Research protocol: part 1

Title: Adjunct omeprazole or resveratrol in non-transfusion dependent thalassemia patients with secondary hemochromatosis: a randomized, double-blind, placebo-controlled trial

General Information

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Sponsor/Source of Funding

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Rationale & Background Information

Thalassemia is the most common hereditary disorder, with a global prevalence of 18.28 per 100,000 people [1]. In Thailand, approximately 1% of the population is affected by this condition [2]. The pathogenesis of thalassemia arises from defective globin chain production, which is regulated by alpha- and beta-globin genes. This results in reduced or absent synthesis of globin chains, leading to clinical manifestations such as anemia, jaundice, hepatosplenomegaly, and growth retardation. The clinical symptoms and severity of thalassemia are categorized into two groups based on the necessity of blood transfusions: transfusion-dependent thalassemia (TDT) and non-transfusion-dependent thalassemia (NTDT) patients [3]. Accordingly, TDT patients develop iron overload due to regular blood transfusions with approximately 0.8 mg of iron per 1 mL of transfused blood [4] along with the lack of an effective iron excretion mechanism in the body [5]. In comparison, iron overload in NTDT patients mostly results from increased intestinal iron absorption and ineffective erythropoiesis of which chronic hypoxia leads to an increased erythroferrone (ERFE) secretion, suppressed hepcidin production and enhanced intestinal iron absorption [6–8]. Although iron overload in NTDT patients does not significantly increase mortality due to cardiac iron deposition [9–12], it poses risks for hormone deficiencies, osteoporosis, pulmonary hypertension, and liver disease, which is a leading cause of death [13–15]. Among the mechanisms contributing to iron overload, one critical pathway involves increased intestinal absorption of dietary iron which is 90% non-heme and 10% heme iron. Non-heme iron absorption requires conversion to ferrous iron, mediated by gastric enzymes and an acidic environment [16]. Therefore, agents/compounds that reduce gastric acidity may decrease iron absorption are relevant.

Proton pump inhibitors (PPIs), widely used to reduce gastric acid, inhibit the proton-potassium ATPase in parietal cells at the final step in gastric acid secretion, thereby reducing gastric acidity [17]. Lower gastric acidity may be insufficient to solubilize dietary iron, interfere with intestinal iron absorption and potentially induce iron deficiency [18, 19]. Previous studies have demonstrated the use of PPIs, including pantoprazole and esomeprazole, in managing iron overload in thalassemia patients and observed a reduction in systemic iron accumulation [20–23]. Additionally, dietary factors such as calcium-rich foods and polyphenols can reduce iron absorption [24]. Resveratrol that is an antioxidative polyphenolic compound [25, 26] can reduce oxidative stress, improve hepatic iron-overload states [27, 28], decrease intestinal iron absorption [29] and increase fetal hemoglobin (HbF) levels in beta-thalassemia patients [30].

Nowadays, the standard treatment for iron overload in thalassemia involves iron chelation therapy which standard iron chelators including deferasirox (DFX), deferiprone (DFP) and deferoxamine (DFO) are used in Thailand. However, parenteral administration of DFO, limited

accessibility to DFX, and severe side effects or suboptimal outcomes with DFP may hinder effective management in NTDT patients with iron overload. Given these limitations and the underlying mechanisms of iron overload, this study aims to investigate the effects of omeprazole as PPI and resveratrol as an adjuvant on dietary iron absorption in NTDT patients. We hypothesize that these interventions could further reduce systemic iron levels through their proposed mechanisms.

Research Hypothesis

Adjunctive omeprazole or resveratrol in combination with iron chelators could reduce serum ferritin compared with placebo.

Study Goals and Objectives

Primary Objective

To evaluate the efficacy of adjunct omeprazole or resveratrol in combination with iron chelators in reducing serum ferritin compared to placebo

Secondary Objectives

1. To evaluate the efficacy of adjunct omeprazole or resveratrol in combination with iron chelators in reducing labile plasma iron (LPI), non-transferrin bound iron (NTBI) and serum iron (SI) compared to placebo
2. To study the association between adjunct omeprazole or resveratrol in combination with iron chelators and the levels of ERFE and hepcidin (Hcd)
3. To study the association between resveratrol and changes in HbF levels
4. To assess the adverse effects of adjunct omeprazole or resveratrol with iron chelators

Ethics

This study has been approved by the Human Experimentation Committee Research Institute for Health Sciences and authorized by Associate Professor Nimit Morakote, Ph.D., a Chairman of the Committee, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand, Certificate Number: 476/2024 (English version) and 476/2567 (Thai version), Date: 17th December 2024. Adherence to ethical guidelines was of prime importance throughout the study process. All patients will be fully informed about the particulars of the study and willingly provide their signatures on the consent forms before any study procedures are performed. This study follows the guidelines of the Helsinki Declaration 2008, revised in 2013: Ethical Principles for Medical Research Involving Human Subjects. Subjects' rights have been protected by an appropriate Institutional Review Board and written informed consent will be granted from all subjects.

