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Status of Thiamin deficiency in boarding school children from seven districts in Bhutan with previous history of peripheral neuropathy outbreaks: a cohort study

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ABSTRACT

Introduction: Peripheral neuropathy outbreaks have been a common occurrence amongst boarding schoolchildren from seven districts in Bhutan. Thiamin deficiency has always been suspected to be the cause but the status of the vitamin has never been established. This study aims to find the status of thiamin and dietary intake of micronutrients in boarding schoolchildren from seven districts with previous history of peripheral neuropathy outbreaks. **Methods:** Whole blood thiamin and dietary intake of micronutrients were assessed in 448 school children for four study periods (SP). Baseline data (SP1) was collected when the school children just joined the school at the start of the school aca- demic year. SP2 was the first half of the school year and the data was collected just before the midterm break. SP3 was the short summer break and SP4 the second half of the school academic year. **Results:** 50.58% of the school children were found to be thiamin deficient at baseline which increased to 90.1% in SP2. The percentage of thiamin deficient school children increased to 91.8% in SP3 and then decreased to 79.82% in SP4. The requirements for vitamin B1, B12, vitamin A and iron were never met by dietary intakes in all the study periods. **Conclusions:** In conclusion, this study found a high prevalence of Thiamin deficiency in schoolchildren at baseline and the number of school children with Thiamin deficiency increased when in schools. The school children also had inadequate dietary intake of many micronutrients.

Keywords: Beriberi; Micronutrient deficiency; Outbreak; Peripheral neuropathy; Thiamine.

INTRODUCTION

Thiamin or Vitamin B1 is a water soluble vitamin of the B-complex group. The human body does not produce endogenous Thiamin and dietary sources include animal sourced proteins, whole grain cereals, beans and nuts. The human body requires a minimum of 0.33 milligrams of Thiamin for every 1,000 kilocalories consumed[1]. The half-life of Thiamine is between 9 to 18 days, and as a result, body stores can deplete within two to three weeks[2]. Deficiency of Thiamin will cause a disease called beriberi; if the deficiency is accompanied with nervous system involvement then it is termed as dry beriberi which is characterized by a variety of symptoms like paresthesia of the extremities, weakness, wasting of muscles and peripheral neuropathy. On the other hand if the Thiamine deficiency has cardiovascular involvement, it is then called as wet beriberi. Wet beriberi is characterized by edema (especially of the legs, but also involving the trunk and the face), high cardiac output, ventricular failure, sinus rhythm, dilatation of arterioles and pulmonary congestion with pleural effusions. In wet beriberi death from congestive heart failure may occur abruptly[3].

In December of 2011, beriberi was suspected to be the cause of two deaths among the school children from Orong higher secondary school, located 65 km from Eastern Indo-Bhutan border town of Samdrup Jongkhar, following an outbreak of peripheral neuropathy (PN)[4]. PN outbreaks have been a common occurrence in Bhutan, especially among boarding school children from seven high risk districts[5].

Although, micronutrient deficiency, especially Thiamin deficiency, has been suspected to be the cause of PN outbreaks among school children[4,5]; the status of the vitamin has never been established. Therefore, this study was planned with aims to find the status of Thiamin in the boarding schoolchildren from districts of Bhutan with previous history of PN outbreaks. The results of the study would be used as evidence to form strategies to prevent PN outbreaks in the country.

METHODS

Subjects

To get the maximum represented sample size for this study, the following formula was used. A confidence interval of 95% was decided, with margin of error 0.05. Since, such a study on PN was being conducted in the country for the first time, variance (or standard deviation) was not available, and therefore a most forgiving number 0.5 was taken as the standard deviation.

$$n = \frac{Z_\alpha^2 * \sigma(1-\sigma)}{\epsilon^2}$$

Where:

n = Sample size required
Z_α = 1.96 (Taking 95% confidence interval)
σ = Standard deviation (0.5)
ϵ = Margin of Error (0. 05)

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Allowing for a 15% dropout rate the sample size was estimated to be about 450. With 2 refusing to participate in the study after selection, the final sample size became 448. A total of 448 Bhutanese school children were studied from February till November of 2014 from seven districts of Chukha, Lhuntse, Mongar, Pema Gatshel, Samdrup Jongkhar, Trongsa and Zhemgang; with previous history of PN outbreaks. The schoolchildren were divided from among the selected districts based on proportion of boarding students to the total boarding students in each district. The proportions of girls and boys in each school were also calculated and probability proportional to size sampling (PPS) technique was employed to select 19 schools from the selected districts. A minimum of 20 school children aged 9-22 years were selected from each school based on simple random sampling technique. Ages 9-22 years were taken as selection criteria as most previous PN outbreaks occurred in this age group, while school children with acute and chronic diseases including those on multivitamin supplements were excluded from the study.

