International Journal of Food Sciences and Nutrition,
Volume 55, Number 6 (September 2004) 517–525

Taylor & Francis
healthsciences

Determinants of iron status in Malaysian adolescents from a rural community

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Iron deficiency anaemia is the most common micronutrient deficiency worldwide. The prevalence of anaemia in the developing countries is three to four times higher than that in the developed countries. The iron status was assessed in 199 apparently healthy male and female adolescents aged 12–19 years living in a fishing community in Sabah, Malaysia. Data on socio-economic characteristics, lifestyles, anthropometry measurements, iron status, and dietary intake were gathered. Dietary intake of energy, iron, and most nutrients (with the exception of protein and vitamin C) were below the recommended levels for Malaysian adolescents. Three-quarters of the iron was derived from plant foods. The mean haemoglobin value for the male was 13.9 ± 1.3 g/dl with 9.5% having less than 12 g/dl, while the respective figures for the female were 12.4 ± 1.6 g/dl and 28.6%. The mean serum ferritin concentrations for male and female adolescents were 21.5 and 15.4 μg/l, respectively; with 25.7% of the males and 49.5% of the females having deficient levels of ferritin. Dietary intake of total energy and iron, and gender were found to be independent determinants of serum ferritin and haemoglobin levels, accounting for over 40% of the variations for each of these iron indicators. In males, but not in females, the intake of dietary protein and iron, and physical activity were also found to be significant determinants of serum ferritin. The age of subjects and household size were significant determinants of haemoglobin levels for male subjects, but not for female subjects. The findings indicate the importance of adequate intake of energy and dietary iron for improving the iron status of adolescents.

Introduction

Iron deficiency anaemia (IDA) is the most common micronutrient deficiency in developed and developing countries, including Malaysia (Tee *et al.*, 1999). It is normally characterised by low haemoglobin (Hb) and serum ferritin concentrations. The prevalence of anaemia in developing countries is three to four times higher than that in developed countries (UNICEF/UNU/WHO/MI, 1999). It is usually attributed to poor diet that is low in iron bioavailability and high in iron absorption inhibitors (Tatala *et al.*, 1998).

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ISSN 0963-7486 printed/ISSN 1465-3478 online

DOI: 10.1080/09637480400015786

Several studies have shown that iron deficiency can lead to serious consequences, including both immediate and long-term impairment in motor and cognitive development in infants and children (Lozoff *et al.*, 2000), decline in mental and physical performance in children and adolescents (Pollitt, 1997) and a decline in reproductive performance and work capacity in adults (Allen, 2000; Li *et al.*, 1994).

Pregnant women, pre-school children and young children aged 5–14 years are identified as high-risk groups for IDA because of their high iron requirements (ACC/SCN, 2000). In Asian countries, the prevalence of IDA among adolescents is reported to be more than 40%, especially among females at the onset of menarche (Kurz, 1996). A positive iron balance is difficult to maintain during the period of adolescence due to a large pubertal growth spurt and maturation (Yip, 1994), and this is further exacerbated by low dietary iron intake. Data on anaemia in adolescents are limited in developing countries. IDA in adolescence is known to lead to deleterious effects in a lifecycle fashion, on health in adulthood and during pregnancy, affecting the development of the foetus, which in turn will have health impacts in childhood. There is thus a need to understand the determinants of IDA in adolescence.

Data on IDA in Malaysia are often reported on young children and pregnant mothers. Adolescents were included in a nutritional assessment of rural communities in Peninsular Malaysia, which reported a high prevalence of anaemia among adolescent in fishing and estate communities (Tee *et al.*, 1998). The present study is aimed at identifying the determinants that affect iron status of rural adolescents in the state of Sabah in East Malaysia.

Methods

Location

The state of Sabah was chosen as relatively few nutritional studies have been carried out in East Malaysia. Fishing villages in Tuaran were purposively selected for the study based on reasons of logistics and the homogeneous population with respect to ethnicity, socio-cultural and daily economic activities.

Subjects

There were a total of 225 adolescents living in fishing communities in the Tuaran District of Sabah, who fulfilled the age criteria of 12–19 years. Out of these, mothers of 18 adolescents refused to give consent, while eight adolescents themselves did not agree to participate. Thus a total of 199 adolescents comprising 94 males and 105 females were included in this study. The subjects were in apparently good health without any diagnosed disease. Anthropometric measurements and 24-h dietary recall for 3 days were administered in all the subjects. Blood samples were obtained from only 165 (83%) subjects, as the rest (20 male and 14 female) refused to have their blood drawn. The study was approved by the State Health Department of Sabah, and consent (either verbal or written) was obtained from both parents and the subjects for the study.

