



INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADHESH

Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh

Phone : 871751-60, Telex : 675612 ICDDR B]

Fax : 880-2-883116, 886050, 871568, 871686, Cable : Cholera Dhaka

Memorandum

16 August 1999

To : Dr. Abdullah-hel Baqui
Public Health Sciences Division

From : Professor Mahmudur Rahman
Chairman, Ethical Review Committee

Sub : Protocol # 99-014

This has reference to your memo of 10th August 1999 along with the modified copy of your protocol # 99-014 entitled "A community-based, randomized, controlled trial to assess the efficacy of iron and/or zinc or a micronutrient mix supplementation to reduce anemia and morbidity and to improve growth and development in Bangladesh infants". I am pleased to inform you that the protocol is hereby approved upon appropriate addressing of the issues raised by the Committee in its meeting held on 4th August 1999.

Thank you.

copy:- Division Director (A)
Public Health Sciences Division



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Memorandum

09 August 1999

To : Dr. Abdullah-hel Baqui
Public Health Sciences Division

From : Professor Mahmudur Rahman *am*
Chairman, Ethical Review Committee

Sub : Protocol # 99-014

The Ethical Review Committee met on 4th August 1999 and considered your protocol # 99-014 entitled "A community-based, randomized, controlled trial to assess the efficacy of iron and/or zinc or a micronutrient mix supplementation to reduce anemia and morbidity and to improve growth and development in Bangladesh infants". After a thorough review and discussion, the Committee made the following observations to be addressed in your protocol:

- a) on face sheet 2(a), 4 (c) and 4(h) should be marked 'Yes'.
- b) you should include provision of compensatory treatment in case of complications caused due to drawing of blood.
- c) discrepancy between project summary and consent form should be corrected.
- d) you should mention who will draw the venous blood from the subjects.
- e) in some case monthly follow up has been proposed whereas in some place every-other month follow up has been proposed. You should correct this discrepancy.

You are requested to modify the protocol incorporating the above observations and **resubmit** a copy of it to the Chair for necessary action.

Thank you.

copy:- Division Director (A)
Public Health Sciences Division



CENTRE
FOR HEALTH AND
POPULATION RESEARCH

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August 10, 1999

To : Professor Mahmudur Rahman
Chairman, ERC

From : Dr. Abdullah H. Baqui 
PI, Protocol # 99-014

Subject: : Revised protocol #99-014 for approval

Attached please find a revised version of the protocol entitled: "A community-based, randomized, controlled trial to assess the efficacy of Iron and/or Zinc or a micronutrient mix supplementation to reduce anemia and morbidity and to improve growth and development in Bangladeshi infants". During the revision process, we have addressed all the comments made by your committee. We have corrected the face sheet, mentioned in the protocol that compensatory treatment will be provided in case of complications caused due to blood drawing, corrected all discrepancies, and mentioned that venous blood will be drawn by a medically qualified person.

The revised protocol may pleased be approved.

Best regards.

cc: Director, PHSD

Revised on: 10/8/99

(FACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator: Dr. Abdullah H. Baqui

Trainee Investigator (if any): _____

Application No. 99-014

Supporting Agency (if Non-ICDDR,B) _____

Title of Study: A community-based, randomized, controlled trial to assess the efficacy of Iron and/or Zinc or a micronutrient mix supplementation to reduce anemia and morbidity and to improve growth and development in Bangladeshi infants

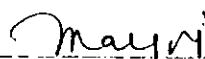
Project Status: _____

☒ New Study☐ Continuation with change☐ No change (do not fill out rest of the form)

Circle the appropriate answer to each of the following (If Not Applicable write NA)

1. Source of Population:
 - (a) Ill subjects ☒ Yes ☒ No
 - (b) Non-ill subjects ☒ Yes ☐ No
 - (c) Minor or persons under guardianship ☒ Yes ☐ No
 2. Does the Study Involve:
 - (a) Physical risk to the subjects ☒ Yes ☒ No
 - (b) Social risk ☒ Yes ☒ No
 - (c) Psychological risks to subjects ☒ Yes ☒ No
 - (d) Discomfort to subjects ☒ Yes ☐ No
 - (e) Invasion of privacy ☒ Yes ☒ No
 - (f) Disclosure of information damaging to subject or others ☒ Yes ☒ No
 3. Does the Study Involve:
 - (a) Use of records (hospital, medical, death or other) ☒ Yes ☐ No
 - (b) Use of fetal tissue or abortus ☒ Yes ☒ No
 - (c) Use of organs or body fluids ☒ Yes ☐ No
 4. Are Subjects Clearly Informed About:
 - (a) Nature and purposes of the study ☒ Yes ☐ No
 - (b) Procedures to be followed including alternatives used ☒ Yes ☐ No
 - (c) Physical risk ☒ Yes ☐ No
 - (d) Sensitive questions ☒ Yes ☐ No
 - (e) Benefits to be derived ☒ Yes ☐ No
 - (f) Right to refuse to participate or to withdraw from study ☒ Yes ☐ No
 - (g) Confidential handling of data ☒ Yes ☐ No
 - (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure ☒ Yes ☐ No
 5. Will Signed Consent Form be Required:
 - (a) From subjects ☒ Yes ☒ No
 - (b) From parents or guardian (if subjects are minor) ☒ Yes ☐ No
 6. Will precautions be taken to protect anonymity of subjects ☒ Yes ☐ No
 7. Check documents being submitted herewith to Committee:
 - ☐ Umbrella proposal - Initially submit an with overview (all other requirements will be submitted with individual studies
 - ☒ Protocol (Required)
 - ☒ Abstract Summary (Required)
 - ☐ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw) (Required)
 - ☐ Informed consent form for subjects
 - ☐ Informed consent form for parent or guardian
 - ☐ Procedure for maintaining confidentiality
 - ☐ Questionnaire or interview schedule*
- * If the final instrument is not completed prior to review, the following information should be included in the abstract summary
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy
 2. Example of the type of specific questions to be asked in the sensitive areas
 3. An indication as to when the questionnaire will be presented to the Committee for review

We agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.



Principal Investigator

Trainee

RESEARCH PROTOCOL

1. Title of Project: **A community-based, randomized, controlled trial to assess the efficacy of Iron and/or Zinc or a micronutrient mix supplementation to reduce anemia and morbidity and to improve growth and development in Bangladeshi Infants**

2. Investigators :

2a. Name of the Principal Investigator including position and qualifications:

Dr. Abdullah H Baqui, MBBS, MPH, DrPH
Senior Epidemiologist and Head, Child Health Program (CHP)
Public Health Sciences Division (PHSD), ICDDR,B

2b. Name of Co-Principal Investigators including position and qualifications:

Dr. George Fuchs, MD
Interim Director, ICDDR,B

Dr. K. Zaman, MBBS, MPH, PhD
Epidemiologist, CHP, PHSD, ICDDR,B

2c. Co-investigators:

Professor Lars Ake Persson, Director, PHSD
Dr. Shams El Arifeen, CHP, PHSD
Dr. Jena Hamdini, Clinical Sciences Division
Mr. MA Wahed, Laboratory Sciences Division
Dr. Md. Yunus, Head, MHRP, PHSD
Dr. Nigar Shahid, CHP, PHSD
Mr. J Chakraborty, MHRP, PHSD

2d. Collaborating Investigators including position and qualifications:

Dr. Robert E Black, MD, MPH
Professor & Chairman, Department of International Health
Johns Hopkins University

Dr. Maureen Black, PhD
Professor, University of Maryland

3. Name of the Division/ Branch / Programme of ICDDR,B under which the study will be carried out: Child Health Programme, PHSD

4. Dates of Proposed Period of Support (Day, Month, Year):

August 1, 1999 - January 31, 2001

5. Cost Required for the Budget Period:

5a. Direct Cost : US\$ 200,596

Total Cost : US\$ 250,745

6. Sponsor(s): USAID/Washington and Nutricia Foundation

7. Approval of the Project by the Division Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

Professor Lars Ake Persson



July 1, 1999

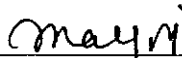
Name of the Division Director Signature

Date of Approval

8. Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

9. Signature of PI:



Date: July 1, 1999

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PROJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application.

Diets given to infants in most developing countries are often deficient in multiple micronutrients including iron and zinc. Consequently, many of these infants develop multiple micronutrient deficiencies, particularly iron and zinc deficiency by six months of age. Iron deficiency during early childhood can cause anemia and impairs the child's physical and mental development. Recent controlled trials have shown a therapeutic effect of zinc on diarrhoeal episodes, preventive effects on diarrhoea and ALRI morbidities, positive effects on growth, immune function, and child development. Thus, in countries where there is no immediate solution to correct micronutrient deficiencies through dietary approaches, it is important to define the need and feasibility of micronutrient supplementation of infants 6 months onwards to prevent anemia, reduce morbidity, and to improve growth, mental, psychomotor and behavioural development.

