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ABSTRACT:
Thiamine deficiency may be associated with severe acute malnutrition (SAM) and contribute to its clinical signs and complications. This hospital-based prospective study aimed to determine if thiamine deficiency is present in children with SAM in Port Moresby General Hospital (PMGH). Convenient sampling was used to select 208 children aged between 2 months and 13 years (median age 15 and IQR 11-32 months) presenting to the Children's Emergency Department requiring venepuncture for routine testing who had additional blood taken for assay of thiamine levels. Nutritional status was determined using the WHO weight for height (WFH) z-scores. Details of socioeconomic background and diet were recorded. Thiamine levels were determined using enzyme-linked immunosorbent assay (ELISA) test kits. A total of 158 (76%) of the 208 samples were satisfactory for analysis; 86 (54%) from children with normal WFH, 26 (17%) from those with moderate malnutrition and 46 (29%) from those with severe malnutrition. Thiamine levels were normally distributed overall. The mean was 34.18 $\pm$ 5.8 ng/ml. This was within the reference level of 16-48ng/ml. There was no statistically significant difference in levels between the normal, moderately and severely malnourished children (33.6 $\pm$ 5.6, 35.3 $\pm$ 5.7 and 34.4 $\pm$ 5.7 ng/ml). The assay of thiamine levels in serum using ELISA is not the best method for determining thiamine deficiency and further studies using whole blood and high-performance liquid chromatography are needed.

Keywords: Thiamine deficiency, children, severe malnutrition, serum, micronutrients

INTRODUCTION:

Severe acute malnutrition (SAM) in children under 5 years of age was the second commonest cause of admission to hospitals in Papua New Guinea (PNG) in 2019. It was either a direct cause of admission or an associated morbidity in 8.1 % of children with a case fatality rate of 10.4% [1].

SAM is defined by a very low weight for height (<-3 z-scores of the median WHO growth standards) and/or by presence of nutritional oedema (kwashiorkor; defined as bilateral pitting oedema) [2]. SAM is caused by

macronutrient deficiency, although micronutrient deficiency is almost always present as well [3]. Heart failure, shock, metabolic disturbances, sepsis, and severe oedema are common complications [4,5].

Thiamine (vitamin B1), a water-soluble vitamin is an essential micronutrient. In its active form thiamine diphosphate (also known as thiamine pyrophosphate or TPP) functions as a vital co-factor to several important enzymes in cellular metabolism [5] and its deficiency may well contribute to the complications of severe malnutrition like heart failure, sepsis, oedema, and refeeding syndrome commonly seen in PNG. Although the prevalence of thiamine deficiency is not well documented worldwide, studies done in Jamaica and Ghana showed thiamine deficiency in 37% and 40% of malnourished children respectively [6,7]. A study from Cambodia reported a prevalence of thiamine deficiency of 38% in children aged 6-12 months using the most conservative cut off level [8]. In contrast malnutrition was not associated with low thiamine levels in a study of children admitted to an intensive care unit in Brazil [9].

In PNG, thiamine deficiency was documented in 6% of boarding school children in the southern region [10]. No studies have been done to document thiamine levels in malnourished children in PNG.

This study aimed to determine the thiamine levels in severely malnourished children admitted to Port Moresby General Hospital (PMGH). This is important because if thiamine deficiency is common this will have implications for the standard management of children with SAM.

METHODOLOGY:

This was a prospective descriptive hospital-based study carried out at the PMGH Paediatric Department. This hospital is a tertiary referral hospital, receiving referrals from other provincial centres as well as the National Capital District (NCD), Central and Gulf provinces. PMGH admits over 4200 children per year.

Children between 2 months and 13 years of age were enrolled by convenience sampling at presentation to the Children's Emergency Department. Those who had SAM who were already on ready to use therapeutic food (RUTF) and/or milk feeds (F75 or F100) that contain thiamine were excluded from the study.

On presentation, each child had anthropometric measurements of weight, height or length, mid-upper arm circumference, and head circumference taken [11].

The nutritional statuses of the children were determined using the WHO Anthro Calculator for children less than 2 years of age, and the standard WHO Weight for Height Charts for children above 2 years [11,12]. They were then categorized as normal, moderate malnutrition or SAM:

- Normal: a WFH/L between -2 & +2SD

- Moderate malnutrition: a WFH/L between -2 & -3SD is moderate malnutrition
- SAM: a WFH/L <-3SD, and/or presence of oedema

Written informed consent to participate in the study was obtained from the parent or guardian. A questionnaire was used to collect their demographic details including a dietary history based on the food consumed in a typical day. Clinical findings such as skin and hair changes were recorded. The maternal consumption of substances potentially interfering with their children's thiamine levels was also noted.