Multiple iron status indicators namely, serum ferritin, transferrin saturation (TS), Hb and mean corpuscular volume (MCV) were used to determine the iron status of the subjects. Serum ferritin is recognised as a fairly sensitive indicator of changes in body iron stores (Expert Scientific Working Group, 1985) and is the most commonly used of indicators of iron stores, whereas the Hb level is used to detect the late stage of iron deficiency.

Socio-economic background and lifestyle

The subjects were interviewed using a pre-tested structured questionnaire, which included general and socio-economic characteristics such as household income, household size, parental occupation and parental education levels. Data on lifestyle factors such as cigarette smoking, alcohol consumption and physical activity level were also gathered. The physical activity level was estimated by recording the types of physical activity, the intensity and duration of each activity. Menstrual status such as age at menarche was recorded for those female

adolescents who agreed to have their blood drawn.

Anthropometry measurements

Measurements of body weight and height were made according to the standard procedures of the World Health Organization (1995). Body weight was measured using an electronic digital scale (TANITA Corporation, Tokyo, Japan) with an accuracy of 0.1 kg, calibrated for a 5 kg calibrator daily. Height was determined using a microtoise tape (Seca bodymeter 208; Hamburg, Germany), calibrated for an accuracy of ± 0.1 cm. The average of at least two measurements was computed. The body mass index was calculated by dividing the weight (kg) by the square of the height (m).

Biochemical and haematological profiles

A volume of 5 ml fasting venous blood was taken from the subject, out of which 2 ml was drawn into tubes containing ethylenediamine tetraacetic acid for the analysis of full blood count, while the remaining 3 ml was collected into an iron-free tube for the analysis of serum ferritin, serum iron and total iron binding capacity (TIBC) concentrations. Hb and red cell indices were measured with an electronic counter (Cell-Dyne® 1700; Abbott Diagnostics Division, Abbott Park, Illinois, USA). The full blood count analysis was performed within 4 h after blood collection as recommended by the International Committee for Standardization in Haematology (1988a,b). Blood for biochemical measurements was allowed to clot at room temperature (25°C), after which the serum was obtained by centrifugation at 3000 *g* for 15 min. The serum was separated and stored at −20°C for later analyses. Serum ferritin concentrations were measured in a microparticle enzyme immunoassay procedure (Automated immunoassay IM_X system Analyzer; Abbott Diagnostics Division), whereas serum iron and TIBC were measured by a modification of the automated Technicon Ferene-S method (Technicon RA-XT system, Bayer Diagnostics Division, Tarrytown, NY, USA). The biochemical analyses were made in Kota Kinabalu, Sabah within 1 month of blood collection. The within-day assay variations for Hb, MCV, serum ferritin, serum iron and TIBC, expressed as the coefficient of variation (CV), were 0.6%, 0.7%, 4.0%, 4.7% and 5.5%, respectively.

The cut-off points of haematological and biochemical indicators for iron status were used to determine the proportion of abnormal value among adolescent subjects. For Hb, the cut-off point for anaemia is Hb < 12 g/l for females and is Hb < 12 g/l (age < 15 years) and < 13 g/l (age 15–19 years) for males, whereas for haematocrit it is < 36% and < 41% for females and males, respectively (Gibson, 1993). The cut-off values for red cell indices were defined to be deficient for both males and females as follows: mean corpuscular haemoglobin (MCH) < 27 pg; mean corpuscular concentration (MCHC) < 320 g/l (Gibson, 1993) and MCV < 78 fl (age 11–14 years) and < 80 fl (age > 14 years) (Expert Scientific Working Group, 1985). Cut-off points for serum iron, TIBC and TS were < 10.74 μmol/l (60 μg/dl), > 73.39 μmol/l (410 μg/dl) (Cook & Finch, 1979) and < 16% (Dallman *et al.*, 1996), respectively. Serum ferritin was considered to be deficient when the level was less than the cut-off value of 12 μg/L (Dallman *et al.*, 1996).