The proposed study is a community-based, prospective, double-blind, randomized, controlled trial to evaluate the efficacy of weekly supplementation of iron and/or zinc or a micronutrient mix for six months in infants beginning 6 months of age. Five groups: i) 20 mg iron with 1 mg riboflavin, ii) 20 mg zinc with 1 mg riboflavin, iii) both iron and zinc with riboflavin, iv) a micronutrient mix, and v) riboflavin only (placebo) will be studied.

The following outcome variables will be measured: a) Iron status, b) Zinc status, c) Copper status, d) diarrhoeal morbidity, e) Growth, and f) Cognitive, psychomotor, and behavioural development. Eight hundred infants from selected Matlab villages meeting the eligibility criteria will be enrolled in the study over a six month period. Infants will be stratified by height-for-age z-scores using -2 z-score height-for-age as the cut-off. The infants in each strata will then be randomized to one of the five study groups using a block randomization procedure. Trained CHWs will visit the infants every week to collect morbidity data. The CHWs will feed the infants the assigned supplement during the weekly home visits. To assess growth, weight, length, and MUAC of study children will be measured at enrollment and thereafter every month. Finger prick blood samples will be collected from each study infant at the start and end of the follow-up and will be tested for haemoglobin. About 3 ml venous blood samples will be collected by a trained nurse from 50% of the study infants at the start and end of the follow-up and will be tested for serum transferrin receptor (sTfR), ferritin, zinc, and copper. An assessment of mental, psychomotor, and behavioural developments will be carried out in a 50% sample of study infants at the start and end of the trial (6 months and 12 months) by a trained psychologist using Bailey's Scale of Infant Development, version 2. Dietary data will be collected from a 10% sample of children to assess the dietary intake of zinc, iron and other micronutrients which would help identify any differences in zinc/iron intake in the different treatment groups.

Data will be analyzed to assess the efficacy of various types of supplementation on the outcomes of interest. To examine the potential additive and multiplicative effects of providing zinc, iron, and other micronutrients, multiple regression analysis will be done with dummy variables representing supplementation groups (e.g., zinc versus no zinc, iron versus no iron). To examine additive effects of zinc and iron, the dummy variables will serve as independent variables. To determine possible multiplicative effects, the interaction of dummy coded variables will be examined.

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

Concisely list in order, in the space provided, the hypothesis to be tested in the proposed study. Provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

1. In a population deficient in multiple micronutrients, particularly iron and zinc, long term supplementation of iron and/or zinc or a micronutrient mix in infants 6 months onward will:
 - i) reduce anemia (in infants who will receive iron alone or in combination);
 - ii) reduce the incidence and prevalence of diarrhoeal illness (in infants who will receive zinc alone or in combination);
 - iii) improve growth (in infants who will receive zinc or iron alone or in combination); and
 - iv) improve cognitive, motor, and behavioural development (in infants who will receive iron or zinc alone or in combination);
2. Infants who will receive both iron and zinc supplementation will have better outcomes in terms of all of the above;
3. Infants who will receive the micronutrient mix (MM) supplementation will have similar outcomes as to both iron and zinc supplementation.

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods (**Type within limits**).

1. To evaluate the efficacy of weekly iron supplementation with or without other micronutrients during infancy on (a) serum iron and zinc status, (b) prevention of iron deficiency anemia, (c) physical growth, and (d) mental development;
2. To evaluate the efficacy of weekly zinc supplementation with or without other micronutrients on (a) serum zinc and copper status, (b) diarrhoeal morbidity, (c) physical growth, and (d) mental, motor, and behavioural development;
3. To examine the inter-relationship between zinc, iron, and other micronutrient supplementation on infants' morbidity, growth, and development;
4. To determine how the efficiency of supplementation with a micronutrient mix (MM) compares with supplementation with zinc and/or iron only;
5. To define the feasibility, acceptability, and potential side effects of long term weekly supplementation.

Background of the Project including Preliminary Observations

Describe the relevant background of the proposed study. Discuss the previous related works on the subject by citing specific references. Describe logically how the present hypothesis is supported by the relevant background observations including any preliminary results that may be available. Critically analyze available knowledge in the field of the proposed study and discuss the questions and gaps in the knowledge that need to be fulfilled to achieve the proposed goals. Provide scientific validity of the hypothesis on the basis of background information. If there is no sufficient information on the subject, indicate the need to develop new knowledge. Also include the **significance and rationale** of the proposed work by specifically discussing how these accomplishments will bring benefit to human health in relation to biomedical, social, and environmental perspectives. (Do not exceed 5 pages, use continuation sheets).

Introduction

Diets given to infants in most developing countries are often deficient in multiple nutrients including iron and zinc. Consequently, many of these infants develop iron deficiency by six months of age (Rahman, 1989; Talukder et al., 1987; Saha et al, 1989). Iron deficiency during early childhood can cause anemia and impairs the child's physical and mental development (Booth and Aukett, 1997; de Andraca et al, 1997). Thus, in countries where there is no immediate solution to correct iron deficiency through dietary approaches, it is important to define the need and feasibility of iron supplementation of infants 6 months onwards to prevent anemia and to improve growth and mental development.

Zinc deficiency is also highly prevalent in developing country children (Sazawal et al. 1995; Sachdev, 1988; Sarker et al., 1985). There is concern that oral iron supplementation may adversely affect zinc absorption. Recent controlled trials have shown that therapeutic use of zinc reduces duration and severity of acute and persistent diarrhoeal episodes (Sazawal et al, 1995; Sachdev 1988, Sachdev 1990). Zinc supplementation has also been shown to prevent acute and persistent diarrhoea, dysentery, and ALRI (Sazawal et al, 1997; Ninh et al, 1996; Ruel et al, 1997; Sempertegui et al, 1996). Zinc also improves growth (Walravens et al 1983; Khanum et al 1988) and the immune function of supplemented children (Fraker et al 1979; Fraker JP 1983; Sazawal et al 1997). Recent controlled trials also found an effect of zinc supplementation on motor development (Maureen Black, personal communication) and on child's response (Ashworth et al, 1998).

Vitamin A deficiency was also common in Bangladesh but periodic vitamin A supplementation in children is now a national program. Deficiencies of other micronutrients such as vitamins D, E, K, niacin, B1, B6, folic acid, riboflavin, pantothenic acid, vitamin B12, ascorbic acid, iodine, copper, manganese, fluoride, selenium, calcium are also presumably common. Although very limited, there is some evidence that supplementation with a micronutrient mix may be more beneficial than supplementation of a single nutrient for some outcomes, e.g. linear growth (Rosado, 1999). However, this is an area which is grossly under studied.

Iron: functions, deficiency, absorption of dietary iron and recommended intake:

Iron is a constituent of hemoglobin, myoglobin, and a number of enzymes and therefore, is an essential nutrient for humans (Bothwell et al., 1979). In addition to these functional forms, as much as 30% of the body iron is found in storage forms such as ferritin and a small amount is

associated with the blood transport protein transferrin. Body iron content is regulated by the changes in the amount of iron absorbed by the intestinal mucosa. The absorption is influenced by body stores (Bothwell et al., 1979; Cook et al., 1974), by the chemical nature of iron in the ingested food (Layrisse et al., 1968) and by a variety of dietary factors that enhance or interfere with iron absorption (Gilloly et al., 1983; Hallberg, 1981). Although the intestinal mucosal regulation is efficient, this regulation may not be adequate to prevent iron deficiency in subjects whose intake is marginal.

Iron deficiency is not always associated with anemia. Three forms of iron deficiency have been identified. In the first stage, the plasma ferritin level is low ($<12 \mu\text{g/L}$) without any functional impairments. In the second stage, the hemoglobin level is normal but transferrin saturation is reduced to less than 16% and usually there is impaired work capacity. The third stage is associated with iron deficiency anemia.

There is no single biochemical indicator available to reliably assess iron deficiency in the general population. Three indicators are often used - serum ferritin, transferrin saturation, and erythrocyte protoporphyrin - and iron deficiency status is considered if at least two of these are abnormal (LSRO, 1985). At times, measurement of ferritin is replaced by mean cell volume (MCV).

Haem iron is highly absorbable but the absorption of non-haem iron is very variable. Nonhaem iron absorption can be enhanced or inhibited by several factors. Ascorbic acid and presence of animal tissues in food enhances the absorption (Gilloly et al., 1983; Cook and Monsen, 1976). In contrast, some dietary substances such as calcium phosphate, phytates and polyphenol in tea decrease nonhaem iron absorption (Gilloly et al., 1983; Monsen et al., 1978).

Because of stored iron (Dahro et al., 1983), the normal term infants can maintain satisfactory hemoglobin levels from human milk without other sources during the first three months of life. From birth to age three years, infants not breast-fed should have an iron intake of 1 mg/kg per day. The RDA for 6 months to 3 years of age 10 mg/day. Low birth weight infants require 2 mg/kg per day, starting no later than 2 months of age (Dallman et al., 1980). Iron deficiency is commonly observed starting at about 6 months of age, because the iron content of milk and commonly used complementary foods are low, the body is growing rapidly and the body reserves of iron are often insufficient to meet needs beyond six months.