Venous blood was collected from the child during cannulation or when routine investigative blood tests were done for the child. None of the children had venepuncture done separately or only for the purpose of this study.

For the assay of thiamine, 1.5-2ml of venous blood was placed in a plain sterile labelled microtainer. The blood was allowed to clot before being placed in a microtainer-box inside a cool-box kept at a temperature of 4-8 degrees C and transported to the Micronutrient laboratory. The blood samples were centrifuged for 10 minutes at about 10 degrees Celsius. Each serum sample was separated into a properly labelled sterile eppendorf vial which was securely closed and stored at -20 degrees C until analysis.

Thiamine levels in serum were assayed using the Aviva Systems Biology Vitamin B1 Enzyme-Linked Immunosorbent Assay (ELISA) kits [13]. Data was entered into a Microsoft Excel spreadsheet and descriptive statistics determined using Stata V.14 and IBM SPSS statistical software.

Ethical clearance was given by the University of Papua New Guinea (UPNG) School of Medicine and Health Sciences (SMHS) Research and Ethics Committee and the study was approved by the PMGH Director of Medical Services

RESULTS:

A total of 208 patients aged between two months and 13 years (median age 15 and IQR 11-32 months) were recruited for this study between June and August 2021. The 208 adequately documented blood samples were sent to the micronutrient laboratory. Of the 208 serum samples obtained, 50 samples were deemed unsuitable for analysis due to various reasons (insufficient and/or hemolysed samples).

Of the 158 serum samples 54.4% (n=86) were from normally nourished children, and 16.5% (n=26) from children with moderate malnutrition (z-score between -2 & -3 SD), and 29.1% (n=46) from children with SAM. The median (IQR) age of the 158 children was 15.5 (12-36) months. Sixty five (median age 12 [9-16] months) were receiving breast milk. The

median age of the 93 not receiving breast milk was 24 (12-48) months.

The overall mean serum thiamine level in the 158 children was 34.1ng/ml and standard deviation (SD) was 5.7ng/ml (minimum 20ng/ml and maximum 49ng/ml). The summary statistics of the thiamine levels in serum from children in the three groups are presented in Table 1. There was no statistically significant difference in thiamine levels between the three groups.

Table 1: Serum thiamine levels (ng/ml) in normally nourished, moderately malnourished, and severely malnourished children

Parameters	**Normal status (N = 86)**	**Moderate status (N = 26)**	**Severe status (N = 46)**
Mean	33.6	35.3	34.4
SD (Standard deviation)	5.6	5.7	5.7
95% CI (Confidence Interval)	32.4- 34.8	33.0 – 37.6	32.7 – 36.8
Range	20.1 – 48.1	26.2 – 47.1	25.6 – 49.0
Median	33.8	34.2	34.0
IQR (Interquartile range)	29.7 – 36.7	31.6 – 39.1	29.0 – 37.1

141 (89%) of the 158 children were living in the Port Moresby suburban area and 88 (56%) were males.

The mean thiamine levels in the severely malnourished children were similar in those breast feeding (33.9 $\pm$ 5.0ng/ml) and not breastfeeding (34.7 $\pm$ 6.3ng/ml). The solid diet mostly consisted of vegetables, fruits, washed white rice, and some animal protein when available. Fortification of all rice sold in PNG is a legal requirement. Cooking practices most often involved washing the white rice before cooking - a practice that results in significant loss of the fortificants in white rice, which include thiamine. None of the mothers practiced changing of rice water during the rice-cooking process.

The family's consumption of different foods on a typical day and the thiamine levels of the children regardless of their nutritional status are shown in Table 2. The serum thiamine levels are similar. Thiamine levels are within the normal reference range regardless of diet.

