Thiamin-fortified fish sauce as a means of combating infantile beriberi in rural Cambodia

Food, Nutrition and Health, University of British Columbia, Canada
Child & Family Research Institute, Canada

in partnership with

Helen Keller International, Cambodia
Ministry of Health, Cambodia
Ministry of Planning, Cambodia

Background

Beriberi is a micronutrient deficiency disease caused by a lack of thiamin (vitamin B_1) in the diet (1–3). In infants, beriberi presents with symptoms of heart failure and is fatal unless thiamin treatment is initiated immediately (1,2,4). Infantile beriberi is common in Southeast Asia (5); in Cambodia it is thought to kill more than 680 infants each year (personal communication, Kantha Bopha Hospital, Cambodia). Since beriberi is eminently preventable, it is an ideal target for intervention. In Cambodia, low thiamin intake stems from poor dietary diversity as well as the high consumption of white rice, which contains very little thiamin, and foods like raw fish that have anti-thiamin activity (2,6,7). Our team recently identified that nearly 60% of rural Cambodian women of childbearing age are deficient or marginally deficient in thiamin, despite showing no clinical signs (8).

Infantile beriberi presents during the exclusive breastfeeding period (typically 4-6 months) when infants are entirely dependent on breast milk for nutrition (9). As such, maternal thiamin status must be improved in order to increase the thiamin concentration of breast milk (10). To prevent infantile beriberi, a thiamin intervention in rural Cambodia is required to increase dietary thiamin intake by women of childbearing age. We propose that thiamin-fortified fish sauce has the potential to increase dietary thiamin intake of breastfeeding mothers, which in turn will improve the thiamin intake of their infants, preventing beriberi.

Thiamin fortification of a food staple is an ideal intervention as it is passive: unlike supplementation or dietary changes, participants do not need to change their eating behaviour in order to increase thiamin intake (11). Fish sauce is a traditional condiment that is consistently consumed in relatively high quantities by the entire Cambodian population, making it an ideal vehicle for thiamin. Since iron-fortified fish sauce has recently become available in Cambodia, this will act as the control sauce, while high and low concentration thiamin-iron-fortified sauces will be used for the intervention in this pilot study. Fish sauce will be provided to pregnant Cambodian women and their families in Prey Veng province for six months to determine the efficacy of the fish sauce at increasing erythrocyte thiamin diphosphate concentrations.

Study Objective

We will conduct a double-blind randomized controlled pilot study to determine the efficacy of thiamin-fortified fish sauce to increase the erythrocyte thiamin concentration of pregnant Cambodian women (between 3-8 months pregnant at baseline) and their household to a level consistent with a low risk of infantile beriberi as compared to a control fish sauce (fortified only with iron).

Inclusion and Exclusion Criteria

To participate in this study, individuals must:

- be the female head of their household,

- be between 18-45 years of age,
- be between 3-8 months pregnant with a singleton fetus at baseline,
- know her approximate due date,
- have no prior history of preeclampsia, pre-term delivery, or birth defects,
- be planning to exclusively breastfeed her child for 6 months,
- be living in Prey Veng province, Cambodia, and not planning to move in the next six months,
- not be receiving any other intervention (for example, homestead food production),
- agree to exclusively feed her entire household the study fish sauce for six months,
- be willing to provide venous blood samples at baseline and endline, a breast milk sample at endline, and to allow for a blood sample to be taken from her infant (aged ~3 months) at endline, and
- not be taking any supplement that contains thiamin.

Consent

All consent forms will be translated into Khmer. All participants will consent on their own behalf. Where the participant cannot read the consent form, the enumerator will read it to her. Participants will have at least 48 hours to decide if they want to participate. The interviewer will return to the house within 48 hours to obtain verbal and signed (signature or thumb print) consent, at which point the trained enumerator will administer the study questionnaire, take anthropometric measurements, and provide instructions on blood sampling.

At baseline and endline, all women will be asked to travel to the local health centre for a venous blood draw. As remuneration for their time, women will receive one sarong (valued at ~US$2.50), and will be provided with US$2.00 to reimburse travel expenses to and from the local health centre.

Research Method

Control and Intervention Groups

In this double-blind randomized placebo controlled pilot study, 90 women of childbearing age in Prey Veng province, Cambodia who meet the study inclusion and exclusion criteria will be randomly assigned to one of three groups: low concentration thiamin-iron-fortified fish sauce, higher concentration thiamin-iron-fortified fish sauce, or iron-fortified fish sauce (placebo).

Women in all groups will receive an unlimited supply of fish sauce for their entire household for six months. All other fish sauce will be removed from the home at the start of the study, and a research associate will visit homes every two weeks to check in, complete monitoring and evaluation, and replenish fish sauce as necessary.

Questionnaire and Anthropometry

All women will complete a questionnaire at baseline (t=0) and endline (t=6 months) to collect demographic data, as well as information on the consumption of fish sauce, and to identify dietary behaviours. A trained, Khmer-speaking enumerator will conduct this interview in the participant's home.