Zinc: functions, deficiency, absorption of dietary iron and recommended intake:

Zinc is an essential element for humans. It is a constituent of enzymes involved in most metabolic pathways (Hambridge et al., 1986). Although large amounts of zinc are deposited in bone and muscle, the body pool of readily available zinc is small with a rapid turn over. Zinc status is subject to strong homeostatic regulation. Small amounts of zinc are more efficiently absorbed and individuals with poor zinc status absorb zinc more efficiently. However, this mechanism is not adequate to protect children from zinc deficiency in the face of sustained low intake and symptoms of zinc deficiency can occur quickly in children with marginal zinc status.

The bioavailability of zinc in different foods varies widely (Inglett, 1983). Meat, eggs and seafood are good sources of available zinc. Whole grain products that contain high concentration of phytates and dietary fibres adversely affect zinc availability. The simultaneous ingestion of equal

amounts of iron and zinc reduces zinc absorption, but no such effects occurs with haem iron or when the food source of zinc is used (Solomon and Jacob, 1981). Thus, it is unlikely that the iron-zinc interaction has a major influence on zinc requirements under most dietary conditions (Solomon and Cousins, 1984).

Since the biochemical assays used to measure body zinc status lack sensitivity and specificity, it is difficult to reliably estimate the prevalence of zinc deficiency (Hambridge, 1991). Because the body pool of readily available zinc is small with a rapid turn over, estimates of zinc status fluctuates. Plasma zinc, hair zinc, and dietary reports are often used to estimate zinc status. Given the limitations of biochemical assays, Hambridge (1991) recommended to use response to supplementation as a reliable method for confirming zinc deficiency at the population level. In a recent study in India, low plasma level of zinc was associated with greater effect of supplementation (Sazawal, 1996).

Full term infants consuming only human milk do not show any signs of zinc depletion in the first few months of life (Hambridge et al., 1979). Therefore, their zinc requirement must be satisfied by the breast milk and the liver stores. During the first month of life, breast-fed infants consume an average of 2 mg of zinc per day. Zinc intake through breast milk is not adequate beyond six months of age and needs to be augmented by zinc in solid foods. Zinc deficiency defined by serum zinc levels below $10.0 \mu\text{mol/l}$ is highly prevalent in developing country children including in Bangladeshi children with rates as high as 38-50% (Sarker SA et al, 1985; Sachdev et al., 1988; Sazawal S et al., 1995).

Although the RDA for infants 6-11 months is 5 mg per day, the amount of zinc needed in young infants to maintain a positive zinc balance in areas with a high prevalence of zinc deficiency is unknown. Most efficacy trials of zinc in infancy used a dose of 10 mg of elemental zinc per day. While an inadequate dose may fail to produce the desired effects, there is concern that excessive amounts of zinc can be harmful (Chandra, 1984; Chandra, 1987). There are two categories of adverse effects: direct effect of zinc toxicity and effects of zinc on other micronutrients, specially copper. Acute toxicity, resulting in nausea, vomiting, abdominal cramps, diarrhoea has been observed following ingestion of 2 gram or more of zinc in the form of sulphate (Prasad, 1976). Hypocupremia, microcytosis, and neutropenia was reported in patients given zinc in quantities 10 to 30 times the RDA for several months (Prasad et al., 1978). Impairment of various immune responses was reported in healthy adults after zinc supplementation with amounts 20 times the RDA for six weeks (Chandra, 1984). On balance, there is no evidence that zinc supplementation in the range of 10-20 mg per day or up to 70 mg twice a week causes any toxic effect or clinically significant copper deficiency (Bates et al., 1993).

Other micronutrients: functions, deficiency, and recommended intake:

Thiamin (vitamin B₁) as thiamin pyrophosphate is a coenzyme required for catabolism of carbohydrate (McCormick, 1988). Dietary sources of thiamin include wheat germ, oatmeal, organ meats (liver, heart, kidney), legumes and nuts. The RDA for thiamin in infants aged 6-11 months is 0.4 mg. Thiamin deficiency occurs most frequently in areas where the diet consists mainly of unenriched white rice and white flour. The clinical condition associated with thiamin deficiency is called beriberi. The characteristics signs include mental confusion, anorexia, muscular

weakness, ataxia, oedema, muscular wasting, tachycardia and enlarged heart (Inouye and Katsura, 1965; Platt, 1967).

Riboflavin (vitamin B₂) is a water-soluble vitamin that catalyses many oxidation-reduction reactions (McCormick, 1988). It is readily absorbed in the proximal small intestine and excreted through urine. Dietary sources of riboflavin are liver, kidney, fish, milk, yoghurt, cheese, mushrooms, and broccoli. The RDA for infants aged 6-11 month is 0.5 mg. Deficiency symptoms of riboflavin include angular stomatitis, cheilosis, seborrheic dermatitis and normocytic anaemia.

Pyridoxine (vitamin B₆) is involved in the metabolism of amino acids. It is a water soluble vitamins and widely distributed in plants and animal tissues. The richest sources of pyridoxine are chicken, fish, kidney, liver, eggs. The RDA is 0.6 mg for infants aged 6-11 months. Clinical signs of deficiency include epileptiform convulsions, dermatitis and anaemia.

Vitamin B₁₂ is a cobalt-containing porphyrin, termed cobalamin. It can be converted to cobalamin coenzymes which are necessary in metabolism. Vitamin B₁₂ is supplied primarily by animal products. Plant foods are essentially devoid of vitamin B₁₂. The RDA for vitamin B₁₂ is 0.5 µg. Vitamin B₁₂ deficiency results in macrocytic megaloblastic anaemia, in neurological symptoms due to demyelination of the spinal cord and brain and the optic and peripheral nerves.

Vitamin C (Ascorbic acid) is a water soluble vitamin that acts as an antioxidant. Vegetables and fruits (orange and other citrus fruits) contain relatively high concentration of vitamin C. The RDA has set for infants at 35mg/day. Its deficiency leads to scurvy, a serious disease characterized by weakening of collagenous structure that results in widespread capillary hemorrhage (Hornig, 1975; Woodruff, 1975).

Vitamin D (calciferol) is a fat soluble vitamin. Exposure of the skin to ultraviolet light catalyses the synthesis of vitamin D. It is essential for the proper formation of the skeleton for mineral homeostasis. The RDA for vitamin D has been set 10 µg (400 IU). Dietary sources of vitamin D include fish, liver, oils, eggs. Rickets (deformation of the skeleton) is the characteristic results of deficiency of vitamin D in children.

Vitamin E, a fat soluble vitamin is a potent antioxidant. It prevents propagation of the oxidation of unsaturated fatty acids. The richest sources of vitamin E are vegetable oils, wheat germs and nuts. Meat, fish, animal fats and most fruits have little vitamin E. The RDA is 4 mg of α -tocopherol. Its deficiency signs include reproductive failure, muscular dystrophy and neurological abnormalities.

Vitamin K is involved in the regulation of blood clotting. The major sign of vitamin K deficiency is the defective coagulation of blood (Suttie, 1985). Green leafy vegetables are the best dietary sources. A recommended dietary allowance is 10 µg.

Calcium is a major constituent of bone. About 99% of body calcium is present in the skeleton. Its absorption is enhanced by vitamin D and impaired by foods containing oxalate or phytate. The extra skeletal calcium plays an essential role in nerve conduction, muscle contraction, blood clotting and membrane permeability. Dietary sources include dairy products, soft bones of fish, vegetables and nuts. The RDA for infants aged 6-11 months is 600 mg.

Phosphorus is present mostly in the bone. It plays an important role in many chemical reactions in the body. Meats, poultry and fish are major sources of phosphorus. The RDA is 500 mg. Its deficiency results in bone loss and is characterized by weakness, anorexia, malaise and pain.

Selenium acts as an antioxidant and has a close metabolic interrelationship with the antioxidant vitamin E (Hoekstra, 1975). Sea foods, kidney and liver are good sources of selenium. Fruits and vegetables contain little selenium. Gross selenium deficiency may result cardiomyopathy characterized by cardiomegaly, heart failure and arrhythmias (Chen et al, 1980). The RDA is 15 µg.

Micronutrients, infection, and immunity:

There is increasing evidence from recent controlled trials of the potential role of zinc in the improvements of immune status and in the prevention and treatment of diarrhoea, malaria, and pneumonia. A number of recent studies documented a significant preventive effect of zinc supplementation on diarrhoeal morbidity. A community-based, double blind, randomized trial in India observed a 26% lower diarrhoeal incidence and a 35% lower prevalence in children aged >11 months who received daily zinc supplementation for a six months period (Sazawal et al, 1997). A trial of zinc supplementation (10 mg/day) in growth retarded Vietnamese children observed a 71% lower diarrhoeal incidence (Ninh, 1996). A similar trial in Mexico found a 36% lower incidence of diarrhoeal episodes in zinc supplemented (20 mg/day) children (Rosado et al, 1997). Sazawal et al (1996) also observed that zinc supplementation reduces the incidence of persistent diarrhoea in zinc deficient children and reduces the risk of dysentery in boys. A report from Guatemala also indicated that zinc supplementation reduces the incidence of all diarrhoea (Ruel et al. 1997).