Table 2: Mean serum thiamine levels in children from families that consumed and those that did not consume food items listed on a typical day

Food Items	Consumed		Not Consumed	
	N = 158			
	N (%)	Thiamine (ng/ml)	N (%)	Thiamine (ng/ml)
Fruits	110 (69.6)	34.10	48 (30.4)	34.02
Vegetables	131 (82.9)	33.88	27 (17.1)	35.03
Unwashed rice	102 (64.6)	33.71	56 (35.4)	34.76
Washed rice	31 (19.6)	34.05	127 (80.4)	34.09
Brown rice	3 (1.9)	32.42	155 (98.1)	34.11
Meat	14 (8.9)	35.23	144 (91.1)	33.97
Fish	48 (30.4)	33.86	110 (69.6)	34.18
Eggs	89 (56.3)	34.07	69 (43.7)	34.09
Biscuits	118 (74.7)	33.86	40 (25.3)	34.13
Processed snacks	54 (34.2)	33.65	104 (65.8)	34.30

Most of the mothers (95%, n=150) in this study chewed betelnut on a daily basis some up to or more than 5 betel nuts in a day. A large proportion of these mothers drink 1-2 cups of strong tea or coffee in a day (95%, n=150). In our sample, there were no significant effects of these consumption practices in mothers on thiamine levels in their children; however the comparator samples were small.

None of the children had clinical signs of beriberi or thiamine deficiency on admission.

DISCUSSION:

Several thiamine reference ranges have been published [7-9], with different metrics and measurement methods – including erythrocyte transketolase pyrophosphate effect, erythrocyte thiamine diphosphate concentrations, and whole blood thiamine level. Our study found the mean (SD) serum thiamine level in the 158 children was 34.18 (5.8) ng/ml (minimum 20.1, maximum 49.3). This was within the reference range for whole blood thiamine levels used in the Brazilian study using a high-performance liquid chromatography (HPLC) assay on whole blood: 16–48 ng/mL [9].

In our population sampled there was no difference in serum thiamine levels between well-nourished and severely malnourished children and all levels found were within the normal range. However, it is important to understand a major limitation of our study - that we measured the levels of thiamine detected by an ELISA assay on serum. This is not the ideal assay method and has a poor sensitivity [14 - 16]. Less than 10% of whole blood thiamine is contained in plasma [14 - 16] and plasma thiamine concentration reflects recent intake

rather than body stores. Whole blood thiamine assay method is more accurate, as 90% of thiamine in the body is in the form of thiamine diphosphate (TDP) in red blood cells. This is ideally measured by HPLC, which is expensive and was not readily available [14].

To our knowledge, there have been no other studies of thiamine levels in serum using ELISA. In studies from Jamaica reporting subclinical thiamine deficiency in 7% of normal children and 37% of malnourished children and from Ghana which reported thiamine deficiency in 40% of malnourished children, the erythrocyte transketolase assay was used [6,7]. A Brazilian study using HPLC to measure whole-blood thiamine concentrations in children admitted to an intensive care unit found no association of low thiamine levels with malnutrition [9]. There was however an association with levels of C-reactive protein, an inflammatory marker. An ultra-HPLC assay was used to measure thiamine levels in red cells in the Cambodian study which reported low thiamine levels in 38% of malnourished children aged between 6-12 months [8]. Accurate measurement of thiamine levels is clearly difficult and it is perhaps not surprising that there is a scarcity of literature on the subject. Thiamine deficiency is generally diagnosed based on the classical clinical findings but the relation of subclinical thiamine levels to the morbidity and mortality associated with malnutrition remains unclear.

Betel nut, strong tea and coffee are known to contain thiaminase with the potential to lower thiamine levels [17]. Betel nut chewing is a common social habit in PNG. Our study was not designed to examine the effect of these thiaminase containing substances on the levels of thiamine in breast milk and the children of breast feeding mothers.

Whilst acknowledging that the method used for measurement of thiamine levels was not ideal, none of the children exhibited clinical signs of thiamine deficiency. It is known that increased intestinal reabsorption of thiamine takes place under deficient conditions [18]. This mechanism may be operating in our study population. It is also possible that the diet in this predominantly urban population provides adequate amounts of thiamine. However, in the only other study of thiamine levels in the PNG population, 6.4% of boarding school students had marginal to severe deficiency detected with HPLC measurement of thiamine pyrophosphate in whole blood [10].

CONCLUSION:

Our study which used an ELISA assay for measuring thiamine levels in serum did not show a difference in levels between normally nourished and severely malnourished children. This was unexpected, and since thiamine deficiency has both subclinical and clinical adverse effects and is easily treatable, further

studies using different methodology are indicated.

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