Study design

The study was designed to collect data from four study periods (SP) spread through the school academic year to obtain the status of the vitamin from the two halves of the school academic year as well as from the short midterm break. Baseline data, connoted as SP1, was obtained in February when school children first joined their schools. SP2 was the first half of the school academic year and the data for this period was collected when the school children were just about to leave for the summer break in late May. SP3 was the period during the school children's short mid-term break and the data for this study period was collected when the school children had just arrived from their mid-term breaks in mid July. SP4 was the second half of the school academic year and the data for this study period was collected just before the commencement of their final exams in November.

Data collection and biochemical process

Ethical clearance was sought from Research Ethical Board of Health (REBH). Questionnaires on the subject's personal and family information including income source, supporter type and family size were completed by health workers trained as enumerators for the study. Dietary information was taken using 24 hr recall method by trained dieticians. Height and weight were measured from all the subjects.

Fasting blood sample was collected by lab technicians from all the subjects in all the study periods. Whole blood was collected into EDTA tubes and transported to public health laboratory maintaining unbroken cold chain during transportation. Whole-blood B1 was measured by HPLC-based method (Immundiagnostik AG, Bensheim, Germany) with the reference range for normality as 32–95 ng/mL[6].

Statistical analysis

Data was entered in EPIDATA software version 3.1 and transferred to R version 3.1.1 and STATA version SE 12 for final analysis. Results are presented as means and percentages. Student's t-test for mean comparison between 2 groups and one-way analysis of variance (ANOVA) were used for comparisons between groups. Thiamin deficiency was defined as blood thiamin concentration < 32 ng/mL. A *p*-value <0.05 (two sided) was considered significant.

RESULTS

A total of 448 school children consented to participate in the study as shown in Table 1. of which, slightly over half (55.6 %) were male. The average age of participants was 15.9 years. 78.8 % of the participants received support (supports for children's

Table 1. Socio-demographic characteristics

Characteristics	n	% /Average
Total	**448**	**100**
Gender		
Male	249	55.6%
Female	199	44.4%
Age (mean years)	448	15.9 (SD=2.684)
Male (years)	249	16.1 (SD=2.836)
Female(years)	149	15.6 (SD=2.462)
Supporter		
Both parents	353	78.8 %
Single parent	80	17.9 %
Others	15	3.4 %
Education of supporter		
None	252	56.3 %
Non-formal/Religious	96	21.4 %
Primary and Less	65	14.5 %
Secondary and Above	35	7.8 %
Main work of the supporter		
Salaried	53	11.8 %
Self-employed	153	34.2 %
Non-salaried	242	54 %

Table 2. Status of Thiamin Deficiency through the study periods

Characteristics		Study Period 1	2	3	4	ANOVA (*p*-Value)
Thiamin Deficiency	Percentage	50.58	90.13	91.8	79.82	-
	95%CI	(46.85 , 54.3)	(87.13 ,93.13)	(89.03,94.57)	(76.27,83.38)	
Thiamin (ng/ml)	mean	32.61	23.12*	23.57*†	27.94*‡	
	95%CI	(31.74 ,33.47)	(22.5 , 23.74)	(23 , 24.15)	(27.21 , 28.67)	0.0000

** Significant at p-value 0.05 compare to Study Period 1, † Significant at p-value 0.05 compare to Study Period 2 & ‡ Significant at p-value 0.05 compare to Study Period 3*
***Note:** Significance test was not conducted between Study Period 2 and 4*

learning in terms of monetary support and involvement in the children's development) from both parents and a majority of the participants reported their supporters as either self employed (34.2 %) or non-salaried (54%) for the main work.

In the first study period half the participants (50.58 %) were found to be deficient in thiamin as shown in Table 2. In the second study period, the number of participants who were deficient in thiamine rose to 90.1% remaining almost the same in the third study period (91.8%). The fourth study period saw a small decrease in the number of participants with thiamin deficiency ultimately reaching to 79.82%.

When the means of whole blood thiamin level were compared through the study periods using ANOVA, significant differences were found within the groups. Significance test between two study periods was conducted using students t-test; where a significant decrease in the blood thiamin from the first study period (32.61 ng/ml, 95% CI: 31.74-33.47) to the second study period (23.12 ng/ ml, 95% CI 22.5-23.74) was observed.