A number of recent trials have documented the therapeutic effect of zinc in diarrhoea. Sazawal et al. (1995) examined the effect of zinc supplementation (20 mg elemental zinc/day) in children aged 6 to 35 months with acute diarrhoea in a community-based, double blind, controlled trial. Zinc supplementation was associated with significant overall reduction of 23% in the risk of continued diarrhoea on a given day and a 37% reduction in the severity of diarrhoeal episodes when zinc was provided early in the episode. When supplementation was initiated within 3 days of the onset of diarrhoea, there was a 39% reduction in the frequency of episodes lasting more than 7 days after the beginning of the treatment. Similar reduction in diarrhoeal episode duration and severity were observed with zinc supplementation during acute diarrhoea in Bangladesh (Roy et al., 1997) and during acute and persistent diarrhoea in hospitalized children in India (Sachdev et al. 1988, Sachdev et al 1990).

The link between diarrhoea and zinc is well established. Diarrhoea leads to zinc loss and abnormalities of zinc metabolism. Substantial amount of zinc is lost during acute diarrhoea. It was observed that daily losses of zinc in children with diarrhoea could be as high as 159 µg/kg/day, compared with 47 µg/kg/day in control children (Castillo-Duran et al, 1988).

The possible mechanisms for the effect of zinc supplementation on the duration and severity of diarrhoea include improved absorption of water and electrolytes by the intestines (Ghishan, 1984; Patrick et al., 1980; Golden et al., 1985; Patrick, 1978), regeneration of gut epithelium (Bettger

et al., 1981; Elems and Jones 1980; Arcasoy, 1990; Moran, 1985; Weaver et al., 1985; Roy et al., 1992). increased levels of enterocyte brush-border enzymes (Gebhard et al., 1983; Jones et al., 1981), and enhanced immunologic mechanisms for the clearance of infection. Zinc supplementation improves immunity (Fraker et al., 1979; Fraker, 1983; Sazawal et al. 1997) and thereby may promote rapid clearance of diarrhoeal pathogens from the intestine. It is also possible that improved appetite and dietary intake result in shorter duration of diarrhoea (Brown, 1994).

The literature on the association of zinc and respiratory morbidity is limited and mixed (Lombeck et al., 1988; Van Wuuwe et al 1986, Bondestam et al 1985). Two studies reported a reduction in respiratory morbidity (Sempertegui et al., 1996; Ninh et al., 1996) while two others did not (Rosado et al., 1997; Ruel et al 1997). A recent community-based controlled trial conducted in India documented a substantial efficacy of zinc supplementation in reducing pneumonia morbidity. Zinc supplementation was associated with 56% reduction of the incidence of pneumonia and 51% reduction in the days spent with pneumonia (Sazawal et al, 1998). A possible mechanism for the effect of zinc supplementation on reduction of pneumonia morbidity could be enhanced immune status. Another possible mechanism of zinc supplementation is a direct antiviral effect (Eby et al., 1984; Al-Nakib et al., 1987).

The association between iron deficiency, immunity and infection is more controversial. Observational studies revealed that iron deficiency impairs immune functions (Kulapong et al., 1974; Hasan et al., 1989). However, findings of the clinical trials that related iron supplementation with morbidity provided conflicting results. Some investigators reported a decline in morbidity (Angels et al., 1993; Chwang et al., 1988) while other found no such association (Javaid et al., 1991; Moffat et al., 1994).

Micronutrients and Growth:

Although it is well recognized that zinc deficiency produces delayed growth, the benefits of zinc supplementation on incremental growth is controversial. Some studies found a positive effect of zinc supplementation on growth (Gibson et al., 1992; Walravens and Hambridge, 1976; Walravens et al., 1983; Xue-Cun et al., 1985), whereas others did not find that effect (Carter et al., 1969; Cavan et al., 1983; Meeks Garden et al., 1998; Rosado, 1999). This discrepancy can be at least in part explained by the differences in the population (age group, geographic location etc) studied. The benefits in terms of growth was particularly marked in low birth weight and stunted infants. In a recent meta-analysis in which 25 zinc supplementation studies were reviewed, Brown (1995) found a pooled effect of 0.2 SD on height and 0.26 SD on weight. Both effects were positive and small but nevertheless significant. In Bangladesh, the low birth weight rates are very high and majority of the children are either undernourished or marginally nourished (Baqui et al, 1993; Shams El Arifeen, unpublished data). Thus, the benefits of zinc supplementation on growth may be substantial. The catch-up growth following zinc supplementation may be due to improved appetite (Brown, 1994), better absorption of nutrients (Ghishan, 1984, Patrick et al 1980, Golden et al 1985, Patrick 1978) and increased protein synthesis after zinc supplementation (Khanum et al. 1988).

The effect of iron supplementation on growth is also controversial. Angeles et al (1993) demonstrated a positive effect of iron and ascorbic acid on linear growth compared with supplement of ascorbic acid alone. The effect was attributed to a reduction in morbidity observed

in the iron supplemented group. Others have not found an effect of iron supplementation on growth (Migasena et al., 1972; Gershoff et al., 1988; Rosado, 1999).

The lack of effect on linear growth observed in some population with zinc or iron supplementation could be due to two reasons. One possibility could be that the prevalence of iron or zinc deficiency in some of those population was very low precluding any added benefits from supplementation. Alternatively, the children in some setting could be deficient in several other nutrients, which may in itself have impaired growth or impaired the potential positive effects of zinc and/or iron. A recently concluded study in Mexico reported effects on linear growth in infants/children supplemented with a micronutrient mix containing vitamins A, D, E, K, niacin, B-1, B-6, folic acid, pantothenic acid, iodine, copper, manganese, fluoride, selenium, ascorbic acid, riboflavin, vitamin B-12, iron and zinc Rosado, 1999). Further evaluation of this strategy in a variety of setting will be useful.

Micronutrients and Child Development:

A number of studies observed delayed/inadequate cognitive and motor development in infants/children with iron deficiency and improvements in developmental scores after iron supplementation (Lozoff et al., 1982a; Lozoff et al., 1982b; Lozoff et al., 1987; Walter et al., 1983; Walter et al., 1989). However, a large iron supplementation trial in Chile did not find any differences in mental and motor test scores at 12 months between supplemented and non-supplemented infants (Lozoff et al., 1996). The Chilean trial excluded all newborns below 3 kg. The evidence to-date suggest that the effect of iron supplementation on cognitive development is more pronounced in babies with small-for-gestational age. In Bangladesh, about three-quarter of the babies fall in this category (Sham El Arifeen, unpublished data).

Recent data suggest that zinc deficiency may also be associated with deficits in activity, attention, and motor development, particularly in children in under-privileged communities and/or born with small-for-gestational age. Zinc supplementation was found to be associated with improved motor development among very low birth weight infants (Friel et al., 1993), more vigorous activity among Indian infants (Sazawal et al., 1996), and higher level functional activity and responsiveness among Guatemalan and Brazilian infants/children (Bently et al., 1997; Ashworth et al., 1998). In contrast, Cavan et al (1993) did not find any association between zinc supplementation and measures of attention through standardized test performance among school age children. Thus, it seems that the timing and duration of supplementation is important.

Although both iron and zinc deficiency have been associated with deficits in cognitive development, to-date, no published studies examined the inter-relationship between the two micronutrients on this outcome. A UNICEF supported multi-country study is currently examining this relationship where the supplements are being given daily.

The mechanism of iron and/or zinc deficiency related cognitive deficits are not clear. There may be both structural and interactional components (Pollitt, 1987; Maureen Black, personal communication). In the first few years of life, rapid development occurs in the central nervous system and any nutritional deficits at this time can lead to neurological or processing problem.

Programmatic and policy relevance of the proposed research:

Despite many controversies in the iron and zinc literature, the balance of evidence suggest that both iron and zinc supplementation should be beneficial for infants, particularly for those who born with small-for-gestational age (SGA) and/or those raised in deprived communities. Thus, in developing country infants such as in Bangladeshi infants, where SGA rates high, infectious diseases are highly prevalent, and dietary insufficiencies of multiple micronutrients, particularly zinc and iron are highly prevalent, assessment of the efficacy of supplementation with iron and/or zinc or a micronutrient mix would be important. Most of the earlier studies assessed the effects of iron or zinc separately. As many of the micronutrients have synergistic (e.g. vitamin C enhances nonhaem iron absorption) and antagonistic (e.g. iron interferes with zinc absorption, zinc interferes with copper absorption, calcium interferes with iron absorption) effects, it would be important to study the separate and joint effects of these micronutrients. To our knowledge, only two published studies to-date examined the combined effects of iron and zinc supplementation on morbidity and/or growth (Rosado, 1999; Thu BD et al., 1999). The first study found that zinc supplementation reduced morbidity but neither zinc nor iron supplementation affected growth but supplementation of a micronutrient mix did have a significant effect on linear growth. The second study observed a significant increase in linear growth but only in stunted children. Unlike vitamin A, the total available body store of iron and zinc are small and maintenance of iron and zinc-sufficient status requires long term supplementation in frequent dosage.