After questionnaire administration, a trained enumerator will measure the height and weight of the participant using standardized techniques. Within 72 hours of birth (t~3 months), anthropometric measurements (length and weight) will be completed on the participant's infant. Within this first month of the study, all participants and their families will be invited to attend an educational workshop on dietary thiamin intake, thiamin deficiency and beriberi, and infant and young child feeding (IYCF).

Blood Samples

At baseline and endline, a trained phlebotomist from the National Institute of Public Health (NIPH) will draw 10mL of non-fasted blood from all participants via venipuncture into an evacuated EDTA-coated tube at the local health centre. At endline, a pediatric phlebotomist will collect a blood sample (4mL) from the ~3 month old infant. All samples will be transported on ice daily to the NIPH for processing and storage in a -80°C freezer. Erythrocyte thiamin diphosphate (TDP) concentration will be measured in each sample. Thiamin status will be classified as: thiamin sufficient: TDP > 90nmol/L, marginally thiamin deficient: 90 ≤ TDP ≤ 70 nmol/L, and thiamin deficient: TDP <70 nmol/L (3,4).

This is a very low risk study; however, the blood collection procedure may cause some discomfort and slight bruising or, very rarely, an infection at the site of the needle poke. After the blood draw participants will immediately be given a bandage to cover the spot where the blood was taken.

Breast Milk Samples

At endline a breast milk sample will be obtained from the mother in her home (12). Using a battery powered breast pump, the mother will express one full breast into an amber polypropylene container. The samples will be immediately transported on ice to the NIPH where they will be warmed to room temperature, swirled gently to homogenize the contents, then aliquoted and frozen at -20°C.

Recruitment and Enrollment

All villages in Prey Veng province that are not already involved in an active intervention (for example, homestead food production, micronutrient powder intervention etc) will be randomized. A Khmer-speaking research assistant will contact the Village Chief and/or Village Health Volunteer in the first village on the randomized list and determine the number of eligible women in that village (based on the inclusion and exclusion criteria outlined above). The research assistant will move down the randomized list of

villages until 90 participants have been enrolled. We expect enrollment from approximately 10 villages.

Once the participant completes the baseline questionnaire she will be randomly assigned to one of the three treatment groups: low concentration thiamin-iron-fortified fish sauce, higher concentration thiamin-fortified fish sauce, or placebo: iron-fortified fish sauce. The bottles and labels of the three fish sauces will be identical in appearance, except for a code that differentiates the sauces.

Based on previous studies our team has conducted in Prey Veng province, we expect the majority of eligible women in the randomized villages will choose to participate in the study.

Sample size estimate

This pilot study is part of a larger study that will run concurrently in Prey Veng province. Here, 276 non-pregnant women of childbearing age (n~90 per group) will participate in the same double-blind randomized control trial.

Estimates for this pilot study indicate we need a sample size of n=30 women per group to detect a 30% difference between fortified and control groups at endline, assuming a minimum baseline of 38 nM, SD of 18 nM, 80% power, and alpha=0.05.

Statistical Analysis Plan

Descriptive Statistics

Participant characteristics will be summarized as the number of participants and percentage for categorical variables and means and standard deviation for continuous variables.

Outcome Variable

The main outcome of this study is maternal erythrocyte TDP at endline (t=6 months). The effect of treatment on maternal and infant erythrocyte TDP as well as breast milk thiamin concentration, as continuous variables, will be determined using a general linear model. Erythrocyte TDP or breast milk thiamin will be the dependent variable and treatment will be a factor. In the case of maternal erythrocyte TDP, baseline maternal TDP will be entered as a covariate.

Data Collection and Storage

A unique identifier will be assigned to each participant. This identifier will not derived from personal identifiers.

All electronic data files will be stored on password-protected computers and/or secure servers accessible only to members of the research team. Archived electronic data files

and any hard copies of data, consent forms, questionnaires or other papers containing data will be stored in locked filing cabinets in locked storage rooms at Helen Keller International (HKI), Cambodia. Access to the documents will be limited to the principal investigator and co-investigators.

De-identified data will be sent from Cambodia to the University of British Columbia (UBC) and the Child and Family Research Institute (CFRI). Data will be sent by email over a password-protected spreadsheet. All co-investigators and research assistants working on the project will have access to the data. Responsibilities concerning privacy and confidentiality will be discussed with the research assistants.

Paper and archived electronic data will be stored in locked filing cabinets in locked research rooms at HKI for at least 5 years following publication of research findings. After this time, they will be physically destroyed (e.g., paper copies will be shredded; CD's will be made unusable).

Dissemination of Information

Upon completion of this study, data will be compiled and analyzed. It will be shared with the scientific community at pertinent scientific conferences, and a manuscript of the findings will be prepared for a peer-reviewed nutrition journal. This information will also be shared with the Cambodian National Nutrition Working Group, and the National Sub Committee for Food Fortification.

We plan to analyze all blood samples within two months of the endline sample collection. Our team will then return to the villages to share study findings with all participants at a community meeting (general results shared), and then separately to share individual thiamin results one-on-one. All participants will have already participated in an educational workshop highlighting the importance of dietary thiamin intake, and the signs of thiamin deficiency and beriberi. Individuals with suboptimal thiamin status will be encouraged to speak with their healthcare provider, and will also be counseled using the materials that were previously used in the educational workshop.

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