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**Approved Protocol**

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**EFFECT OF IRON FORTIFIED FOODS ON HEMATOLOGICAL  
RESPONSE AND ZINC STATUS: SYSTEMATIC REVIEW OF  
RANDOMIZED CONTROLLED TRIALS**

**Prof. H. P. S. Sachdev**

*Senior Consultant, Pediatrics and Clinical Epidemiology, Sitaram Bhartia Institute of  
Science and Research, B-16 Qutab Institutional Area, New Delhi 110016, India.*

E-mail: [hpssachdev@gmail.com](mailto:hpssachdev@gmail.com)

**Dr. Tarun Gera**

*Consultant Pediatrics, Fortis Hospital, Delhi, India.*

E-mail: [tarun256@yahoo.com](mailto:tarun256@yahoo.com)

Correspondence to: Prof. H.P.S. Sachdev, E-6/12 Vasant Vihar, New Delhi 110057,  
India. E-mail: [hpssachdev@gmail.com](mailto:hpssachdev@gmail.com)

## **Rationale**

Anemia, the major clinical manifestation of iron deficiency, is a widespread problem throughout the world and has both health and economic consequences. The most extreme effects are increased maternal mortality, poor cognitive development in children, and lower worker productivity (1,2). Anemia affects nearly one-third of the world's population (3). Infants and young children are particularly affected with global estimates suggesting that 35% of all preschoolers are anemic (4). The problem is more widespread in South Asia (53%) than in other regions of the world (4). More specifically, estimates from India show anemia prevalence to be 74% among children 9–36 months old (5).

Dietary strategies to counter anemia include the use of iron supplements, food fortification, or possibly dietary modification. Food fortification is often propagated as the most realistic way to increase iron intake on a widespread and sustainable basis and is presently implemented in the USA, Britain, and parts of Latin America. Currently biofortification of crops is being intensively evaluated as a viable and possibly superior alternative to food fortification. As there is a paucity of relevant human biofortification trials, experiences from food fortification trials in various populations will be invariably utilized for evaluation of newer biofortified crops, both for formulating and addressing research issues and for drawing inferences about their utility in combating iron deficiency anemia.

Evidence indicates that hemoglobin and ferritin are currently the most efficient indicators of population response to iron interventions (6). However, relevant randomized controlled trials provide conflicting evidence regarding the utility of iron-fortified foods in improving the iron status and reducing anemia in populations. A recent systematic review of randomized controlled iron supplementation trials in children documented that iron fortified foods (primarily infant milk substitutes and complementary foods) resulted in a substantially lower average hemoglobin response in comparison to oral medicinal iron supplementation (0.25 vs 0.74 g/dl) (7). Use of electrolytic or reduced iron fortified wheat flour for two years in Sri Lanka did not have any effect on hemoglobin

concentration (8). Conversely, the use of NaFeEDTA fortified fish sauce in Vietnam resulted in improvements in hemoglobin (0.2 and 1.0 g/dl) and reduction in anemia (20 and 25%) in non-pregnant women with interventions lasting for 18 and 6 months, respectively (6). Similarly, the use of micronized ferric pyrophosphate fortified salt in children in Africa for 10 months resulted in a significant ( $p < 0.01$ ) hemoglobin increase (1.6 g/dl), which reduced anemia from 30% at baseline to 5% (9).

The success of iron fortified food intervention depends on several factors including consumption pattern of the fortified food; effect of the fortificants on the taste and appearance of food vehicle; shelf life of the fortified food; bioavailability of the iron fortificants; and the baseline iron status of the population. For example, it is reported that iron bioavailability in foods fortified with reduced iron compounds, which are relatively inexpensive and have good organoleptic properties, is low (10,11). Evaluation of more expensive iron compounds has therefore been proposed as suitable fortificants.

A new dimension has been added to the iron fortification of foods with the emergence of zinc as an important micronutrient for child health (12). Iron and zinc deficiencies may frequently co-exist in populations that consume diets with insufficient amounts of animal-source foods (13). Because they have chemically similar absorption and transport mechanisms, iron and zinc have been thought to compete for absorptive pathways (14). In view of the biological and functional consequences of zinc deficiency, it is important to determine if iron fortification of foods creates or aggravates zinc deficiency, particularly in vegetarian populations subsisting on borderline zinc nutriture.

Food fortification is a very expensive intervention, particularly for widespread population use. Policy makers, programme implementers, and the target population need to carefully weigh the benefits and safety of this intervention before recommending it for populations. A usual perusal of the available literature is likely to be confusing with conflicting conclusions, as pointed out above. The need for a focused systematic review and meta-analysis is obvious for guiding policy and relevant research. The present proposal has therefore been formulated to fulfill this objective.

## **Objectives**

This systematic review and meta-analysis of randomized controlled trials will focus on the following objectives:

- (i) To evaluate the effect of iron fortified foods and biofortified crops on efficient indicators of population response (6), namely, hemoglobin and serum ferritin.
- (ii) To evaluate the possible predictors of a positive hemoglobin response, particularly focusing on food vehicle, iron fortification compound, iron content, baseline hemoglobin and ferritin levels, and duration of fortification.
- (iii) To evaluate the effect of iron fortified foods and biofortified crops on zinc status.