A UNICEF supported multi-country study is currently examining this relationship where the supplements are being given daily. From the programmatic point of view, this would not be easy. We propose to provide iron and/or zinc supplement weekly instead of daily. The need to identify target populations that will benefit from supplementation, the formulation of the supplement, the timing, dose, interactions between different micronutrients, duration, and frequency of supplementation are all seem to be important. With large scale supplementation program, factors such as cost, availability, and distribution of supplements and compliance with prescribed supplement intake often reduce the program's effectiveness (Schultink et al., 1996). Therefore, alternative supplementation strategies need to be investigated to raise the effectiveness. Supplementation on a weekly basis instead of daily is cheaper and may be easier to manage. A number of studies including a recent study by Bui et al (1999) concluded that daily and weekly supplementation of iron or zinc have similar effects in preschool children.

In addition, we propose to supplement the infants in the fifth study arm of our study with a micronutrient mix (MM) mainly because the programs are moving in that direction. Two arguments are often provided to justify supplementation with a micronutrient mix: a) population deficient in iron and/or zinc are likely to be deficient in other micronutrients and therefore if there is another limiting micronutrient, the effect of giving extra zinc or iron may not be seen; and/or b) if programs are giving supplement why not give all the micronutrients since the additional cost is minimal. However, there is very little evidence to-date that supplementation with a MM will provide any added benefit. A recently completed three cell controlled trial in a cohort of 6-35 month old children in Lima, Peru comparing the protective efficacy of six month supplementation of zinc alone, zinc with a MM and a placebo on fever, cough, diarrhoea and ALRI morbidity reported that for all the illness variables the zinc group had the lowest incidence/prevalence and the MM group had the highest (even higher than the placebo group) incidence/prevalence (Penny et al. unpublished manuscript). The investigators of this study concluded that they were unable

to determine which component of their MM was responsible for the apparent detrimental effect but suspected that it could be due to iron. The Peru study did not have an iron only group. Because of public health significance of this finding, the efficacy of different formulation needs to be further investigated before deciding which formulation would be most beneficial. If iron and/or zinc or a micronutrient mix is proved to be beneficial, it would be attractive to combine them into a single preparation to improve cost-effectiveness and justify the strategy.

Research Design and Methods

Describe in detail the methods and procedures that will be used to accomplish the objectives and specific aims of the project. Discuss the alternative methods that are available and justify the use of the method proposed in the study. Justify the scientific validity of the methodological approach (biomedical, social, or environmental) as an investigation tool to achieve the specific aims. Discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Point out safety procedures to be observed for protection of individuals during any situations or materials that may be injurious to human health. The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment of the project. **(Do not exceed ten pages, use continuation sheets).**

Study Design:

A community-based, prospective, double-blind, randomized, controlled trial is proposed to evaluate the efficacy of weekly supplementation of iron and/or zinc or a micronutrient mix for six months in infants beginning 6 months of age. Five groups: i) iron with riboflavin, ii) zinc with riboflavin, iii) both iron and zinc with riboflavin, iv) a micronutrient mix, and v) riboflavin only (placebo) will be studied.

The following doses will be used:

- i) Iron group: 20 mg of elemental iron and 1 mg of riboflavin every week.
- ii) Zinc group: 20 mg of elemental zinc and 1 mg of riboflavin every week.
- iii) Both iron and zinc group: 20 mg of elemental iron, 20 mg of elemental zinc, and 1 mg of riboflavin every week.
- iv) Micronutrient mix: In addition to 20 mg of elemental iron, 20 mg of elemental zinc, and 1 mg of riboflavin, 2 RDA of the vitamins C, D, E, K, niacin, B-1, B-6, B-12, folic acid, pantothenic acid, iodine, calcium, copper, manganese, selenium every week.
- v) Riboflavin group: 1 mg of riboflavin weekly.

As vitamin A supplementation is a national program, infants in all groups will receive 100,000 IU of vitamin A at the beginning of the study. This will allow us to evaluate the efficacy of various micronutrient formulations independent of the efficacy of vitamin A.

Study Population:

The trial will be carried out at Matlab, a rural field research area of ICDDR, Bangladesh, originally developed for field evaluation of cholera vaccines. To support field studies, a

Demographic Surveillance System (DSS) was established in 1966. This system currently operates in 144 villages containing about 210,000 population. The DSS gathers vital event information such as births, deaths, migrations on a regular basis through home visits. The demographic database is computerized and regularly updated. Matlab is fairly representative of rural Bangladesh. In 1997, the crude birth and crude death rates were 25.2 and 7.3 per 1,000 populations respectively. The infant mortality rate was 64.7 per 1,000 live births (ICDDR,B-DSS Annual Report, 1997).

Study Subject:

Infants from selected Matlab villages meeting the following inclusion and exclusion criteria will be enrolled in the study:

Inclusion criteria:

- a) Infants between 5-6 months of age at enrollment;
- b) Not fed on infant formulas; and
- c) Permanent resident of the selected villages.

Exclusion criteria:

- a) Severely malnourished infants (weight-for-age <60% of the NCHS reference median) requiring hospitalization;
- b) Severely anemic infants (Haemoglobin <90 gram/L) requiring immediate therapy; and
- c) Infants with neurological disorders, physical handicaps, and chronic illness that may affect feeding, activity and cognitive development.

Outcome Variables and Sample Size Determinations:

The following outcome variables will be measured:

- a) Iron status: Hemoglobin (Hb), Serum ferritin, Serum Transferrin receptor;
- b) Zinc status: Serum zinc;
- c) Copper status: Serum copper;
- d) Morbidity: incidence and prevalence of diarrhoea;
- e) Growth: weight gain and linear growth; and
- f) Child Development: mental, motor, and behavioural development.

Sample sizes were calculated to detect assumed differences between the treatment and comparison groups with 80% power and 95% significance level. The table below provides sample size requirements by various study outcomes. As many earlier studies found an effect on growth and morbidity only in stunted children, we plan to enrol children in the trial stratifying by height-for age z-score. The sample size calculation has taken in to account the need for stratified analysis. Allowing for drop-outs due to out-migration, refusal etc., a sample size of 160 infants in each study groups would be sufficient.

Table. Main outcome variables, incidence/prevalence/proportion in the control group, expected reduction in the treatment group and data collection methods

Outcome Variables	Expected level in the control group	Expected level in the treatment group (expected % improvement/reduction)	Sample size required in each group (after making allowances for 10% drop outs)*
Haemoglobin (g/L)	110.0±15.0	120±15.0 (10%)	106
Zinc (μmol/L)	11.0 ±2.5	13.0±2.5 (18%)	60
Copper (mg/L)	0.9 ±0.1	0.8 ±0.1	40
Diarrhoeal morbidity Incidence/child/year	5.0±2.0	4.0 ±2.0 (20%)	150
Growth			
Effect upon weight gain (mean±sd)	-2.0 ±0.8 z-score weight for height	-1.6±0.8 z-score weight for height	140
Effect upon linear growth (mean±sd)	-2.0 ±0.8 z-score weight for height	-1.6±0.8 z-score weight for height	140
Child development			
Bayley Mental (raw score)	55.0±5.0	60.0±5.0	60
Bayley Motor (raw score)	35.0±5.0	40.0±5.0	60

* Calculated sample sizes have been multiplied by 2 to allow stratified analyses plus allowances for 10% drop-out has been made.

Selection of Study Subjects and Randomization:

On average, about 450 infants are born in the Matlab DSS villages each month. At any given time there will be about 400-500 infants of 5 months of age in the DSS villages. However, to keep the study logistics simpler and to avoid contaminations from other interventions, the study will be limited to a sample of villages in the surveillance area. About 130-140 infants will be enrolled in each month. Therefore, the enrollment of the required number of infants (n=800) will need about 6 months time. A list of eligible infants will be obtained from the DSS database in each month. The infants weight and length will be measured and percentages of NCHS reference median and z-scores weight-for-age and height-for-age will be calculated for each infants. Infants with wight-

for-age <60% of the reference median will be excluded from the study and referred to Matlab Hospital for care. Infants with hemoglobin levels <90 gram/L will also be excluded and referred for care. The remaining infants will be stratified by height-for-age z-scores using -2 z-score height-for-age as the cut-off. The infants in each strata will then be randomized to one of the five study groups using a block randomization procedure. As equal number of infants from each strata will be included in the study, some over sampling of stunted infants may be required.