Methods

Study Design

Phase 3, randomized, double-blind, placebo-controlled trial

Scope of Research

Non-transfusion thalassemia patients with secondary iron overload visiting Out-patient department numbers 9 or 23 at the Division of Hematology, Department of Internal Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand between 10th February 2025 and 30th April 2026.

Study Population

Inclusion Criteria

- Age 20-65 years
- Non-transfusion dependent thalassemia [alpha or beta] diagnosis by HPLC, PCR or gene mutation
- Hemochromatosis with serum ferritin 300-1000 ng/mL or > 1000 ng/mL with maximum iron chelator (DFP at 75 mg/kg/day or DFX at 20 mg/kg/day)
- Concurrent iron chelators without changing dose during 3 months before enrollment and no tendency to adjust the dose of iron chelators between study
- Inform consent

Exclusion Criteria

- Pregnancy or breastfeeding
- Chronic inflammation or other conditions that might confound serum ferritin interpretation
- Alanine transaminase more than 5 times upper limited of normal, creatinine clearance < 60 mL/min
- Can not receive oral medication
- Currently receive omeprazole or resveratrol
- allergy to omeprazole or resveratrol

Discontinuation Criteria

- Have more or equal than grade 3 adverse events
- Have conditions that are not suitable for continuation of study
- Protocol violations that significantly affect data collection or interpretation
- Pregnancy
- Need to increase the dose of iron chelation while currently in study
- Have indication for proton pump inhibitor or resveratrol such as gastrointestinal bleeding
- Withdrawal of informed consent

Sample Size Calculation

Expected standardized difference of 0.22 from the Eghbali et al. [22] study which found that adjunct pantoprazole with iron chelators could lower serum ferritin more than iron chelators alone by decreasing serum ferritin from 1444±613 ng/mL to 1197±956 ng/mL and 1928±694 ng/mL to 1501±385 ng/mL respectively. If alpha = 0.05 and beta = 0.2, the estimated sample size is 20 persons in each study group [31] as shown in Table 1.

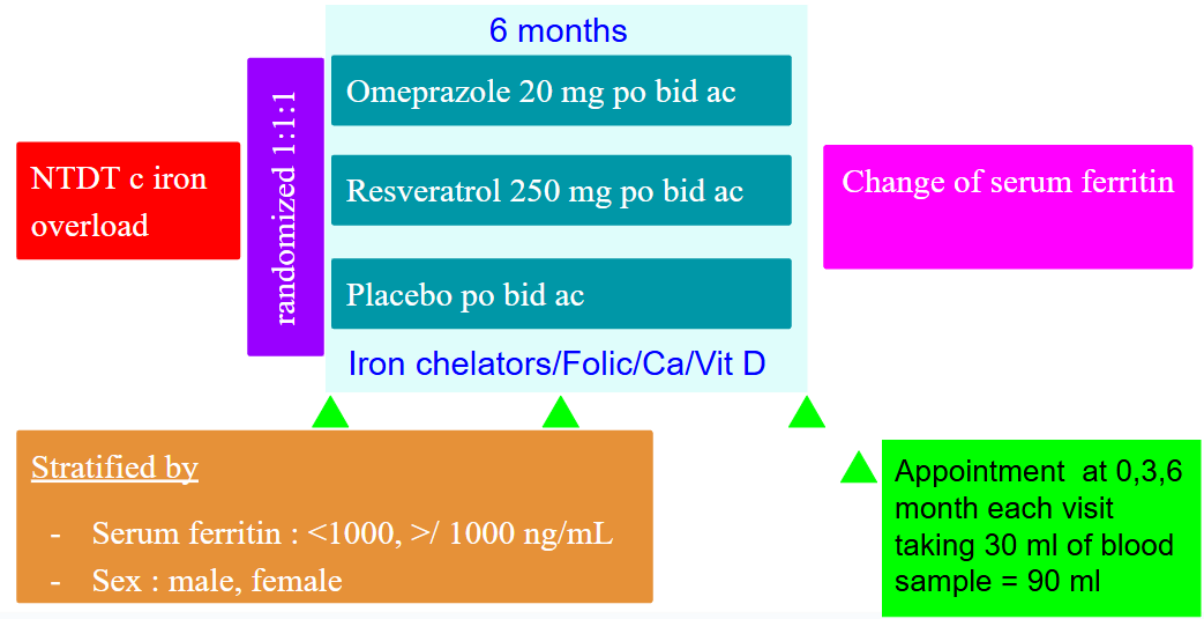
Table 1 Estimated stepped rules of thumb for required trial sample size/treatment arm [31].