## **Methods**

### *Types of Trials*

The systematic review will comprise randomized controlled trials with variations in design including random allocation of individuals or clusters; multi-arm trials, factorial trials and cross over trials for the first period of measurement only. Quasi-randomized controlled trials (individual or cluster allocation done on the basis of a pseudo-random sequence, for example, odd/even house number or date of birth, alternation) will also be eligible for inclusion.

### *Types of Participants*

Apparently healthy (non-diseased) individuals, families or communities irrespective of age and gender considerations. Trials conducted exclusively in specifically diseased participants will be excluded, for example, in acquired immune deficiency syndrome, tuberculosis, *etc.*

### *Intervention*

Iron supplementation through the route of food fortification or biofortification. Food for the purpose of this systematic review will be defined as a usually consumed dietary item in the population, either in a raw or cooked form. Use of iron as separate additive to

dietary items, for example as sprinkles or syrups, will *not* be considered as food fortification.

### *Comparison*

Participants provided similar food without iron fortification. Trials with simultaneous fortification of additional micronutrients would be included if the only difference between the intervention and comparison arms is iron fortification. Similarly, trials employing simultaneous co-interventions like health education and/or drugs (for example, deworming or antimalarials) would be included if the only difference between the intervention and comparison arms is iron fortification.

### *Outcome Measures*

*Primary outcomes* will include:

1. Hemoglobin (g/L)
2. Anemia (% , as defined in individual trials)
3. Serum ferritin ( $\mu\text{g/L}$ )
4. Iron deficiency (% , as defined in individual trials)
5. Serum or plasma zinc ( $\mu\text{mol/L}$ )

*Secondary outcomes* will include:

1. Serum transferrin receptor (units reported in individual trials)
2. Transferrin saturation (%)
3. Total iron binding capacity (units reported in individual trials)
4. Serum or plasma iron (units reported in individual trials)
5. Zinc protoporphyrin (units reported in individual trials)
6. Adverse effects (any, as defined in individual trials)
7. Malaria (as defined in individual trials)
8. Diarrhea (as defined in individual trials)
9. Pneumonia or acute lower respiratory infections (as defined in individual trials)
10. Upper respiratory infections (as defined in individual trials)
11. Weight for age in children below 5 years age (z scores or crude weight)
12. Height for age in children below 5 years age (z scores or crude height)

13. Weight for height in children below 5 years age (z scores)
14. Cognitive or mental development in children below 18 years of age (specific tests reported in individual trials)
15. Motor development in children below 18 years of age (specific tests reported in individual trials)

#### *Method for identification of trials*

Aim to identify all relevant trials regardless of language or publication status.

#### *Databases*

Computerised bibliographic databases, including Medline, Embase, Web of Science, Cochrane Controlled Trials Register and IBIDS using appropriate key words. The following provisional search strategy for PubMed will be further refined: (("iron"[MeSH Terms] OR iron[Text Word]) AND ("food"[MeSH Terms] OR food[Text Word])) OR (iron fortificant[All Fields] OR iron fortificants[All Fields] OR iron fortification[All Fields] OR iron fortified[All Fields] OR iron fortifying[All Fields]) OR (iron supplement[All Fields] OR iron supplementation[All Fields] OR iron supplementations[All Fields] OR iron supplemented[All Fields] OR iron supplementing[All Fields] OR iron supplements[All Fields]) AND (Clinical Trial[ptyp] OR Randomized Controlled Trial[ptyp] OR Meta-Analysis[ptyp] OR Review[ptyp]). Analogous search strategy will be used for the other databases. A lateral search using the related articles link in PubMed will also be done for ten most recent key references included from the search strategy.

#### *Researchers and Organizations*

Websites of organizations like FAO, IFPRI, INACG, Micronutrient Initiative, and Micronutrient Forum and contacting researchers working in the field and the key organizations. Additional searches will be attempted in the Food and Nutrition Library (United Nations System Standing Committee on Nutrition (SCN) c/o World Health Organization, 20 Avenue Appa, CH 1211, Geneva 27, Switzerland), Iron Deficiency Project Advisory Service Web Site under food vehicles ([www.idpas.org](http://www.idpas.org)), and Nutrition

Research website search option  
([http://www.elsevier.com/wps/find/journaldescription.cws\\_home/525483/description#description](http://www.elsevier.com/wps/find/journaldescription.cws_home/525483/description#description)).

### *Reference Lists*

The reference lists of the identified articles will also be reviewed to search for citations that are not listed in the computerised databases. This will be supplemented by hand searches of reviews and proceedings of past three years of Micronutrient Forum international conferences or meetings.