Description of the Intervention:

The duration of follow-up will be six months from entry into the trial and the onset of supplementation. During this time, each study infant will receive weekly dosage of the assigned supplement or the placebo in the form of syrup. The weekly dose of iron will be 20 mg of elemental iron in the form of ferrous sulphate with riboflavin. The weekly dose of zinc will be 20 mg of elemental zinc in the form of zinc acetate with riboflavin. The five types of supplement: iron with riboflavin, zinc with riboflavin, iron and zinc with riboflavin, a micronutrient mix which will also contain iron, zinc, and riboflavin (exact formulation has been described in the design section), and riboflavin only (placebo) will be presented in the same type of bottle and labeled in a way so that the various types of supplements cannot be differentiated. The use of riboflavin as a placebo should help mask the flavour of B vitamins. Riboflavin was successfully used as placebo in a similar trial that has just been completed in India (Robert Black, personal communication). Blind supplementation will be ensured by coding the 5 treatment groups as A, B, C, D, and E and by putting the syrups in bottles having a corresponding code. Before the beginning of the study, the acceptability of the syrups will be tested in 20 infants. The study workers will feed the supplements to study infants using a sterile syringe during the weekly visits. If an infant is absent on the schedule visit day, the infant will be re-visited within 2-3 days. Each week, the worker will record the infants supplement intake status.

Data Collection:

Baseline Enrollment and measurements:

A list of eligible infants residing in the study villages including their household identification numbers, socio-economic, and demographic information will be obtained from the DSS database. The baseline enrolment will include obtaining written consent of the parents of the study infants, one week retrospective morbidity data, feeding information, and anthropometric measurements. Baseline data will be used: (a) to describe the population, (b) to determine baseline differences between the treatment and comparison groups, and (c) to adjust for baseline differences, if any during data analysis.

Estimation of iron, zinc and copper status:

Finger prick blood samples will be collected from each study infant at the start and end of the follow-up and will be tested for haemoglobin. Venous blood samples will be collected from 50% of the study infant by a trained nurse at the start and end of the follow-up and will be tested for serum transferrin receptor (sTfR), ferritin, zinc, and copper. About seven infants will be enrolled per day and 3-4 of them will be brought to one of the ICDDR,B health sub-centres for enrollment and specimen

collection. About 3 ml blood will be collected in a non-heparin-treated, trace element free tubes. Disposable plastic syringes will be used for blood collection. Hb concentrations will be measured in duplicate in 5 μ l blood immediately after samples are collected according to the cyanomethoglobin method (INACG, 1985) using a portable photometer (Computer Minilab 3: Bayer Diagnostic, Munich, Germany). The remaining blood will be transported on a ice pack to Matlab laboratory within four hours of collection. In the Matlab laboratory, serum will be separated by centrifuging the blood and will be stored at -20°C. The specimens will be transported to ICDDR,B Dhaka laboratory in cold boxes where the specimens will be analyzed.

Iron status will be assessed by analyzing serum ferritin using an immunoradiometric assay (IRMA, Diagnostic Products Corporation, CA, USA) and serum transferrin receptor by an enzyme immunoassay double antibody sandwich method R&D Systems, Minneapolis, USA). Serum zinc and copper will be analyzed using by atomic absorption spectroscopy (Smith et al., 1979).

Anthropometry:

To assess growth, weight and length of study children will be collected by a team of trained health assistant and community health worker. Measurements will include weight, length, and mid-upper arm circumference (MUAC) at enrollment and thereafter every month. Naked weight will be obtained to the nearest 50 grams on a Salter scale standardized with a one kg weight prior to each weight measurement. Recumbent lengths will be measured to the nearest 0.1 cm with a locally constructed length board with a footplate and a head bar. MUAC will be measured to the nearest millimeter by TALC insertion tapes. The mean of two consecutive measurements will be recorded as the observed value. To ensure reliability and accuracy of the anthropometric data, WHO measurement and standardization protocols will be followed. The anthropometric data will be compared to the NCHS reference data and the nutritional status will be assessed by Z scores (Waterlow JH, 1972).

Morbidity Surveillance:

To assess the effect of supplementation on diarrhoea morbidity, a weekly morbidity surveillance will be instituted. Trained Community Health Workers (CHWs) will visit each infant in their homes every week and collect and record information on the occurrence, type, and severity of diarrhoea and other morbidities by interviewing mothers. Diarrhoea will be defined as three or more loose stools in a 24 hour time or one or more loose stools containing blood. A diarrhoeal episode will be considered new if there is three or more diarrhoea free days before the beginning of the episode.

Data on dietary intake of zinc and iron:

Dietary data will be collected from a sample of children to assess the dietary intake of zinc which would help identify any differences in zinc intake in the different treatment groups and would help with external validity of the study.

Individual dietary data will be collected for a 10% systematic sample of the study children. Trained field interviewers will ask each mother of the sampled children to report all foods eaten

by her child in the 24 hours preceding the interview. Using pre-measured utensils and spoons, the interviewers will determine and record the types and amount of all foods that were consumed during the previous day. Nutrient contents including zinc and iron contents of the foods consumed will be calculated using food composition tables relevant for Bangladesh (Nutrient contents of Indian food. C. Gopalan, National Institute of Nutrition, Hyderabad, India).

Assessment of Child Development

An assessment of mental and psychomotor developmental will be carried out in a 50% sample of infants at the start and end of the trial (6 months and 12 months) by a trained psychologist using Bailey's Scale of Infant Development, version 2 (Bayley, 1993). After administration of Bayley's Scales, the examiner will rate the infants behaviour using a modified version of Bayley and Wolke (Wolke, 1990) on a 9-point scale. These ratings include infants activity (very still=1, overactive=9), emotional tone (unhappy=1, radiates happiness=9), approach or responsiveness to examiner in the first 10 minutes (avoiding=1, friendly and inviting=9), cooperation with test procedures (resist all suggestions=1, always complies=9), and vocalization (very quiet=1, constant vocalization=9). This scale was used by one of the investigators (Dr. Jena Hamdini) in her earlier studies and she found a very high inter-observer correlations (approach: $r=0.92$; emotional tone: $r=0.96$; activity: $r=0.88$; cooperation with test procedures: $r=0.96$; and vocalization: $r=0.97$).

The scale rate the infants on behaviors that have been shown to be sensitive to nutritional factors, such as responsivity, vocalization, cooperation, emotional tone, and activity. The testing will be done in one of the ICDDR,B health sub-centres. The results of the tests will be presented as the raw mental and psychomotor scores, the Mental Development Index (MDI), the psychomotor Development Index (PDI), and the Behavioral Rating Scale (BRS).

Data on Compliance of supplementation, and Adverse Events (if any)

As the supplements will be directly fed by the community health workers (CHWs) during home visits, the compliance of supplementation should be high. The CHWs will record the supplementation status of the study infants every week. The occurrence of adverse effects such as diarrhoea, vomiting, black stools etc. will be monitored and recorded.

Methods to Ensure Data Quality:

To ensure data quality, the study supervisors and investigators will make spot checks. In addition, a 5% sample of study children will be re-interviewed and re-measured within two days of the original interview/measurement.

Data Management:

All questionnaires and data forms will be reviewed by the investigators for accuracy, consistency and completeness. Whenever necessary, the CHWs will make additional field visits to clarify inconsistencies or collect missing information. After editing, the data will be entered in databases using on-line custom-designed data entry programs. Necessary range and consistency checks will be in-built. Data will be periodically checked by running and reviewing frequency distributions and cross-tabulations.

Data Analysis:

Baseline characteristics of the different treatment and comparison groups will be examined for group comparability. Any significant baseline differences will be controlled for during data analysis.

The distribution of data will be examined and if the data is not normally distributed, decisions about need for data transformation and on appropriateness of statistical tests will be made. The effect on diarrhoea morbidity in the six month follow-up period will be evaluated by comparing incidence and prevalence rates of diarrhoea in the different treatment and comparison groups. Relative rates for the effect of zinc and/or iron or MM supplementation on incidence will be estimated using poisson regression method. Odds ratio for prevalence will be estimated using marginal logistic regression models. As each month of observation will be treated as the unit of analysis, each child will potentially contribute up to 6 observations. To account for correlation between multiple observations, Generalized Estimating Equation (GEE) method will be used. The monthly weight and length measurements will be used to calculate growth velocities and changes in z-score weight-for-height, weight-for-age, and height-for-age. Differences in the mean growth velocities and z-scores between the intervention and comparison groups will be compared using appropriate statistical tests. For the growth velocity, two stage random effects models appropriate for longitudinal data will be used. For the weight and height increments both fixed and random effects will be modeled. Proc Mixed (SAS release 6.12) will be used for random effect modeling.

To examine the potential additive and multiplicative effects of providing zinc, iron and other micronutrients, multiple regression analysis will be done as described above with dummy variables representing supplementation groups (zinc versus no zinc, iron versus no iron). To examine additive effects of zinc and iron, the two dummy variables will serve as independent variables. To determine possible multiplicative effects, the interaction of dummy coded variables will be examined.

