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Comparison of home fortification with two iron formulations among Kenyan children: Rationale and design of a placebo-controlled non-inferiority trial

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ABSTRACT

Introduction: Home fortification powders containing iron and other micronutrients have been recommended by World Health Organisation to prevent iron deficiency anaemia in areas of high prevalence. There is evidence, however, that home fortification at this iron dose may cause gastrointestinal adverse events including diarrhoea. Providing a low dose of highly absorbable iron (3 mg iron as NaFeEDTA) may be safer because the decreased amount of iron in the gut lumen can possibly reduce the burden of these adverse effects whilst resulting in similar or higher amounts of absorbed iron.

Objective: To show non-inferiority of home fortification with 3 mg iron as NaFeEDTA compared with 12.5 mg iron as encapsulated ferrous fumarate, with haemoglobin response as the primary outcome.

Design: 338 Kenyan children aged 12–36 months will be randomly allocated to daily home fortification with either: a) 3 mg iron as NaFeEDTA (experimental treatment), b) 12.5 mg iron as encapsulated ferrous fumarate (reference), or c) placebo. At baseline, after 30 days of intervention and within 100 days post-intervention, blood samples will be assessed for primary outcome (haemoglobin concentration), iron status markers, *Plasmodium* parasitaemia and inflammation markers. Urine and stool samples will be assessed for hepcidin concentrations and inflammation, respectively. Adherence will be assessed by self-reporting, sachet counts and by an electronic monitoring device.

Conclusion: If daily home fortification with a low dose of iron (3 mg NaFeEDTA) has similar or superior efficacy to a high dose (12.5 mg ferrous fumarate) then it would be the preferred choice for treatment of iron deficiency anaemia in children.

1. Introduction

Home fortification aims at supplementing local diets by adding micronutrient powders to semi-solid, ready-prepared foods (http://www.hftag.org/). The World Health Organisation (WHO) recommends daily universal home fortification with iron for children aged 6–23 months in populations where the prevalence of anaemia in children under 5 years of age is ≥ 20% [1]. Prevalence values within this range indicate a moderate-to-severe public health problem, which is the situation in virtually all developing countries [2].

The WHO-recommended iron dose for home fortification (10–12.5 mg iron as ferrous salt for children aged 6–23 months, [1]) corresponds to the dose that was previously established for iron supplementation in this age range [3]. There is evidence from randomised controlled trials among young children in low-income countries to suggest that home fortification with iron-containing micronutrients may cause an excess burden of diarrhoea, and increased numbers of potentially pathogenic enterobacteria, with a concurrent increase in gut inflammation [4]. Other gastrointestinal adverse effects of oral iron supplementation, such as epigastric discomfort, nausea and constipation, are common, are dose-dependent and are likely to reduce adherence [5].

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Compared to the conventional daily dose (12.5 mg as ferrous salt), home fortification or supplementation with a low dose of highly absorbable iron (3 mg iron as NaFeEDTA) may result in similar or higher amounts of absorbed iron [6,7] but the decreased amount of iron in the gut lumen can possibly reduce the burden of adverse gastro-intestinal effects.

There is substantial evidence that iron interventions in young children can also increase rates of malaria and possibly respiratory disease [8–11]. Because adverse events associated with such systemic diseases are likely to depend on the absorbed amount of iron, the risks may be similar when comparing a daily dose of 3 mg iron as NaFeEDTA and 12.5 mg as ferrous salt. WHO has recommended that iron interventions should be implemented in conjunction with measures to control malaria [1].

We aimed to show non-inferiority of home fortification with 3 mg iron as NaFeEDTA compared with 12.5 mg iron as encapsulated ferrous fumarate in young Kenyan children protected for 3–4 weeks against malaria by chemoprevention.

2. Study methodology

2.1. Study site

The study will be conducted from January–December 2014 in the administrative units of Kanyawegi, Osiri and Ojolla in Kisumu-West District, a rural area at an altitude below 1,300 m, adjacent to Lake Victoria, Kenya. This area covers 395 square kilometres with a population of approximately 12,000 people, of whom 20% are children aged below five years. The majority of the population consists of subsistence farming families but inadequate and unreliable rainfall patterns have immensely affected agricultural activities in the area [12]. The local diet is mainly based on maize and vegetables. Animal foods, which are rich sources of iron, are rarely consumed and often sold in the urban markets to boost income. Malaria transmission is perennial and stable [13], with most infections being due to *Plasmodium falciparum* [14]. The prevalence of *P. falciparum* infection in children aged 1–4 years has been reported to range between 39% and 63% [15]. The area is endemic for *Schistosoma mansoni*, with a prevalence of infection in infants of 14% [16]. Hookworm and *Trichuris trichiura* infections are also common in young children [17]. Co-infection of hookworm, *T. trichiura* and *P. falciparum* has been associated with low haemoglobin concentrations in pre-school children [18].