Standard difference	80% powered main trial	90% powered main trial
Extra small (δ <0.1)	50	75
Small ($0.1 \leq \delta < 0.3$)	20	25
Medium ($0.3 \leq \delta < 0.7$)	10	15
Large ($\delta \geq 0.7$)	10	10

Study Intervention

- Divide the patients into three study groups in a 1:1:1 ratio using stratified randomization based on gender (male and female) and serum ferritin (<1,000, equal to 1,000 or >1,000 ng/mL). The randomization will be used web-based randomization.
- Allocation concealment will use sealed envelopes to ensure concealment, A staff member not involved in this study will place the study drugs into opaque, sealed envelopes and label them into unique codes. The patients will receive the study drug corresponding to the code on their envelope, without knowing the content. The code lists will be securely stored and remain inaccessible to the research team during the trial.
- This study will use a double-blind technique for patients and outcome assessors. Three study compounds including omeprazole, resveratrol and placebo will be prepared and filled in gelatin capsules with the same appearance as follows; 20 mg omeprazole capsule, 250 mg resveratrol capsule and 20 mg maltodextrin. The capsules will be packed in a white plastic bottle (30 capsules each) and labeled codes under the bottles.
- The patients in Group 1 will take an omeprazole capsule orally twice daily (total 40 mg/day) in the morning and evening before meal for 6 months.
- The patients in Group 2 will take a resveratrol capsule orally twice daily (total 500 mg/day) in the morning and evening before meal for 6 months.
- The patients in Group 3 will take a placebo capsule orally twice daily in the morning and evening before meal for 6 months.
- All groups will receive standard treatments including iron chelators, folic acid supplementation, vitamin D supplementation, calcium supplementation and lifestyle modifications throughout the study.

Research Design



- The patients will be appointed at the first visit, 3 months and 6 months to evaluate clinical signs, blood parameters and side-effects. If there are side effects more or equal to grade 3, the doctors may decrease the dosage of the study drug according to the following table.

Side effects	Dose adjustment
Grade ≥3 Nausea, vomiting, diarrhea, bloating	Decreased study drug 1 level [from twice daily dose to once daily dose]

- In each visit, the patients will be requested to collect their blood (30 mL) which will be separated into EDTA-whole blood (5 mL) and clotted blood (25 mL). Whole blood will be used for complete blood count (CBC) analysis and Hb typing. Clotted blood will be separated into the serum to be used for quantitation of kidney function biomarkers such as blood urea nitrogen (BUN) and creatinine (Cr); electrolytes (e.g., Na^+, K^+, Ca^{2+}, Mg^{2+} and phosphates); liver function biomarkers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP); and iron parameters such as serum ferritin (sFt, SI), total iron-binding capacity (TIBC), NTBI, LPI, Hcd and ERFE according to the below table.

Activities	1st visit	2nd visit	3rd visit
History taking and physical examination			

Blood for CBC, BUN, Cr, electrolytes, AST, ALT, ALP, sFt, %TS, TIBC, SI, Hb typing, LPI, NTBI, Hcd and ERFE			
Blood for quantitation of serum vitamin B12, vitamin D, Ca^{2+} and Mg^{2+}, and determination of Hb type			
Adverse effect record			