Study Schedule:

August 1999	: Recruit and train staff, select study villages, and start randomizing infants into treatment groups
September 1999 - February 2000	: Enrollment, baseline measurements, implementation of the intervention, and follow-up of study infants
March 2000 - August 2000	: Continue intervention and follow-up
September 2000 - January 2001	: Complete analysis and report preparation

Project title: A community-based, randomized, controlled trial to assess the efficacy of Iron and/or Zinc or a micronutrient mix supplementation to reduce anemia and morbidity and to improve growth and development in Bangladeshi Infants

TOTAL BUDGET

(USAID/W: \$ 200,745/-. and Nutricia Research Foundation: \$50,000)

Total project period: 18 months (August 1999 - January 2001)

<u>SALARY</u>	Pay	# of	% of	Monthly	Year-1	Year-2	Sub-total	USAID	Nutricia	Total
<u>Position</u>	<u>Level</u>	<u>Staff</u>	<u>effort</u>	<u>Rate</u>	<u>(12 months)</u>	<u>(6 months)</u>	<u>for</u>	<u>Part</u>	<u>Part</u>	<u>budget</u>
							<u>Year 1-2</u>	<u>(US \$)</u>	<u>(US \$)</u>	<u>(US \$)</u>
Dr. Abdullah H. Baqui	P-5/7	1	20%	10,036	24,086	12,645		27,909	8,823	36,732
Dr. George Fuchs*		1	5%	-	-	-		-	-	-
Dr. Md. Yunus	NO-D/21	1	5%	1,995	1,197	628		1,825		1,825
Mr. J. Chakrabarty	NO-C/14	1	5%	1,390	834	438		1,272		1,272
Dr. Shams El Arifeen	NO-C/7	1	20%	1,307	3,137	1,647		4,784		4,784
Dr. K. Zaman	NO-C/11	1	40%	1,382	6,634	3,483		3,848	6,268	10,116
Dr. Jena Hamadani	NO-B/11	1	10%	1,075	1,290	677		1,967		1,967
Medical Officer**	NO-A/7	1	25%	790	2,370	207		1,031	1,546	2,577
Data Analyst/Manager	NO-A/6	1	45%	769	4,153	2,180		4,915	1,418	6,333
Office Manager	NO-A/2	1	40%	634	3,043	1,598		3,415	1,226	4,641
Psychologist**	GS-6/1	1	100%	475	5,700	499		6,199		6,199
Programmer	GS-6/2	1	25%	506	1,518	797		2,315		2,315
FRO (New)**	GS-5/1	1	100%	364	4,368	764		5,132		5,132
Medical/Nurse Assist.**	GS-4/1	1	100%	267	3,204	280		0	3,484	3,484
Health Assistant**	GS-3/7	2	100%	328	7,869	689		8,557		8,557
Data Mgt.Assistant Gr-II	GS-3/1	1	100%	224	2,688	1,411		4,099		4,099
Comm. Health Worker Spl Level (CHW)**		12	100%	85	12,240	1,071		6,655	6,656	13,311
Existing/Regular CHWs**	Spl Level	60	5%	105	3,780	0		3,780		3,780
Consultant					5,000	5,000		5,500	4,500	10,000
SUB-TOTAL:					93,110	34,015	127,125	93,204	33,921	127,125
TRAVEL COSTS										
Dhk-Balt-Dhk (2 trips)					5,500	5,500		11,000		11,000
Conveyance cost					2,400	1,700		4,100		4,100
SUB-TOTAL:					7,900	7,200	15,100	15,100	0	15,100
SUPPLIES & OTHER COSTS										
		<u>Qty</u>	<u>Unit price</u>							
Zinc and iron Syrup					4,000	2,000		3,746	2,254	6,000
Portable photometer		2	500		1,000	0		1,000		1,000
Bayley's scale		1	1200		1,200	0		1,200		1,200
Uni-scale		2	100		200	0		200		200
Length boards		3	35		105	0		105		105
Computer/printer/UPS/accessories etc.					2,850	0		2,850		2,850
Office & field supplies					1,000	500		1,125	375	1,500
Communications					350	250		400	200	600
Printing & Publications of forms					1,000	500		1,125	375	1,500
Service charges (daily wagers/CSAs)					2,667	1,333		3,250	750	4,000
SUB-TOTAL:					14,372	4,583	18,955	15,001	3,954	18,955
INTER-DEPARTMENTAL SERVICES										
Laboratory tests					22,000	12,600		34,600		34,600
Transport (land & water)					1,200	500		425	1,275	1,700
Guest house costs					1,500	750		1,950	300	2,250
Medical Illustration					244	122		117	250	367
Xerox, Mimeograph, Library					300	200		200	300	500
SUB-TOTAL:					25,244	14,172	39,417	37,292	2,125	39,417
TOTAL DIRECT COSTS:					140,626	59,970		160,596	40,000	200,596
OVERHEAD @25%:					35,157	14,993		40,149	10,000	50,149
TOTAL PROJECT COSTS:					175,783	74,963		\$200,745	\$50,000	\$250,745

* Covered by ICDDR,B

** Field-based staff have been budgeted for 13 months (one month training + 6 months enrollment + 6 months follow-up)

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Budget Justifications

The total duration of the proposed study will be 18 months. About one month will be required for preparatory activities including recruitment and training of study staff. The study infants will be enrolled over a period of 5-6 months and they will be followed for 6 months. Therefore, the study will involve a total 13 months of field work. Accordingly, the field based staff have been budgeted for a period of 13 months. Data management and analysis will be ongoing activities of the project. The investigators and data analyst have been budgeted for another 5 months to complete data analysis and report preparation.

Justification for field-based staff

Psychologist (one) - for developmental assessment

Medical Assistant (one) with support from the part-time medical officer and the investigators will be responsible for field implementation and day to day supervision of field work and will be responsible for collecting blood samples and data on developmental milestones.

Health Assistants (2) - will be responsible for enrollment of the infants in the study, baseline data collection and collection of two-monthly anthropometric data.

Community Health Workers (12) - will be responsible for weekly morbidity surveillance and collection of dietary data.

Data Staff:

Data Manager/Analyst (45% time) - will be responsible for designing systems for data management/ entry and cleaning; ensuring timely availability of clean data files and analysing data as instructed by the investigators.

Data Management Assistants (1) - responsible for data entry and cleaning.

Consultants: Expert assistance of a child developmental specialist will be needed.

Supplies: One computer, two portable photometer (Computer Minilab 3: Bayer Diagnostic, Munich, Germany), Bayley's Scale, Salter Scale, Length Board, stationaries, printing of forms etc.

Laboratory test: Budget prepared based on the actual unit cost of tests in the ICDDR,B laboratory.

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A community-based, randomized, controlled trial to assess the efficacy of Iron and/or Zinc or a micronutrient mix supplementation to reduce anemia and morbidity and to improve growth and development in Bangladeshi Infants

Abstract summary for the ethical review committee

Diets given to infants in most developing countries are often deficient in multiple micronutrients including iron and zinc. Consequently, many of these infants develop multiple micronutrient deficiencies, particularly iron and zinc deficiency by six months of age. Iron deficiency during early childhood causes anemia and impairs the child's physical and mental development. Recent controlled trials have shown a therapeutic effect of zinc on diarrhoeal episodes, preventive effects on diarrhoea and ALRI morbidities, positive effects on growth, immune function, and child development. Thus, in countries where there is no immediate solution to correct micronutrient deficiencies through dietary approaches, it is important to define the need and feasibility of micronutrient supplementation of infants 6 months onwards to prevent anemia, reduce morbidity, and to improve growth, mental, psychomotor, and behavioural development.

The proposed study is a community-based, prospective, double-blind, randomized, controlled trial to evaluate the efficacy of weekly supplementation of iron and/or zinc or a micronutrient mix for six months in infants beginning 6 months of age. Five groups: i) 20 mg iron with 1 mg riboflavin, ii) 20 mg zinc with 1 mg riboflavin, iii) both iron and zinc with riboflavin, iv) a micronutrient mix, and iv) riboflavin only (placebo) will be studied.

The following outcome variables will be measured: a) Iron status, b) Zinc status, c) Copper status, d) diarrhoeal morbidity, e) Growth, and f) Cognitive, psychomotor, and behavioural development. Eight hundred infants from selected Matlab villages meeting the eligibility criteria will be enrolled in the study over a six month period. Infants will be stratified by height-for-age z-scores using -2 z-score height-for-age as the cut-off. The infants in each strata will then be randomized to one of the five study groups using a block randomization procedure. Trained CHWs will visit the infants every week to collect morbidity data. The CHWs will feed the infants the assigned supplement during the weekly home visits. To assess growth, weight, length, and MUAC of study children will be measured at enrollment and thereafter every month. Finger prick blood samples will be collected from each study infant at the start and end of the follow-up and will be tested for haemoglobin. About 3 ml venous blood samples will be collected by a trained nurse from 50% of the study infants at the start and end of the follow-up and will be tested for serum transferrin receptor (sTfR), ferritin, zinc, and copper. An assessment of mental, psychomotor, and behavioural developments will be carried out in a 50% sample of study infants at the start and end of the trial (6 months and 12 months) by a trained psychologist using Bailey's Scale of Infant Development, version 2. Dietary data will be collected from a 10% sample of children to assess the dietary intake of zinc, iron and other micronutrients which would help identify any differences in zinc/iron intake in the different treatment groups.

