 2002).

Although, the high prevalence of infected children, using CRP as indicator was not observed, the number of children with higher WBC level than normal range was high, 23.5% in the younger age group and 4.1% in the older age group. Around 7.5% of children with anemia and high Ft >30 µg/L (3 of 40) had higher than normal proportion of monocytes. Excess WBC in the peripheral blood may be indicative of various disease states, including inflammation (acute or chronic) from bacteria virus or parasites (Abramson and Melton, 2000).

Although, this study did not collect stool samples for parasitic examination nor test food and water contamination, the observation showed that participants with poor personal hygiene and inappropriate management of food and drinking water may be at risk of infection and parasite infestation (Pruss-Ustun *et al.*, 2004; Yamamah *et al.*, 2007). Poor hygiene and contaminated food and drinking water could be one of the causes of their illness, especially in young children (Esrey *et al.*, 1985; Adeoye *et al.*, 2007). Poor hygiene related to defecation and not wearing shoes make children at risk for infestation, especially hookworm which is one specific cause of anemia (Stoltzfus and Dreyfuss, 1998). Signs and symptoms of infectious diseases such as fever, tinea, ringworm and diarrhea were found, which was in line with previous studies (Saksirisampant *et al.*, 2004; Tienboon and Wangpakapattanawong, 2007). It might be concluded that infectious and parasitic diseases would be another cause of anemia in this population.

Conclusion: This study found a high prevalence of stunting and anemia in Thai hill-tribe children and adolescents in DWC. Several possible causative factors might be inadequate nutrient intakes, particular low iron intake, inappropriate food management and monotonous staple food, as well as thalassemia trait.

Another possible cause of anemia in this population might be bad sanitation and poor personal hygiene, which lead to infectious and parasitic diseases and anemia. Management of food sanitation, hygienic environment and promotion of iron rich food consumption in the welfare centers or schools would be the proper resolutions to reduce the prevalence of anemia.

ACKNOWLEDGEMENT

This project was funded by National Research Council Thailand (NRCT). We thank Dr. Ratchanee Kongkachuichai for her assistance and technical guidance for biochemical analysis. We thank Dr. Jitnarin N. and Dr. Harold Furr for their help in editing the manuscript. Special thanks are given to the Dean of DWC for permitting the research team to conduct the study. We also thank all children for participation in the study.

Coauthor contributions: S.P.P. developed the concept, monitored the data collection and supervised this project. S.P.P and P.O. designed the experiment. P.O. and I.T. collected the data in the field. O.P. performed blood biochemical analysis and analyzed the data. Y.U. advised tools for anthropometric assessments and data analysis and edited manuscript.

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