2.2. Study design

This study concerns a randomised, double-blind, non-inferiority trial comparing daily home fortification for 30 days with 3 mg iron as NaFeEDTA (investigational intervention), 12.5 mg iron as encapsulated ferrous fumarate (reference) and placebo. We conceived it as an explanatory trial to evaluate the efficacy of daily home iron fortification under maximal compliance.

2.3. Sample size determination

Sample size calculations are based on procedures for non-inferiority trials as recommended by USA Food and Drug Administration [19,20].

1. Based on a meta-analysis [21] we estimated the expected effect of 12.5 mg iron as ferrous fumarate on haemoglobin concentration relative to placebo. The lower limit of the 95% CI thus obtained (9.3 g/L) was used as M_1, the minimum anticipated effect of 12.5 mg iron as ferrous fumarate (Fig. 1; left panel).
2. Next, we set M_2 as the margin specified to preserve 50% of the anticipated minimum effect of 12.5 mg ferrous fumarate. This margin (haemoglobin concentration of 4.7 g/L) can be interpreted as the largest loss of effect compared to 12.5 mg ferrous fumarate (inferiority) that would be acceptable, and is below an effect for 5 g/L iron as NaFeEDTA that we considered to be of minimum importance for public health.
3. We set the sample size at 339 children (estimating 113 children per intervention group) so that the lower limit of the 95% CI around the difference in haemoglobin concentration between the two iron formulations (i.e. 12.5 mg ferrous fumarate and 3.0 mg iron as NaFeEDTA) would lie above M_2 (Fig. 1; right panel).

2.4. Recruitment

The research assistants will hold meetings with local authorities, community health workers and parents to inform them about study aims and procedures. The community health workers will compile a list of parents with children aged 1–3 years residing within the three administrative units, and invite parents to bring these children for screening to the research clinic, where they will be asked to sign an informed consent form (Appendices 1, 2).

At the screening visit, research assistants will collect vital data and information on household characteristics: a) date of birth as recorded in the birth certificate or health card held by the mother or, if not available, from records of the Expanded Program of Immunization held by local clinics; b) anthropometric data that include weight measured to the nearest 100 g using a Salter scale (UNICEF, catalogue 0145555, Copenhagen, Denmark) that is calibrated daily using a 5 kg weight. During measurement, the child will wear neither clothes nor shoes; standing height (children $\geq$24 months or $\geq$ 85 cm) or recumbent length (children $\leq$24 months or $\leq$ 85 cm) will be measured within 0.1 cm using wooden measuring boards (UNICEF, catalogue 0114500); and mid-upper arm circumference, a marker of wasting, using a measuring tape (UNICEF, catalogue 145600) within 0.1 cm.

Medical staff will conduct medical examinations and collect the following data: a) a parent-reported 48-h history of illness including fever, diarrhoea, vomiting or breathing distress; b) parent-reported history of signs of major systemic disorders; c) parent-reported use of specific medicines (antiretroviral drugs, rifampicin, carbamazepine, phenytoin or phenobarbital); c) parent-reported drug allergies, or 30-day history of using drugs (antimalarials, benzimidazoles, praziquantel) that might interfere with the study treatment protocol.

Clinical officers will ask parents to bring children for re-screening two weeks later if the child has a 48 h history of antimalarial drug use, or has received treatment for malaria. Children with axillary temperature $\geq$37.5 °C plus demonstrated blood infection (rapid dipstick tests positive for malaria) or minor illnesses will be treated immediately and also asked to return after two weeks for re-screening.

Phlebotomists will collect venous blood (4 mL) in tubes containing Li-heparin. We will determine haemoglobin concentration (HemoCue 301, Ängelholm, Sweden) and zinc protoporphyrin:haem ratio (AVIV, model 206D, Lakewood NJ, USA) in whole blood as a marker of iron-deficient erythropoiesis, each in triplicate. We will assay *Plasmodium* antigenaemia by rapid tests (see section 'Laboratory analyses' below). We will transfer aliquots of whole blood (125 μL) to DNA collection cards (FTA Mini Card, catalogue WB120055, Little Chalfont, UK) for storage at ambient temperature and subsequent detection by PCR of *Plasmodium* infection; and we will prepare thick and thin blood smears to allow for detection and counting of *Plasmodium* parasites.

An aliquot (1.0 mL) of blood will be centrifuged (600 × *g*, 10 min). Plasma (500 μL) will be transferred to a microtube, centrifuged (2000–3000 × *g*, 15 min), transferred to a cryovial, and stored immediately in liquid nitrogen (−196 °C). The erythrocyte sediment (500 μL) will be washed and centrifuged (600 × *g*, 8 min) three times with isotonic phosphate-buffered saline (Medicago, Uppsala, Sweden; catalogue 09-9400-100) to allow measurement in triplicate of the erythrocyte zinc protoporphyrin:haem ratio. Measurement of zinc protoporphyrin:haem ratio in washed erythrocytes is considered a more valid measurement when compared to whole blood because the