To ensure data quality, the study supervisors and investigators will make spot checks. In addition, a 5% sample of study children will be re-interviewed and re-measured within two days of the original interview/measurement. All questionnaires and data forms will be reviewed by the investigators for accuracy, consistency and completeness. Data will be entered in databases using on-line custom-designed data entry programs. Necessary range and consistency checks will be in-built. Data will be periodically checked by running and reviewing frequency distributions and cross-tabulations.

Baseline characteristics of the treatment and comparison groups will be examined for group comparability. Any significant baseline differences will be controlled for during data analysis. The frequency distribution will be examined to assess the distribution of data. If the data is not normally distributed, decisions about need for data transformation and on appropriateness of statistical tests will be made.

Data will be analyzed to assess the efficacy of various types of supplementation on the outcomes of interest. The effect on diarrhoea morbidity in the one year follow-up period will be evaluated by comparing incidence and prevalence rates of diarrhoea in the various treatment groups. Appropriate statistical methods will be used to account for within child correlations. The bi-monthly weight and length measurements will be used to calculate growth velocities and change in z-score weight-for-height, weight-for-age, and height-for-age. Differences in the mean growth velocities and z-scores between the intervention and comparison groups will be compared using appropriate statistical tests.

To examine the potential additive and multiplicative effects of providing zinc, iron, and other micronutrients, multiple regression analysis will be done with dummy variables representing supplementation groups (e.g., zinc versus no zinc, iron versus no iron). To examine additive effects of zinc and iron, the dummy variables will serve as independent variables. To determine possible multiplicative effects, the interaction of dummy coded variables will be examined.

If iron and/or zinc or a micronutrient mix is proved to be beneficial, it would be attractive to combine them into a single preparation to improve cost-effectiveness and justify the strategy.

Strategies to address ethical issues:

1. Infants between 6-11 months of age will be studied in this study because many infants develop iron and/or zinc and/or other micronutrient deficiencies beginning 6 months of age and deficiencies at this age can adversely affect child's health including physical and mental development. Thus, they are the ones who are most likely to benefit from this intervention.
2. There is no real risk involved in this study except the minor risk of infection related to blood collection. Treatment will be provided free of cost if any complication arises due to drawing of blood. Earlier studies showed that the proposed doses of iron, zinc and other micronutrients are completely safe.
3. Sterile non-touch techniques will be used to collect blood samples to reduce the risk of infection.
4. Identity of all study participants will remain confidential. Records will be used by study staff only in connection with carrying out their obligations relating to the clinical trial and every effort

will be made to keep the records as confidential as possible. All data forms will be kept in a locked file cabinet. Data will be analyzed and published using subjects' identification number only without reference to any name or other identity.

5. The mother/caretaker of the study infants will have the study explained to them and will be asked if they agree to participate in the study. Those who agree to participate will be required to sign the consent form. No information regarding potential risk will be withheld.
6. The mothers/ caretakers of study children will be interviewed to collect data on diarrhoea and ARI morbidities in the study infants every week. In addition, dietary data will be collected from a sample of study children once every two months. This interview will not take more than 15 minutes.
7. Children who will receive the supplements are likely to experience fewer episodes of diarrhoea and ARI and improved physical growth and mental development. In addition, if the proposed intervention is proved to be beneficial and incorporated with the existing program, it will significantly improve child health and survival.
8. Matlab Demographic Surveillance System records will be used to select the study subjects. About 3 ml blood will be collected 2 times from 50% of the study infants.

CONSENT FORM

Study Title: A community-based, randomized, controlled trial to assess the efficacy of Iron and/or Zinc or a micronutrient mix supplementation to reduce anemia and morbidity and to improve growth and development in Bangladeshi Infants by *Abdullah H Baqui et al.*

Elements of informed consent:

Studies have shown that diets given to infants in most developing countries are often deficient in multiple micronutrients including iron and zinc. Consequently, many of these infants develop multiple micronutrient deficiencies, particularly iron and zinc deficiency by six months of age. Iron deficiency during early childhood causes anemia and impairs the child's physical and mental development. Controlled trials have shown a therapeutic effect of zinc on diarrhoeal episodes, preventive effects on diarrhoea and ALRI morbidities, positive effects on growth, immune function, and child development. Thus, in countries where there is no immediate solution to correct micronutrient deficiencies through dietary approaches, it is important to define the need and feasibility of micronutrient supplementation of infants 6 months onwards to prevent anemia, reduce morbidity, and to improve growth, mental, psychomotor and behavioural development.

This randomized, community-based, controlled trial is to test the efficacy of supplementation of various formulations of micronutrients in infancy. As a parent of a child you are requested to allow your child to participate in this study. The study is sponsored by ICDDR,B. It is essential that you understand that: i) taking part in the study is entirely voluntary and ii) you may withdraw from the study at any time without loss of benefits to which you are otherwise entitled.

If your child participates in this study s/he will receive one of the following supplements every week for six months beginning six months of age: i) 20 mg iron with 1 mg riboflavin, ii) 20 mg zinc with 1 mg riboflavin, iii) both iron and zinc with riboflavin, iv) a micronutrient mix, or v) riboflavin only (placebo). A trained CHW will visit your child every week to collect morbidity data. This CHW will feed the child the assigned supplement during the weekly home visits. To assess growth, weight, length, and MUAC of study children will be measured at enrollment and thereafter every month. Finger prick blood samples will be collected from each study infant at the start and end of the follow-up and will be tested for haemoglobin. About 3 ml venous blood samples will be collected from 50% of the study infants at the start and end of the follow-up and will be tested for serum transferrin receptor (sTfR), ferritin, zinc, and copper. An assessment of mental, psychomotor, and behavioural developments will be carried out in a 50% sample of study infants at the start and end of the trial (6 months and 12 months) by a trained psychologist using Bailey's Scale of Infant Development, version 2. In addition, dietary data will be collected from a sample of children to assess the dietary intake of zinc and iron which would help identify any differences in zinc/iron intake in the different treatment groups. Your child may or may not be selected for collection of venous blood, mental and psychomotor assessment, and collection of dietary data. The identity of your child will remain confidential in any publications resulting from this study. The records may be reviewed by representatives of the ICDDR,B as part of their responsibility to oversee this study. By signing this consent document, you agree to such inspection and disclosure.

Consent: The study described above has been explained to me, and I voluntarily consent to participate in it. I, _____, RID _____, age _____ having full capacity to consent do hereby give consent for my child to participate in this study.

Signature of ^{LTI}parent: _____ Date: _____

Name of witness: _____ Signature of witness: _____

ප්‍රකාශිත

පාලනකර ප්‍රකාශිත කළාට ම ප්‍රසිද්ධතාවය (කෙසේ නිකුත්
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මේ පාලනකර නිකුත් ප්‍රතිපත්තිමය ප්‍රතිපත්තිමය ප්‍රතිපත්තිමය
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(11) ଦାମଦାନୀ ଏହା ଧ୍ୟାନ ସମୟ ସିଦ୍ଧିରୁ ଶୁଦ୍ଧି ମାତ୍ରରେ
 ଶୁଦ୍ଧ ସଫଳତା ନିମନ୍ତେ ନିମ୍ନ ଧ୍ୟାନ । (ମାତ୍ରରେ ଧ୍ୟାନରୁ
 ସମସ୍ତ ସଫଳତା ଧ୍ୟାନ ଶୁଦ୍ଧି ଥିବାରୁ ।

(iv) ଶାନ୍ତିନଗର ସ୍ଥିତିରୁ ମଧ୍ୟମ ଗଣତନ୍ତ୍ର (v) ଶୁଣିବାକୁ ବିରୋଧାଭିମୁଖ ।

১৮৮৩ খ্রিঃ ১০২৫ বঙ্গাব্দে জন্মগ্রহণ করেন।

ଅନୁଷ୍ଠାନର ସମସ୍ତ କର୍ମଚାରୀଙ୍କୁ ଏହି ନିୟମାବଳୀର ଅନ୍ତର୍ଗତ ଥିବା ବିଷୟରେ ଜଣାଇ ଦିଆଯାଉଛି ।

ସ୍ୱାକ୍ଷର _____ ତାରିଖ _____

ସ୍ୱାକ୍ଷର _____ ପଦବୀ _____

ଅନୁଷ୍ଠାନର ସମସ୍ତ କର୍ମଚାରୀଙ୍କୁ ଏହି ନିୟମାବଳୀର ଅନ୍ତର୍ଗତ ଥିବା ବିଷୟରେ ଜଣାଇ ଦିଆଯାଉଛି ।

ସ୍ୱାକ୍ଷର _____ ତାରିଖ _____

ସ୍ୱାକ୍ଷର _____

ସ୍ୱାକ୍ଷର _____ ପଦବୀ _____