Dietary intake assessment

The subjects were asked to record their dietary intake for 3 days, comprising two weekdays and a weekend, in order to minimise day-to-day variability of an individual's food intake. Subjects were asked for detailed description of all foods and beverages consumed including brand names, the process of preparation and cooking methods. For composite dishes, the amount of each ingredient used in the recipe and the amount consumed by the subjects were recorded. Local recipes of commonly eaten foods in the community were obtained. The subjects were instructed on estimating portion sizes based on food models and household measures. At the end of the 3 days, all the subjects returned their intake records and each record was checked for completeness and accuracy. All food amounts were analysed for energy and nutrient content using the nutrient conversion computer programme 'Diet 4', which is based

on the Malaysian food composition table (Tee *et al.*, 1997). The subjects were also asked if they taking dietary supplements.

Statistical analysis

All variables were tested for normality against a standard normal distribution using the Kolmogorov–Smirnov test and test of homogeneity of variance before any statistical comparisons were made. The iron status indicators used namely, serum iron, TIBC, TS, haemoglobin, haematocrit, MCH, and MCHC were normally distributed and their means were used as the measure of central tendency. However, medians and 95% confidence interval (CI) were used for the serum ferritin and MCV, as their distributions were not normally distributed. The Pearson correlation coefficient test was used to determine the relationship between haematological and biochemical indices for continuous variables. A stepwise regression analysis was performed to ascertain the significance of dietary and non-dietary factors in relation to the iron status indicators. The dependent variable plasma ferritin was transformed (natural log) to improve the original skewed distribution. Results from the gender-specific models and gender-combined models analyses were expressed as standardised regression coefficients, which represent the predicted difference in the dependent variable corresponding to a difference of one standard deviation of the independent variable used. Statistical significance for all the tests was defined at $P < 0.05$. All statistical analyses were carried out using SPSS software (version 10.0; SPSS Inc., Chicago, IL, USA).

Results

The anthropometric characteristics and dietary intake of the adolescents are presented in Table 1. Female adolescents had significantly higher mean values for body mass index, whereas male adolescents showed significantly higher physical activity level and energy intake. The female adolescents showed a significantly higher intake of dietary vitamin A and iron than the males, with

Table 1. Physical characteristics and nutrient intake of adolescents ($n = 199$)

Characteristic	*Male (n = 94)*	*Female (n = 105)*
Age (years)	14.6 ± 1.8	15.7 ± 2.4[b†††]
Height (m)	1.5 ± 0.1	1.5 ± 0.1
Weight (kg)	41.6 ± 10.6	42.1 ± 7.5
Body mass index (kg/m^2)*	17.8 ± 3.0	18.9 ± 2.8[b††]
Household size	8.4 ± 2.6	7.8 ± 2.7
Household income per capita (RM**)	93.5 ± 53.6	98.9 ± 75.8
Physical activity (hours/week)	4.5 ± 2.9[b††]	3.2 ± 2.6
Total energy intake (MJ)	7.1 ± 0.9[b†††]	6.1 ± 1.0
% of RDA	68	68
Protein (g/MJ)	8.4 ± 1.0	8.6 ± 1.0
% of RDA	114	102
Vitamin A (μg RE/MJ)*	74.5 ± 23.6	81.2 ± 22.9[b†]
% of RDA	74	70
Vitamin C (mg/MJ)	11.8 ± 3.7	11.3 ± 4.6
% of RDA	294	241
Calcium (mg/MJ)	47.4 ± 14.0	50.6 ± 15.5
% of RDA	57	57
Iron (mg/MJ)	1.5 ± 0.3	1.6 ± 0.3[b††]
% of RDA	63	44
Iron of animal origin (mg/MJ)	0.4 ± 0.1	0.4 ± 0.1
Iron of plant origin (mg/MJ)	1.2 ± 0.2	1.3 ± 0.3

Data presented as mean ± standard deviation. RDA, Recommended Dietary Allowances.
*Analysis based on transformed data.
**RM3.80 = US$1.00.
[a]Significantly different between age group according to gender: [†††] $P < 0.001$, [††] $P < 0.01$ and [†] $P < 0.05$.
[b]Significantly different between gender: [†††] $P < 0.001$, [††] $P < 0.01$ and [†] $P < 0.05$.

the nutrients adjusted for energy intake. The mean energy and nutrient intakes were compared with the Malaysian Recommended Dietary Allowances (RDA) (Teoh, 1975), and showed that intake of all nutrients except for protein and vitamin C were below the Malaysian RDA levels. Dietary iron intake was unsatisfactory, with approximately 63% and 44% of the male and female adolescents, respectively, failing to meet the RDA. Three-quarters of the total dietary iron derived from plant-origin foods.