### *Data Collection and Analyses*

The title and abstract of trials identified by the search strategy will be scanned to exclude studies that are obviously irrelevant. The full text of the remaining trials will be retrieved and relevant reports identified. Two investigators will independently assess eligibility from full-text articles and perform data extraction on preformed questionnaires. Differences in opinion (if any) will be resolved by mutual discussion. The data included in the review will be derived from the published manuscript or as provided by the authors for unpublished studies. The authors will be contacted for clarifications, if required (and if possible). Information recorded on questionnaires will include the eligibility criteria; the geographic location; the age of the study population; the baseline hematological status of the supplemented group; fortification dose; duration of supplementation and follow up; food vehicle; and fortification compound.

### *Assessment of Quality and Risk of Bias*

Trial quality and risk of bias will be evaluated by considering six features (Cochrane recommendations): sequence generation, allocation sequence concealment, blinding, incomplete outcome data, selective outcome reporting and 'other' potential sources of bias. This will comprise a description and judgment for each entry in a 'Risk of Bias' table, where each entry addresses a specific feature of the study. The judgment for each entry involves answering the question, with answers 'Yes' indicating low risk of bias,

‘No’ indicating high risk of bias, and ‘Unclear’ indicating either lack of information or uncertainty over the potential for bias (15).

### *Quantitative Data Synthesis*

In factorial trials and in multi-arm designs yielding two or more iron intervention groups (for example, different dose or duration) and a single control group, the data in the intervention groups, including the variation in the intervention characteristic, will be pooled and compared against the single comparison arm to prevent unit-of-analysis error (16). However, for multi-arm designs employing iron salts with wide variation in bioavailability (for example, ferrous sulphate or fumarate versus NaFeEDTA) we will split the ‘shared’ comparison group into two or more groups with smaller sample size, and include two or more (reasonably independent) comparisons. For dichotomous outcomes, both the number of events and the total number of participants would be divided up. For continuous outcomes, only the total number of participants would be divided up and the means and standard deviations left unchanged (16).

For variables usually known to have a skewed distribution (for example serum ferritin), pooling will be performed on natural logarithm transformed values (17). In order to maximize the data input for the pooled outcome measures, we will primarily utilize the post-intervention values (means and SDs) in preference to the changes from baseline, which may not be reported in all trials (18). For the primary outcome variable hemoglobin, we will perform sensitivity analyses with change values. The standard deviations for change are likely to be extracted or imputed (from SE, *t* or *p* values) in several but not all studies. In the remaining trials, these standard deviations will be computed by the following assumptions: (i) correlation of 0.5 between the pre-intervention and post-intervention variances (19); and (ii) pre- intervention and post-intervention samples considered to be independent (no correlation).

For cluster-randomized trials, the stated cluster adjusted values will be used, irrespective of the method employed. In the absence of this information, authors will be requested to



provide the design effect and/or we will perform sensitivity analysis using a design effect inflation of SE (16) based on prudent estimates from other cluster randomized trials.

Data entry and initial analysis will be performed on SPSS ® (Version 13.0) software. Meta-analysis and meta-regression will be performed with user written programmes on Stata ® (version 9.2) software. The presence of bias in the extracted data will be evaluated quasi-statistically using the funnel plot (20). Formal statistical tests for funnel plot asymmetry, namely the Begg's and Egger's methods will also be conducted with the user written "metabias" command in the STATA ® (version 9.2) software (21,22). Pooled estimates of the evaluated outcome measures will be primarily calculated by the generic inverse variance method by the user written "metan" command in STATA ® (version 9.2) software (21,23). This program also computes the formal tests of heterogeneity, namely, the statistic Cochran Q and I-squared (variation in pooled estimate, for example, weighted mean difference attributable to heterogeneity) (24). Binary outcomes will be pooled as RR and 95% CI. If the continuous outcome variable is reported in different units or measurements across trials, for pooled estimates the standardized mean difference (pooling as standard deviations instead of actual measurements) will be used instead of weighted mean difference (for example, for serum transferrin). Pooled estimates will be made using both fixed effects and random effects model assumptions. The random effects model will be preferred if there is evidence of significant heterogeneity (I-squared >25% and/or P<0.05 for Cochran Q).

Sensitivity and sub-group analyses (specified below) will be conducted for the primary outcome hemoglobin to explore predictors of positive response. This will be done by disaggregating results with the user written "metan" command ("by option") in STATA ® (version 9.2) software (21,23). The contribution of these variables to heterogeneity will also be explored by meta-regression using the "metareg" command in STATA ® (version 9.2) software with the restricted maximum likelihood option (25). The specified variables for sub-group analyses and metaregression include: (i) Risk of bias (low versus others); this will also distinguish quasi-randomized trials from properly randomized trials (26), (ii) Age group (only children versus adults included), (iii) Fortification vehicle; (iv)

Fortification compound; (v) Iron consumed as fortificant (mg); (vi) Fortification duration (months); (vii) Compliance estimation (directly observed or replacement versus others); (viii) Baseline hemoglobin ( $\leq 120$  versus  $>120$  g/L); (ix) Baseline serum ferritin (geometric mean  $\leq 20$  versus  $>20$   $\mu\text{g/L}$ ); and (x) Geographic location (developed versus developing countries; and malarial endemic areas versus malarial non-endemic areas if data permits).

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