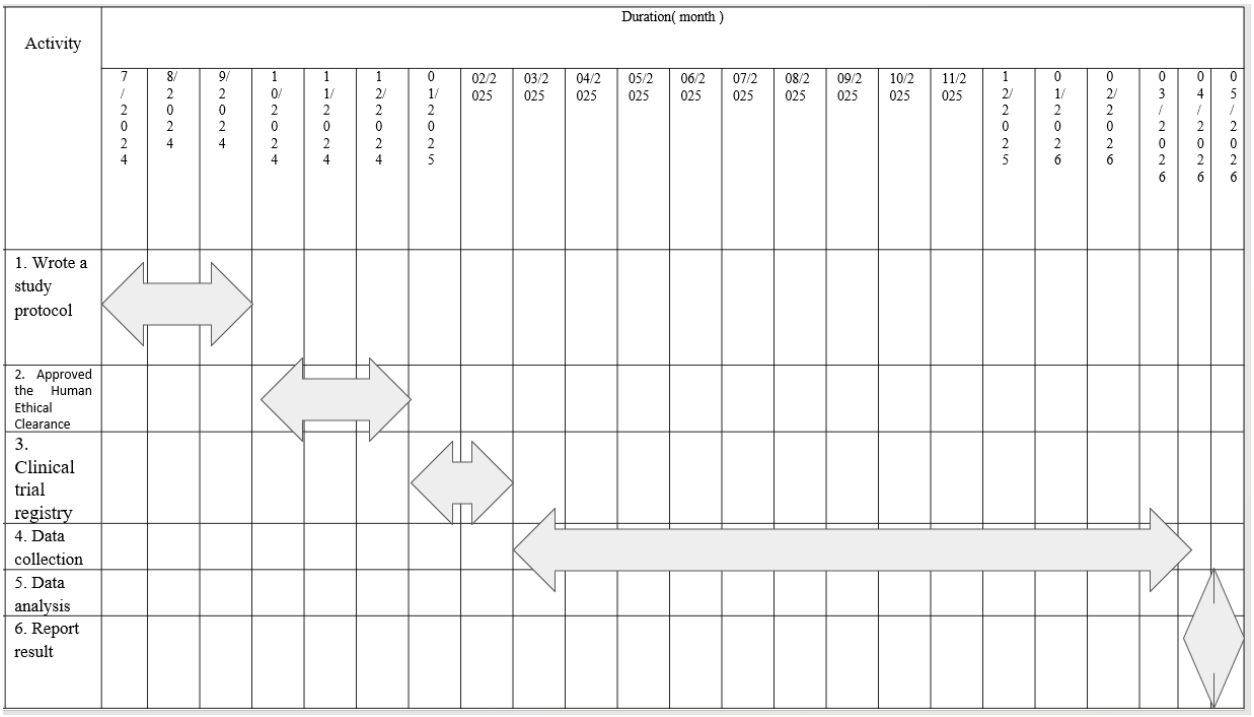
Laboratory Investigation

1) CBC will be analyzed using an automated Beckman Cell Analyzer machine according to the manufacturer's instruction at the Central Laboratory, Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University.
2) Hb typing will be performed at the Hematology laboratory, Department of Internal Medicine, Chiang Mai University.
3) Serum BUN, Cr, electrolytes, AST, ALT, ALP, sFt, SI and TIBC will be quantified using an automated Roche Cobus Biochemical Analyzer machine according to the manufacturer's instruction at the Central Laboratory, Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University.
4) Serum iron parameters will be quantified at the Oxidative Stress Laboratory, Department of Biochemistry, Faculty of Medicine, Chiang Mai University using specific reagents and techniques as described below.
    4.1) LPI using rhodamine B fluorescence probe [32]
    4.2) NTBI using NTA chelation/iron chelator fluorescence beads/flow cytometry [32]
    4.3) Hcd using a sandwich ELISA kit (ABBEXA limited company, Cambridge, UK) according to the manufacturer's instruction [33]
    4.4) ERFE using a sandwich ELISA kit (ABBEXA limited company, Cambridge, UK) according to the manufacturer's instruction [34]

Statistical Analysis

All patients who receive at least 1-time study drug will be analyzed in an Intention-to-treat analysis. Patients who receive at least 80% of the study drug will be analyzed in a Per-protocol analysis. Descriptive statistics will be used for the analysis of demographic data. Primary outcome and secondary outcomes [continuous variable] will be analyzed using Friedman's analysis of variance (ANOVA) test. Secondary outcomes [dichotomous variable] will be analyzed using Chi-square or Fisher exact test for independent data or Cochran's Q test for dependent data. The association between continuous variables will be analyzed using Pearson's or Spearman's test. Accordingly, a P-value <0.05 is considered statistical significance.

Duration of Study Plan



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