The mean Hb value for the males was 13.9 ± 1.3 g/dl with 9.5% having less than 12 g/dl, while the respective figures for the females were 12.4 ± 1.6 g/dl and 28.6% (Table 2). The mean serum ferritin concentrations for male and female adolescents were 21.5 µg/l (95% CI, 3.8–177.5) and 15.4 µg/l (95% CI, 1.6–43.2), respectively, with 25.7% of the males and 49.5% of the females having deficient levels of ferritin. Male adolescents in general showed a better iron status than the female subjects with a lower proportion having deficient levels for the various iron status indicators except haematocrit. Approximately one-half of the females had unsatisfactory levels of serum iron (51.6%), transferrin saturation (50.5%) and serum ferritin (49.5%) compared with their male counterparts. On the other hand, the MCHC among the male and female adolescents showed the lowest proportion with a deficient value (3% versus 7%, respectively) compared with other iron status indicators. Significantly higher values for the males were found in Hb, haematocrit, serum iron, TS and serum ferritin. In contrast, female adolescents had a significantly higher level of TIBC compared with their male counterparts. The iron status indicators namely, the concentrations of Hb, haematocrit, MCV, serum iron, and TS showed significant positive correlations with one another, and negative correlation with TIBC (Table 3).

In a stepwise regression analysis, dietary intake of total energy and iron, physical activity and gender emerged as the most important independent determinants of the serum ferritin level among the subjects, accounting for 30.2% of the variance (Table 4). Among the male adolescents only, dietary intake of energy, iron and protein, and the physical activity level were significant determinants, accounting for 43.8% of the variance in serum ferritin; while among the females, only total energy intake showed a significant effect, accounting for 12.5% of the variance.

The factors that showed a significant effect on Hb concentrations were gender, intake of energy and dietary iron for both genders, explaining about 40% of the total variance for Hb levels. For the male subjects, energy intake, age and household size showed significance; while for the females, intake of

Table 2. Blood biochemical characteristics of the male and female adolescents ($n = 165$)

	Male (n = 74)		*Female* (n = 91)	
Indicator	*Mean ± standard deviation*	*Number (%) deficient**	*Mean ± standard deviation*	*Number (%) deficient*
Hemoglobin (g/dl)	$13.9 \pm 1.3^{a\dagger\dagger\dagger}$	7 (9.5)	12.4 ± 1.6	26 (28.6)
Hematocrit (%)	$40.6 \pm 3.4^{\dagger\dagger\dagger}$	31 (41.9)	36.4 ± 4.2	35 (38.5)
Mean corpuscular volume** (fl)	80.6 (42.1 92.4)***	24 (32.4)	81.4 (46.8 90.7)***	38 (41.8)
Mean corpuscular haemoglobin (pg)	27.6 ± 2.2	25 (33.8)	26.7 ± 3.8	39 (42.9)
Mean corpuscular haemoglobin concentration (g/dl)	342.9 ± 12.3	3 (4.1)	339.6 ± 12.5	7 (7.7)
Serum iron (µmol/l)	$14.4 \pm 5.9^{\dagger\dagger\dagger}$	18 (24.3)	10.3 ± 5.6	47 (51.6)
Total iron binding capacity (µmol/l)	$63.0 \pm 14.3^{\dagger\dagger}$	12 (16.2)	68.8 ± 13.2	33 (36.3)
Transferrin saturation (%)	$24.6 \pm 14.4^{\dagger\dagger\dagger}$	12 (16.2)	15.9 ± 9.5	46 (50.5)
Serum ferritin** (µg/l)	21.5 (3.8 177.5)††† (22.3 ± 26.2)****	19 (25.7)	15.4 (1.6 43.2) (15.5 ± 21.3)	45 (49.5)

*Criteria for deficient values were explained in Methods.
**Analysis based on log-transformed data.
***Data presented as median (95% confidence range).
****Geometric mean ± standard deviation in parentheses
[a]Significantly different from females: $^{\dagger\dagger} P < 0.01$ and $^{\dagger\dagger\dagger} P < 0.001$; independent sample t-test.