Table 3. Dietary intake of nutrients through the study periods

	Req.		Study Period 1	2	3	4	ANOVA P-Value
			Food Recall				
Calories (Kcal)	2100	mean	2554	2387.76*	2533	2447*	0.4436
		95%CI	(2472 , 2637)	(2109 , 2666)	(2306 , 2760)	(2278 , 2616)	
Proteins (g)	48	mean	54.24	47.89*	48.76*†	48.32*	0.0000
		95%CI	(52.92 , 55.56)	(46.98 , 48.8)	(47.46 , 50.07)	(47.72 , 48.91)	
B1 (mg)	0.9	mean	0.77	0.65*	0.71*†	0.57*‡	0.0000
		95%CI	(0.74 , 0.81)	(0.63 , 0.66)	(0.67 , 0.75)	(0.56 , 0.58)	
B6 (mg)	0.9	mean	1.41	1.09*	1.27*†	1.14*‡	0.0000
		95%CI	(1.33 , 1.48)	(1.07 , 1.12)	(1.21 , 1.33)	(1.12 , 1.16)	
B12 (mcg)	0.9	mean	0.61	0.11*	0.42*†	0.07*‡	0.0000
		95%CI	(0.54 , 0.69)	(0.09 , 0.13)	(0.36 , 0.48)	(0.06 , 0.07)	
Iron (mg)	22	mean	6.54	7.19	5.82*†	7.08‡	0.0000
		95%CI	(6.3 , 6.78)	(7.03 , 7.36)	(5.59 , 6.06)	(6.57 , 7.59)	
Folate (mcg)	160	mean	149.77	239.7*	121.23*†	203.39*‡	0.0000
		95%CI	(141.21, 158.34)	(232.37, 247.03)	(112.56, 129.89)	(198.61, 208.17)	
Vit. A (mcg)	500	mean	104.93	94.89*	113.98†	110.86	0.0000
		95%CI	(99.69, 110.16)	(91.67, 98.11)	(108.13, 119.84)	(107.44, 114.28)	

** Significant at p-value 0.05 compare to Study Period 1, †Significant at p-value 0.05 compare to Study Period 2 & ‡ Significant at p-value 0.05 compare to Study period 3.*
***Note:** Significance test was not conducted between Study Period 2 and 4.*

There was a small but statistically significant rise in the blood thiamin level in the third (23.57 ng/ml, 95% CI 23- 24.15) and fourth study periods (27.94 ng/ml, 95% CI 27.21-28.67) respectively. However, when the blood thiamin level was compared between the first and fourth study period, a significant decrease in the blood thiamin level was seen in the fourth study period.

Mean dietary intake of nutrients through the study periods are shown in Table 3. ANOVA test showed significant differences within the groups for intake of dietary nutrients except for calories (p= 0.4436). Dietary intake of B1 and B12 decreased to 0.65 mg (95% CI 0.63-0.66) and 0.11 mcg (95% CI 0.09-0.13) in the second study period from 0.77 mg (95% CI 0.74-0.81) and 0.61 mcg (95% CI 0.54-0.69) respectively. A small but statistically significant increase in the levels of both the vitamins occurred in the third study period (B1 0.71mg, 95% CI 0.67-0.75; B12 0.42 mcg, 95% CI 0.36-0.48) before decreasing again in the fourth study period (B1 0.57mg, 95% CI 0.56-0.58; B12 0.07 mcg, 95% CI 0.06-0.07). Calories and proteins also showed a similar pattern. On the other hand Iron and folate increased to 7.19 mg (95% CI 7.03-7.36) and 239.7 mcg (95% CI 232.37-247.03) in the second study period from 6.54 mg (95% CI 6.3-6.78) and 149.77 mcg (95% CI 141.21-158.34). In the third study period iron decreased to 5.82 mg (95% CI 5.59- 6.06) before increasing to 7.08 mcg (95% CI 6.57-7.59) in the fourth study period. Similarly folate decreased to 121.23 mcg (95% CI 112.56-129.89) in the third study period and increased to 203.39 mcg (95% CI 198.61-208.17) in the fourth study period. Significance test was not conducted between study periods 2 and study periods 4 as it did not serve any purpose.

When the dietary intake of nutrients were compared with recommendations set by joint FAO and WHO recommendations for recommended

The requirements are based on the joint FAO/WHO recommendation for recommended mean daily per capita nutrient intakes to prevent malnutrition in emergency situation[7]. The actual Recommended Daily Allowances (RDA) are higher, mean daily per capita intakes to prevent malnutrition in emergency situation only the requirements for calories, proteins and vitamin B6 were met in all the study periods7. The requirements for vitamin B1, B12, vitamin A and iron were never met.

Almost all the participants were either normal weight {88.98% (95% CI 85.45, 92.5)} or overweight {10.49% (95% CI 7.05, 13.94)} in SP 1 as given in Table 4. Their weight status did not change in SP4 remaining constant through all the study periods.