Table 3. Correlation coefficients between the haematological indices (n = 165)

	Iron status indicator					
	Haemoglobin (g/dl)	*Haematocrit (%)*	*Mean corpuscular volume (fl)*	*Serum ferritin (μg/l)*	*Serum iron (μmol/l)*	*Total iron binding capacity (μmol/l)*
Hematocrit (%)	0.959[a++]					
Mean corpuscular volume (fl)*	0.584[++]	0.557[++]				
Serum ferritin (μg/l)*	0.588[++]	0.589[++]	0.562[++]			
Serum iron (μmol/l)	0.537[++]	0.509[++]	0.431[++]	0.551[++]		
Total iron binding capacity (μmol/l)	−0.334[++]	−0.309[++]	−0.310[++]	−0.541[++]	−0.198[+]	
Transferrin saturation (%)	0.503[++]	0.470[++]	0.381[++]	0.573[++]	0.809[++]	−0.560[++]

*Analysis based on transformed data.
[a]Two-tailed significance at [+] $P < 0.05$ and [++] $P < 0.01$.

energy and dietary iron were the significant factors, accounting for 32% and 33% of the variance, respectively. Dietary factors namely, total energy and dietary iron intake attributed to 34% of the variance in Hb levels, whereas gender only explained an additional of 6% of the variance in the sexes-combined model for Hb. The age at menarche of female adolescents was included in the analyses of these models, but it did not show any significant effect on either serum ferritin or Hb levels.

Discussion

The diet of the low-income rural community in Malaysia is typically plant-based with high fibre, phytate and polyphenol content, and low in the quantity of iron and animal protein. Thus, low bioavailability of dietary iron leading to iron deficiency is a common occurrence in rural populations, particularly in young children and pregnant women. This study sought to identify the determinants that affect iron status of rural adolescents, as this age group is less often studied.

The nutrient intakes of the adolescents were compared with the Malaysian RDA according to their age groups (Teoh, 1975), indicating that a large proportion of the adolescents showed an inadequate intake of energy and selected minerals and vitamins except for protein and vitamin C intake. The high intake of raw local fresh fruits, vegetables and tubers products in the study area throughout the year may be responsible for the high intake of vitamin C. Although several studies have been suggested that vitamin C is regarded as an effective enhancer of non-haeme iron absorption (Bothwell, 1995), there was no significant determinant of vitamin C on iron status when all nutrients were included in the analysis. One possibility that can be suggested is that the diets in the present study were largely derived from plant-based foods high in fibre, phytates and polyphenols that can counteract the positive effect of vitamin C on iron status, especially when its level is high (Bothwell, 1995), by forming an insoluble complex with ferum in the intestinal lumen, which inhibits the iron absorption (Hurrell *et al.*, 1999). Another likely possibility is that vitamin C is considerably destroyed by heating and oxidation during food preparation and processing (Hallberg & Rossamder-Hulten, 1982) and during prolonged storage (Hurrell, 1991). For instance, cabbage was cooked for 3–4 min, which reduced the ascorbic acid content by one-half (Tuntawiroon *et al.*, 1990). Thus, estimates of vitamin C should be based on the actual amount contained in the food as eaten and not in the raw materials.

Positive significant correlations were found among the biochemical and haematological indices of iron status. Total energy and iron intake played a significant and positive effect on body iron stores. These results are in agreement with other studies on iron status in adolescents (Lee *et al.*, 1999), young adults (Milman *et al.*, 1998) and the elderly (Joo *et al.*, 2000). Similar results were also reported in children, women of childbearing

Table 4. Multiple regression models predicting serum ferritin and haemoglobin

	Regression coefficient	*Standard error (SE)*	*Standardised coefficient, β*	*P value*
Serum ferritin (μg/l)				
Male, model I (all characteristics)		Adjusted R^2 = 0.438, SE = 0.2467		
Total energy intake (MJ)	0.194	0.031	0.570	0.000
Total dietary iron (mg/MJ)	0.252	0.106	0.214	0.021
Protein (g/MJ)	0.068	0.031	0.196	0.034
Physical activity (hours/week)	−0.036	0.011	−0.286	0.002
(Intercept)	0.840	0.392		
Female, model II (all characteristics)				
Total energy intake (MJ)	0.132	0.039	0.370	0.001
(Intercept)	0.314	0.245		
Genders combined, model III (all characteristics)		Adjusted R^2 = 0.302, SE = 0.2123		
Total energy intake (MJ)	0.138	0.026	0.417	0.000
Physical activity (hours/week)	−0.027	0.010	−0.198	0.006
Total dietary iron (mg/MJ)	0.266	0.091	0.218	0.004
Gender (0 = female; 1 = male)	0.134	0.061	0.180	0.031
(Intercept)	−0.064	0.192		
Haemoglobin (g/dl)				
Male, model I (all characteristics)		Adjusted R^2 = 0.323, SE = 1.096		
Total energy intake (MJ)	0.342	0.147	0.248	0.023
Age (years)	0.172	0.085	0.217	0.048
Household size	−0.167	0.051	−0.334	0.002
(Intercept)	10.328	1.504		
Female, model II (all characteristics)		Adjusted R^2 = 0.330, SE = 1.270		
Total energy intake (MJ)	0.521	0.140	0.368	0.000
Total dietary iron (mg/MJ)	1.821	0.491	0.368	0.000
(Intercept)	6.179	1.014		
Genders combined, model III (all characteristics)		Adjusted R^2 = 0.401, SE = 1.258		
Total energy intake (MJ)	0.553	0.103	0.386	0.000
Gender (0 = female; 1 = male)	1.144	0.240	0.354	0.000
Total dietary iron (mg/MJ)	1.144	0.359	0.216	0.002
(Intercept)	7.084	0.771		

age and free-living elderly in developed and developing countries (Preziosi *et al.*, 1994; Fleming *et al.*, 1998; Backstrand *et al.*, 2002).

The adolescents may be described as being moderately active. Their physical activity level (expressed as hours per week) was shown to have an inverse relationship with the serum ferritin level, especially among the male adolescents. Several studies have shown that intense physical activity may further exacerbate suboptimal iron nutrition, especially in athletes (Lampe *et al.*, 1986; Haymes & Spillman, 1989; Weaver & Rajaram, 1992). It is hypothesised that intense physical activity increases iron losses through sweat and increased turnover rates of red cell iron (Weaver & Rajaram, 1992).

Several nutritional surveys in rural Malaysian communities showed that socio-economic variables such as household income and family size influence the nutritional status of children (Norhayati *et al.*, 1997; Marjan *et al.*, 2002). This study also found household size to have a significant and negative effect on haemoglobin levels, especially for male subjects. Families of low socio-economic status with many growing children typically receive less food, leading to a poor-quality diet that is deficiency in energy and essential nutrients (Bhargava *et al.*, 2001).

Gender was found to exert a strong influence on serum ferritin and Hb levels. Males have higher levels of serum ferritin and Hb than female subjects, after adjustment for dietary and non-dietary factors. This finding is consistent with previous studies (Samuelson *et al.*, 1996; Milman *et al.*, 1997), indicating that male adolescents have a significantly better iron status than their female counterparts.

It is well known that iron loss via menstrual bleeding is an important contributor to iron deficiency (Fogelholm *et al.*, 1993; Milman *et al.*, 1998). However, in the present study no such association was discernible for the female subjects. This may be due to the wide range for the age of menarche and small sample size.

It is proposed that prevention strategies for IDA should include targeting adolescents, due to their high physical activity and physiological requirements for iron. Targeting adolescents at risk of IDA before childbearing should be part of an ongoing effort to address the problem of anaemia during pregnancy and infancy (Kanani & Poojara, 2000; Zavaleta *et al.*, 2000). Supplementation programmes given through the schools may be a viable intervention. Based on the result from a randomised trial study on female adolescents in a school setting in Malaysia, Tee *et al.* (1999) recommended weekly supplementation of iron and folate as a safe, effective and inexpensive procedure to improve the long-term iron nutrition of female adolescents.

Acknowledgements—This study was funded by the IRPA Programme of the Ministry of Science, Technology and Environment of Malaysia. Appreciation is extended to the Director and staff of Sabah State Health Department, staff of the Hospital Tuaran, District Health Office of Tuaran, Clinical Biochemistry Unit of the Pathology Department, Hospital Queen Elizabeth, and Luyang Polyclinic, Kota Kinabalu, and others for their invaluable support throughout the study. Sincere gratitude is also expressed to all the subjects and their parents for their cooperation.

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