Table 4. Weight status

Characteristics		Study Period 1	Study Period 4
Over Weight	Percentage	10.49	10.58
	95%CI	(7.05 , 13.94)	(6.61 , 14.54)
Normal	Percentage	88.98	88.69
	95%CI	(85.45 , 92.5)	(84.62 , 92.75)
Under Weight	Percentage	0.53	0.74
	95%CI	(-0.38 , 1.45)	(-0.28 , 1.76)

Note: *weight status was calculated using WHO's BMI for age growth chart*

DISCUSSION

The outbreak of PN in the country is a big public health concern. Although never confirmed by laboratory tests, the outbreak investigations that followed have always suspected the cause of the outbreaks to be from deficiency diseases due to inadequacy in the dietary intake of micronutrients, particularly vitamin from the B complex group. The deficiency of Thiamin has caused beriberi outbreaks in many countries[8-12]. The PN outbreaks in Bhutan could very much be beriberi outbreaks, this study's purpose was to find the status Thiamin deficiency among the boarding school children the high risk districts.

The most important finding from the study is the significant rise in the number of students with subclinical Thiamin deficiency in schools. About 90% of the students became thiamin deficient before the mid-term breaks. The status of Thiamin did not change during the two weeks midterm break, with the number of students with Thiamin deficiency remaining almost the same. However, in the second half of the school academic year, the percentage of students with Thiamin deficiency decreased to about 80%. This slight improvement in the Thiamin deficiency status in the school children could probably be explained by the interventions undertaken by Ministry of Education to improve their school feeding program. This period coincided with the implementation of the centralized procurement system (CPS) from July 2014, where 60 % of the student's Nu 1,000 stipend was released to Food Corporation of Bhutan (FCB) to supply 9 non- perishable food items (Rice, Oil, Pulses, Chickpeas, Soya meat, milk powder, sugar, salt and tea leaf). The remaining 40 % of the student's stipend were released to their respective schools for procurement and supply of perishable foods[13]. Despite the efforts by Ministry of Education to improve the school feeding program, this study indicated that thiamin nutrition of the school children were still far from adequate.

The Thiamin deficient school children in this study was higher than that found in a Taiwanese elementary school children with respective prevalence rates of marginal and deficient thiamin states at 10.4% and 7.8% for boys and 9.3% and 7.3% for girls[14]. These differences could be explained by the differences in the dietary pattern of the school children from two countries. In the diet of the Taiwanese school children, pork and its products contributed 35% of Thiamin, followed by rice and its products (12.1%) and soybeans and soy products (10.8%). Each of the remaining food categories provided less than 5% of the Thiamin to the diet of school children[14]. The school children of this study on the other hand, had generally very low intake of Thiamin from dietary sources. The average dietary Thiamin intake was 0.65 and 0.57 mg/day in the first and second half of the school academic year respectively against the requirement of 0.9 mg/ day set by FAO/WHO for emergencies[7]. However, average Thiamin intake was only slightly better with 0.77 mg/ day at baseline and 0.71 mg/day during the midterm breaks.

Although, this study was not powered to represent the whole of Bhutanese population, Thiamin could be a big problem in the general population as half the participants of this study were already deficient in the nutrient when they entered the study. A larger population based study is required to validate the findings of this study

An interesting observation from this study is the prevalence of micronutrient deficiencies in the school children despite being either normal or overweight. Their weight remained constant throughout the study periods. The presence of the micronutrient deficiencies in these apparently health school children are an indication that the problem lies in micronutrient nutrition rather than the total calorie consumption. The total energy intakes by the students were statistically not significant throughout the study periods. However, the mean dietary intake of B vitamins, Iron and vitamin A were all below the minimum requirements in all the study periods. In a review of micronutrient malnutrition by Ramakrishan in 2002[15], developing countries of South East Asia and Sub Saharan Africa were found to be particularly high in the prevalence of these deficiencies. The authors acknowledged the public health concerns caused by deficiencies of micronutrients including zinc, folate and the B vitamins and suggested a need for simple approaches that evaluate and address multiple micronutrient malnutrition.

There are few limitations to the study. First, this study was conducted on only boarding school children from seven districts with previous history of peripheral neuropathy outbreaks, so this population cannot be considered representative of all school children. Secondly, dietary information was collected using 24 hour recall method, which are susceptible to recall biases as they are memory dependent. Thirdly, since this study was the first of its kind in Bhutan, a reference interval of Thiamin level for Bhutanese has never been set; therefore this study used the reference interval given by Immundiagnostik AG, Bensheim, Germany to define Thiamin deficiency. Never the less, Thiamin deficient school children increased during the first and second half of the school academic year when compared with the baseline.

CONCLUSIONS

In conclusion, this study found a high prevalence of Thiamin deficiency in school children from the seven districts in Bhutan with previous history of PN outbreaks. The deficiency of Thiamin got worse when in school. In addition the school children had inadequate dietary intake of micronutrients.

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AUTHORS CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

LD: Concept, design, literature search, data collection and analysis, manuscript writing and review.

TD: Concept, design and review

DP: Analysis, design and literature review

GPD: Concept, design and review

PY: Design, analysis, manuscript writing and literature review

KW: Design, and literature review

Author agree to be accountable for all respects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

None

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